

International society for gastrointestinal hereditary tumours—InSiGHT

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The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) is an international multidisciplinary, scientific organization. Its mission is to improve the quality of care of patients and their families with any condition resulting in hereditary gastrointestinal tumours. This mission will be accomplished by:

1. Encouragement of research into all aspects of gastrointestinal hereditary tumour syndromes.
2. Education of physicians and other healthcare professionals in the molecular genetics and clinical management of gastrointestinal hereditary tumour syndromes.
3. Assistance for institutions and individuals interested in beginning or maintaining a registry for families with gastrointestinal hereditary tumour syndromes.

Provision of a forum for the presentation of data, discussion of controversial areas involved in the care of patients and their families, and facilitation of collaborative studies.

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6th Biennial InSiGHT Meeting 2015

Venue
Instituto de Ensino e Pesquisa
Hospital Sírio-Libanês –São Paulo - Brazil

Support
GETH—Study Group on Hereditary Tumours—www.geth.org.br
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**HOSPITAL
SÍRIO-LIBANÊS**

**Programme—6th Biennial InSiGHT Meeting
2015—São Paulo—Brazil**

June 18th 2015—Thursday

7:00—Registration Opens

8:00—Official Opening

Benedito Mauro Rossi—Chairman
David Uip—Secretary of Health—São Paulo State - Brazil

8:05—Session 1

Chairpersons
Hans Vasen—Finlay Macrae

Henry Lynch Lecture (20 min)

Introduction by Patrick Lynch

What epidemiology tells us about Lynch Syndrome
Mark Jenkins

Oral presentations (60 min)

(presentation: 7 min + questions: 3 min)

103—Impact of Colonoscopy on Risk of Colorectal
Cancer for Members of Lynch Syndrome Families
Driss Ait Ouakrim

6—Vaccination with Monocyte-Derived Dendritic Cells in Lynch
Syndrome Patients: Vigorous T Cell Responses to Neoantigen Fram-
meshift-Derived Peptides
Nicoline Hoogerbrugge

130—Copy Number Variation Analysis in 85 Suspected Lynch
Syndrome Families Reveals Novel Potential Causative Candidate
Genes
Katrin Kayser

13—Prospective Cancer Risks And Survival In Healthy MMR Mu-
tation Carriers Subject To Surveillance Colonoscopy
Pål Møller

85—Bi-Allelic Somatic Mutations as a Cause of Tumour Mismatch
Repair-Deficiency in Colorectal Cancer: Implications for Identifying
Mismatch Repair Gene Mutation Carriers Within Population-Based
Colorectal Cancer
Daniel Buchanan

49—Cancer Risks in Family Members of CMMR-D Patients
Maartje Nielsen

Review (20 min)

Hereditary Breast and Colorectal Cancer
Annika Lindblom

10:00—Break ("include" electronic posters)

10:30—Session 2

Chairpersons
Allan Spigelman—Mark Jenkins

Review (20 min)

Hereditary Diffuse Gastric Cancer
Susan Parry

Oral presentations (60 min)

(presentation: 7 min + questions: 3 min)

55—Evidence of Influence of Aspirin on Mucosal Immune Status and an The Carcinogenic Effects of Obesity Support The Need For The Dose Non-Inferiority Study, CAPP3
John Burn

198—Better Education Is Needed For Both HNPCC Family Members And Their Providers
Dennis James Ahnen

16—Extracolonic Cancer In Lynch Syndrome
Christina Therkildsen

190—Yearly Gastroscopy In MLH1 And MSH2 Mutation Carriers—
an Endoscopy too Far?
Susan Parry

76—Identifying Lynch Syndrome Using Universal Colorectal Cancer Screening: Implications of Patient Age
Matthew F Kalady

14—Time Between Colonoscopies, Colorectal Cancer Incidence and Death in MLH1 Mutation Carriers
Pål Møller

Aldred Scott Warthin Lecture (20 min)
Introduction by Matthew Kalady
The MMR system: from bench to bedside
Gabriel Capellá

12:30—Lunch (including electronic posters)

12:45–13:45
InSiGHT Council Meeting & Lunch (by invitation only)

14:00—Session 3
Chairpersons
Annika Lindblom—James Church

Review (20 min)
GI cancers in Li-Fraumeni Syndrome
Maria Isabel W. Achatz

Oral presentations (60 min)
(presentation: 7 min + questions: 3 min)

201—Results of High/Moderate Cancer Gene Panel Tests in An Ethnically Diverse Patient Population
Monica M. Alvarado

138—Exome Sequencing of an Amsterdam-Positive Family Identifies a Novel Causal Gene For Hereditary Non-Polyposis Colorectal Cancer
Laura Valle

95—Spectrum Of Cancer Phenotypes In Asian Lynch Syndrome Families
Chun Gan

106—Updating The Insight Database To Meet The Challenges The Genome Sequencing Era
John-Paul Plazzer

45—Metachronous Colorectal Cancer in a General National Cohort From 1943 to 2012 and its Relevance as Indicator of Hereditary Colorectal Cancer
Lars Joachim Lindberg

98—Short-Term Risk of Colorectal Cancer For Lynch Syndrome: a Meta-Analysis
Mark Jenkins

Meera Khan Lecture (20 min)
Introduction by Thomas Weber
Towards universal screening for Lynch Syndrome
Ian Frayling

16:00—Break (including electronic posters)

16:30—Session 4
Chairpersons
Patrick Lynch—Gabriela Moeslein

Review (20 min)
Chemotherapy for Hereditary GI Cancer
Paulo Hoff

Oral presentations (25 min)
(presentation: 2 min + questions: 1 min)

81—MLH1 Mutation Type And Frequency In Colorectal Carcinomas Demonstrating Solitary Loss of PMS2 Protein Expression
Daniel Buchanan

137—Specific Bacterial Sequences Determination In Feces Identifies Higher Colorectal Neoplasia Risk Subgroup Among Lynch Syndrome Carriers
Gabriel Capellá

97—Validation of Lynch Syndrome Prediction Models in Asian Populations
Chun Gan

129—Common Genetic Variants Within The TERT Gene and Risk of Colorectal Cancer For DNA Mismatch Repair Gene Mutation Carriers
Daniel Buchanan

160—Uptake of Genetic Testing Among Relatives of Lynch Syndrome Carriers in a United States Cancer Genetics Registry
Elena Martinez Stoffel

172—Germline MLH1 Mutations in Individuals with PMS2 Deficient Tumours
Kara Semotiuk

57—The Forgotten GI Cancers in FAP
Sarah-Jane Yvonne Walton

31—Activated Systemic Dendritic Cell Phenotype In Familial Adenomatous Polyposis (FAP)—Does APC Mutation Affect The Antigen Presenting Cells Of The Innate Immune System?
Gui Han Lee

Discussion of selected cases—interactive (40 min)
Francisco Lopez—Fabio Ferreira
Moderated by Patricia Prolla

18:30—Reception

June 19th 2015—Friday

8:00—Session 5
Chairpersons
Maurizio Genuardi—John Paul Plazzer

Sir Ian Todd Lecture (20 min)
Introduction by Susan Clark
New colorectal cancer predisposition genes identified by NGS
Nicoline Hoogerbrugge

Oral presentations (60 min)

(presentation: 7 min + questions: 3 min)

12—Risk of Extracolonic Cancers For Carriers of Biallelic and Monoallelic Mutations in *MUTYH*
Aung Ko Win

7—Frequency and Phenotypic Spectrum of Germline Mutations in *POLE* and Seven other Polymerase Genes in 266 Patients with Colorectal Adenomas and Carcinomas
Isabel Spier

116—Polymerase Proofreading-Associated Syndrome: *POLE* and *POLD1* Mutations In Hereditary Colorectal Cancer and Polyposis
Laura Valle

71—Mutations in DNA Polymerase Genes (*POLD1* & *POLE*) in Individuals Having Early-Onset Colorectal Cancer and/or Multiple Adenomas
Guy Rosner

208—Macrolide Induced Read-Through of APC Nonsense Mutations In Familial Adenomatous Polyposis
Rina Rosin-Arbesfeld

58—MicroRNA Expression Associated with Desmoid Tumours in FAP
Sarah-Jane Yvonne Walton

Review (20 min)

Cancer risk and *MUTYH* mutations
Stefan Aretz

10:00—Break (including electronic posters)

10:30—Session 6

Chairpersons
Susan Parry—Thomas Weber

Eldon Gardner Lecture (20 min)

Introduction by Allan Spigelman
Management of upper GI tract in FAP
Andrew Latchford

Oral presentations (60 min)

(presentation: 7 min + questions: 3 min)

199—Experience With Pancreas-Sparing Duodenectomy For Familial Adenomatous Polyposis
R Matthew Walsh

24—Risk Modifying Factors in Patients With PMS2 Or *MUTYH* Mutations
Sanne Willy Ten Broeke

80—Expanding The Mutation Spectrum and Phenotype of Polymerase Proofreading-Associated Polyposis: Novel and Previously Reported *POLE* Variants in an Italian Series
Maurizio Genuardi

48—Experiences and Attitudes Towards Directly Approaching Individuals at High Risk of Hereditary Cancer
Helle Vendel Petersen

39—Duodenal Disease In MAP
Sarah-Jane Yvonne Walton

151—Randomized Comparison of Surveillance Intervals in Familial Colorectal Cancer
Simone Désirée Hennink

Debate—interactive (20 min)

Laparoscopic surgery and desmoid risk
Yes—James Church versus *No*—Lucio Bertario
Moderated by Miguel Rodriguez-Bigas

12:30—Lunch (including electronic posters)

12:30–14:00

InSiGHT Variant Interpretation Committee (VIC) Meeting

14:00—Session 7

Chairpersons
Elena Stoffel—Miguel Rodriguez-Bigas

Dick Bussey Lecture (20 min)

Introduction by Gabriela Moeslein
Risk reduction strategies for hereditary CRC
John Burn

Oral presentations (60 min)

(presentation: 7 min + questions: 3 min)

150—Exome Sequencing Identified Potential Causative Candidate Genes for Hyperplastic Polyposis Syndrome
Christina Astrid Trueck

110—Long Term Data For Chemoprevention In Colorectal Disease In Familial Adenomatous Polyposis (FAP)
Andrew Latchford

200—Increasing Incidence Of Colorectal Cancer (CRC) Among Young Adults In The U.S. Challenges Insight And Current Epidemiologic Tools To Explain And Reverse The Trend
Thomas Kenneth Weber

123—*BETA2*-Microglobulin Mutations and NK Cell Mediated Cytotoxicity In Microsatellite Unstable Colorectal Cancer
Matthias Kloor

148—*MMR*-Deficient Crypt Foci as Cancer Precursors in Lynch Syndrome—Evidence From Tumor Histology
Aysel Ahadova

192—Frequency of *CDH1* Germline Mutations in Early-Onset Gastric Cancer in Brazil
Rodrigo Santa Cruz Guindalini

Review—Ileal Pouches

Pouch for clinicians and surgeons—Gabriela Moeslein (10 min)
The aging pouch—James Church (10 min)

16:00—Break (including electronic posters)

16:00–17:00

InSiGHT Business Meeting

17:00—Session 8—Kay Neale Session

Chairpersons
Fabio Ferreira—Fabio Guilherme Campos

Debate—Interactive (20 min)

Does genotype influence surgical decision making in FAP and Lynch syndrome?
Miguel Rodriguez-Bigas and Susan Clark
Moderated by Gabriel Capellá

David Jagelman Lecture (20 min)

Introduction by Maurizio Genuardi

From Leeds Castle Polyposis Group & International Collaborative Group in Hereditary Non-Polyposis Colorectal Cancer—ICG-HNPCC—to InSiGHT
Patrick Lynch

Oral presentations (25 min)
(presentation: 2 min + questions: 1 min)

78—Can Oral Rehydration Therapy Correct The Metabolic Disturbances and Improve Quality Of Life After Colectomy?
Sreelakshmi Mallappa

176—Survival Rate of Patients who Develop Cancer in Rectal Stump After Colectomy And IRA In FAP Patients
Marco Vitellaro

77—Surgical Management of MYH-Associated Polyposis: is More Better?
Matthew F Kalady

38—Ureteric Complications of Intra-Abdominal Desmoids
Sarah-Jane Yvonne Walton

101—Utility of Single Nucleotide Polymorphisms to Guide Risk Appropriate Colorectal Cancer Screening
Mark Jenkins

124—Molecular Alterations in Mismatch Repair-Deficient Crypt Foci in Lynch Syndrome
Matthias Kloor

68—Adenomas in Lynch Syndrome: The Perfect Storm of Colorectal Carcinogenesis
James Michael Church

88—Miss-Rate and Delay in Diagnosis of Serrated Polyposis Syndrome in a Clinical Cohort
Yasmijn Josanne Van Herwaarden

18:30—End of the day

20:30—Dinner

June 20th 2015—Saturday

9:00—Session 9

Chairpersons
Raul Cutait—Andrew Latchford

TOP 3 Abstracts (30 min)
(presentation: 7 min + questions: 3 min)

11—Environmental Modifiers for The Risk of Colorectal Cancer in Lynch Syndrome
Aung Ko Win

96—A Phase 3 Placebo-Controlled Trial of Celecoxib in Pediatric Subjects with Familial Adenomatous Polyposis
Carol Burke

121—Vaccination Of MSI-H Colorectal Cancer Patients with Frameshift Peptide Antigens—a Phase I/IIa Clinical Trial
Matthias Kloor

Jeremy Jass Lecture (20 min)
Introduction by Pål Møller
Serrated Polyposis Syndromes
Randall W. Burt

10:00—**Break (including electronic posters)**

10:30—Session 10

Chairpersons
Inge Bernstein—Susan Clark

Review (20 min)
PTEN Syndromes
Brandie Leach

Open Lecture (20 min)
Introduction by Luiz Fernando Lima Reis
Genetic profile of the Brazilian population
Sergio Pena

Round Table (10 min presentations—70 min)
Global Collaborations: The way forward
Moderated by John Burn and James Church

107—Worldwide Study of Cancer Risks for Lynch Syndrome: International Mismatch Repair Consortium (IMRC)
Mark Jenkins

15—Prospective Cancer Risks and Survival in MMR Mutation Carriers Having Survived First Cancers
Pål Møller

InSiGHT and The Human Variome Project: The Variant Interpretation Committee
Maurizio Genuardi

Colon Cancer Family Register (CCRF)
Finlay Macrae

Mallorca Group—www.Mallorca-Group.Eu
Gabriela Moeslein

Collaborative Group of The Americas
Inherited Colorectal Cancer
CGA-ICC—www.cga-icc.org
Elena Stoffel

181—Grupo de Estudios de Tumores Hereditarios (GETH)
Study Group On Hereditary Tumors—www.geth.org.br



Benedito Mauro Rossi

12:30—Closing Remarks

Benedito Mauro Rossi

Invitation for the 7th. Biennial InSiGHT Meeting—Firenze—Italy
Maurizio Genuardi

6 Vaccination with monocyte-derived dendritic cells in Lynch syndrome patients: vigorous T cell responses to neoantigen frameshift-derived peptides

Nicoline Hoogerbrugge¹, Harm Westdorp^{2,3}, Gerty Schreibelt³, Kalijn F. Bol^{2,3}, Marieke E. B. Welzen⁴, J. Han J. M. van Krieken⁵, Tanya Bisseling⁶, Marjolijn Ligtenberg^{1,5}, Winald R. Gerritsen², Carl G. Figdor³, I. Jolanda M. de Vries³

¹Departments of Human Genetics; ²Medical Oncology; ³Tumor Immunology; ⁴Pharmacy; ⁵Pathology; ⁶Gastroenterology; ⁷Radboudumc - Nijmegen, The Netherlands

Background: Mismatch repair (MMR) deficiency in tumor DNA causes shifts in the translational reading frame resulting in the production of altered peptides. Frame-shift peptides (FSP), such as Caspase-5 and TGF- β RII, are considered 'foreign' by the immune system. MMR-deficient Lynch syndrome-associated tumors, expressing these FSPs are characterized by a strong lymphocyte infiltration. Dendritic cells are (DC) the professional antigen-presenting cells of the immune system and decisive in inducing immunity. This is the rationale for vaccination with monocyte-derived DC (moDC) loaded with FSPs to stimulate T-cells to combat Lynch syndrome-associated tumors.

Patients and methods: Lynch syndrome associated CRC patients within 1 year after diagnosis ($n = 3$) and healthy Lynch mutation carriers ($n = 19$) were vaccinated with DC loaded with CEA and FSP MHC class I binding peptides. Patients received up to three vaccination rounds, consisting of three weekly intradermal and intravenous DC injections. After each vaccination round, the presence of antigen-specific CD8+ T cells was assessed in blood and challenged skin. Injection of minute amounts of the DC vaccine resulted in infiltration of immune cells into the skin. Specificity of these skin-infiltrating lymphocytes was assessed by flow cytometry with tetrameric MHC complexes binding to T cells that recognize the indicated peptides.

Results: In most patients, after moDC vaccinations, both FSP- and CEA-specific CD8+ T-cells were present. Additionally CD8+ T-cells specific for Caspase-5 and CEA were already detectable. The functionality of their skin infiltrating T-cells was demonstrated by their capacity to produce high amounts of IFN- γ upon stimulation with target cells loaded with CEA or one of the FSPs. All patients reported flu-like symptoms during 2 days.

Conclusions: Cellular immunotherapy with DC vaccination against CEA and FSP-antigens appears feasible and immune responses towards Lynch syndrome tumor-specific peptides are induced. Our data emphasize the potency of DC-based immunotherapy to enhance the host's antitumor immunity and underline consideration for cancer prevention in healthy Lynch syndrome mutation carriers. The results warrant further investigation in a follow-up randomized trial.

Keywords: Lynch syndrome · Prevention · Vaccination

7 Frequency and phenotypic spectrum of germline mutations in POLE and seven other polymerase genes in 266 patients with colorectal adenomas and carcinomas

Isabel Spier¹, Stefanie Holzapfel¹, Janine Altmüller², Bixiao Zhao³, Sukanya Horpaopan¹, Stefanie Vogt¹, Sophia Chen³, Monika Morak⁴, Susanne Raeder¹, Katrin Kayser¹, Dietlinde Stienen¹, Ronja Adam¹, Peter Nürnberg², Guido Plotz⁵, Elke Holinski-Feder⁴,

Richard P. Lifton³, Holger Thiele², Per Hoffmann¹, Verena Steinke¹, Stefan Aretz¹

¹Institute of Human Genetics, University of Bonn - Bonn, Germany; ²Cologne Center for Genomics, University of Cologne - Cologne, Germany; ³Departments of Genetics, Howard Hughes Medical Institute, Yale University School of Medicine - New Haven, United States; ⁴MGZ - Center of Medical Genetics - Munich, Germany; ⁵Medizinische Klinik; ⁶Biomedical Research Laboratory, University of Frankfurt - Frankfurt, Germany

Purpose: In a number of families with colorectal adenomatous polyposis or suspected Lynch syndrome (HNPCC), no germline alteration in the APC, MUTYH, or mismatch repair (MMR) genes are found. Missense mutations in the polymerase genes POLE and POLD1 have recently been identified as rare cause of multiple colorectal adenomas and carcinomas,¹ a condition termed Polymerase proofreading-associated polyposis (PPAP). The aim of the present study was to evaluate the clinical relevance and phenotypic spectrum of polymerase germline mutations.

Methodology: Targeted next-generation sequencing of the polymerase genes POLD1, POLD2, POLD3, POLD4, POLE, POLE2, POLE3, and POLE4 was performed (Illumina platform) using a sample of 266 unrelated patients (219 mutation negative polyposis patients and 47 familial colorectal carcinoma (CRC) cases with microsatellite stable tumours meeting the Amsterdam criteria). Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne).

Results: The previously described pathogenic POLE mutation c.1270C>G;p.Leu424Val was detected in four unrelated patients, three of them had a positive family history. We could demonstrate that the mutation segregates with the phenotype in all 14 affected members from whom DNA was available. The mutation was present in 1.5 % (4/266) of all unrelated patients, 4 % (3/77) of all familial cases, and 7 % (2/30) of familial polyposis cases. The colorectal phenotype in 14 affected mutation carriers (age at diagnosis 16–63 years) ranged from typical adenomatous polyposis to a Lynch syndrome-like manifestation, with high intrafamilial variability. The occurrence of multiple CRCs was common. Most patients (63 %) had duodenal adenomas, and one case of duodenal carcinoma was reported. Additionally, various extraintestinal lesions including ovarian cancer and glioblastomas were evident. Nine further putative pathogenic variants were identified in four polymerase genes. The most promising was a de novo missense mutation in the exonuclease domain of POLE (c.1306C>T;p.Pro436Ser).

Conclusion: A PPAP was identified in a substantial number of our well characterized sample of polyposis and familial colorectal cancer patients. Screening for polymerase proofreading mutations should therefore be considered, particularly in unexplained familial cases. The present study broadens the phenotypic spectrum of PPAP to duodenal adenomas and carcinomas, and demonstrated a considerable clinical overlap between tumor syndromes based on mutations in DNA repair genes. In addition, we identified novel, potentially pathogenic variants in four polymerase genes.

(Supported by German Cancer Aid, BONFOR programme of the University of Bonn and NIH Centers for Mendelian Genomics)

Reference

1. Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I, Sherborne A, Chubb D, et al. (2013) Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet.* 45:136–44.

Keywords: Familial colorectal cancer · Gastrointestinal polyposis syndromes · Adenomatous polyposis

9 Risk of colorectal cancer for carriers of both a MUTYH and a DNA mismatch repair gene mutation

Aung Ko Win¹, Jeanette C Reece¹, Daniel D Buchanan¹, Loic Le Marchand², Robert W Haile³, Polly A Newcomb⁴, Noralane M. Lindor⁵, John L. Hopper¹, Steven Gallinger⁶, Mark A Jenkins¹

¹The University of Melbourne - Melbourne, Australia; ²University of Hawaii Cancer Center - Honolulu, United States; ³Stanford University - San Francisco, United States; ⁴Fred Hutchinson Cancer Research Center - Seattle, United States; ⁵Mayo Clinic Arizona - Scottsdale, United States; ⁶Mount Sinai Hospital - Toronto, Canada

Purpose: The base excision repair protein, MUTYH, functionally interacts with the DNA mismatch repair (MMR) system.¹ As genetic testing moves from testing one gene at a time, to gene panel and whole exome next generation sequencing approaches, understanding the risk associated with having germline mutations in these two genes will be important for clinical interpretation and management.

Methodology: From the Colon Cancer Family Registry, we identified 10 carriers who had both a MUTYH mutation (6 with G396D, 3 with R274Q, and 1 with Y179C) and a MMR gene mutation (3 in MLH1, 6 in MSH2, and 1 in PMS2), 375 carriers of a single (monoallelic) MUTYH mutation alone, and 469 carriers of a MMR gene mutation alone. We estimated the risk of colorectal cancer between groups of carriers using a weighted cohort analysis.

Results: Of the 10 carriers, 8 were diagnosed with colorectal cancer and all of their tumors were MMR-deficient. Risk of colorectal cancer for carriers of both a MUTYH and a MMR gene mutation was 21.5-times (95 % CI 9.19–50.1; $p < 0.001$) that for carriers of a MUTYH mutation alone, but not materially different from that for carriers of a MMR gene mutation alone (HR 1.94, 95 % CI 0.63–5.99; $p = 0.25$).

Conclusion: Within the limited power of this study, there was no evidence that the risk of colorectal cancer was higher for carriers of both a MUTYH and a MMR gene mutation than for carriers of a MMR gene mutation alone. Our finding suggests MUTYH mutation testing in MMR gene mutation carriers is not clinically warranted.

Reference

- Gu Y, Parker A, Wilson TM, Bai H, Chang D-Y, Lu AL (2002) Human MutY Homolog, a DNA Glycosylase Involved in Base Excision Repair, Physically and Functionally Interacts with Mismatch Repair Proteins Human MutS Homolog 2/Human MutS Homolog 6. *J. Biol. Chem.* 277(13): 11135–42.

Keywords: MUTYH · Lynch syndrome · Colorectal cancer

10 Childhood cancers in families with and without Lynch syndrome

John A Heath¹, Jeanette C Reece¹, Colon Cancer Family Registry^{3,2}, Steven Gallinger³, Robert W. Haile⁴, Stephen N. Thibodeau⁵, Noralane M Lindor⁶, John L. Hopper¹, Mark A. Jenkins¹, Aung Ko Win¹

¹The University of Melbourne - Melbourne, Australia; ²National Cancer Institute - Washington D.C., United States; ³Mount Sinai Hospital - Toronto, Canada; ⁴Stanford University - San Francisco, United States; ⁵Mayo Clinic - Rochester, United States; ⁶Mayo Clinic Arizona - Scottsdale, United States

Purpose: Inheritance of a germline mutation in one of the DNA mismatch repair (MMR) genes or the EPCAM gene is associated with

a well-defined increased risk of colorectal cancer, endometrial cancer and other adult malignancies (Lynch syndrome)^{0,1}. Information about childhood cancers in Lynch syndrome families has not been reported.

Methodology: Using data from the Colon Cancer Family Registry, we compared the proportion of childhood cancers (diagnosed before 18 years of age) in the relatives of 781 Lynch syndrome families with a pathogenic mutation in one of the MLH1 (n = 275), MSH2 (n = 342), MSH6 (n = 99), PMS2 (n = 55) or EPCAM (n = 10) genes with that in 5073 non-Lynch syndrome families.

Results: A total of 41 cases of childhood cancer occurred in 781 Lynch syndrome families (0.053 cases per family) compared with 179 cases of childhood cancer in 5075 non-Lynch syndrome families (0.035 cases per family; $p = 0.02$). The proportion of relatives with a childhood cancer was not significantly different between Lynch syndrome families (41/17,230; 0.24 %) and non-Lynch syndrome families (179/94,302; 0.19 %; $p = 0.19$). There was no statistical evidence of an increased risk of all childhood cancers, hematologic cancers, brain and central nervous system cancers, Lynch syndrome-associated cancers, and other cancers.

Conclusion: The risk of childhood cancers does not appear to be increased in Lynch syndrome families. Larger studies are required to better define risk of the different childhood cancer types in Lynch syndrome.

Reference

- Win AK, Young JP, Lindor NM, et al. (2012) Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. *J. Clin. Oncol.* 30(9): 958–64.

Keywords: Childhood Cancer · Lynch syndrome · Mismatch repair

11 Environmental modifiers for the risk of colorectal cancer in Lynch syndrome

Aung Ko Win¹, Seyede Ghazaleh Dashti¹, Driss Ait Ouakrim¹, Rowena Chau¹, Daniel D. Buchanan¹, Colon Cancer Family Registry², John L. Hopper¹, Mark A. Jenkins¹

¹The University of Melbourne - Melbourne, Australia; ²National Cancer Institute - Washington D.C., United States

Purpose: People with germline mutations in DNA mismatch repair (MMR) genes have a substantially elevated risk of colorectal cancer (known as Lynch syndrome), but the modifiers of this risk are not well established. Identifying modifiers of cancer risk is important for understanding carcinogenesis as well as for genetic counselling, screening, and risk-reduction strategies.

Methodology: This study included 1992 (1126 female) carriers of a mutation in an MMR gene (730 in MLH1, 941 in MSH2, 215 in MSH6, and 106 in PMS2) who were recruited into the Colon Cancer Family Registry. Using Cox proportional hazards regressions weighted to correct for ascertainment bias, we estimated hazard ratios (HRs) and 95 % confidence interval (CIs) for associations between environmental factors and risk of colorectal cancer for MMR gene mutation carriers.

Results: A total of 758 carriers (38 %) were diagnosed with colorectal cancer at a mean age of 42.5 (standard deviation 10.6) years. A decreased risk of colorectal cancer was associated with multivitamin supplement intake (<3 years: HR 0.64, 95 % CI 0.38–1.06; and ≥3 years: HR 0.46, 95 % CI 0.30–0.71), calcium supplement intake (<3 years: HR 0.54, 95 % CI 0.27–1.06; and ≥3 years: HR 0.49, 95 % CI 0.24–0.98), aspirin (for 1–10 years: HR 0.48, 95 % CI

0.26–0.89; and for >10 years: HR 0.28, 95 % CI 0.26–0.89), oestrogen and progestin hormone therapy use (HR per year 0.69, 95 % CI 0.48–0.99), hormonal contraceptive use (<5 years: HR 0.84, 95 % CI 0.53–1.33; and ≥ 6 years: HR 0.57, 95 % CI 0.37–0.87), and being parous (HR 0.58, 95 % CI 0.37–0.91). An increased risk of colorectal cancer was associated with overall alcohol consumption (for ethanol per 14 g/day: HR 1.05, 95 % CI 1.00–1.11) and liquor/spirits consumption (for ethanol per 14 g/day: HR 1.34, 95 % CI 1.23–1.46). An increased risk of rectal cancer was found to be associated with beer consumption (for ethanol per 14 g/day: HR 1.19, 95 % CI 1.03–1.37). **Conclusion:** Environmental factors are important modifiers of the risk of colorectal cancer in Lynch syndrome.

Keywords: Environmental factors · Lynch syndrome · Colorectal cancer

12 Risk of extracolonic cancers for carriers of biallelic and monoallelic mutations in MUTYH

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Purpose: Germline mutations of the DNA base excision repair gene MUTYH are known to be associated with an increased risk of colorectal cancer. The estimated cumulative risks (95 % confidence interval [CI]) to age 70 years were 75 % (41–100 %) for male and 72 % (45–92 %) for female carriers of biallelic mutation, and 7 % (5–11 %) for male and 6 % (4–9 %) for female carriers of a monoallelic mutation [1]. Because these mutations are rare, risk of cancers other than colorectal cancer (extracolonic cancers) for MUTYH mutation carriers are uncertain.

Methodology: We identified families of carriers of 41 biallelic and 225 monoallelic MUTYH mutations from the Colon Cancer Family Registry that were ascertained through family cancer clinics and population cancer registries. Mutation status, sex, age, and histories of cancer were sought from their 5846 first- and second-degree relatives. Hazard ratios (HR) and age-specific cumulative risks of extracolonic cancers for carriers of biallelic and monoallelic in MUTYH, were estimated using a modified segregation analysis that conditioned on ascertainment of the index carriers to produce unbiased estimates incorporating both genotyped and non-genotyped relatives.

Results: Compared with incidences for the general population, HRs (95 % CI) for biallelic mutation carriers were: urinary bladder cancer, 19 (3.7–97); and ovarian cancer, 17 (2.4–115). The HRs for monoallelic mutation carriers were: gastric cancer, 9.3 (6.7–13); hepatobiliary cancer, 4.5 (2.7–7.5); endometrial cancer, 2.1 (1.1–3.9); and breast cancer, 1.4 (1.0–2.0). The estimated cumulative risks (95 % CI) to age 70 years for biallelic mutation carriers were: urinary bladder, 25 % (5–77 %) for males and 8 % (2–33 %) for females; and ovarian cancer, 14 % (2–65 %). These risks for monoallelic mutation carriers were: gastric cancer, 5 % (4–7 %) for males and 2.3 % (1.7–3.3 %) for females; hepatobiliary cancer, 3 % (2–5 %) for males and 1.4 % (0.8–2.3 %) for females; endometrial cancer, 3 % (2–6 %); and breast cancer 11 % (8–16 %). We did not find evidence of an increased risk for cancers at other sites.

Conclusion: These accurate and most precise to date estimates of both relative and absolute risks of extracolonic cancers for MUTYH mutation carriers can be used to guide clinical management.

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Keywords: MUTYH · Extracolonic cancers · Penetrance

13 Prospective cancer risks and survival in healthy MMR mutation carriers subject to surveillance colonoscopy

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Purpose: Published estimates of the penetrance of Lynch syndrome (LS) vary widely. Most are retrospective and invalidated by ascertainment biases. This collaborative effort seeks to reduce bias by limiting ascertainment to prospectively observed incident cancers in demonstrated mutation carriers.

Methodology: The study design is an open observational trial where all cases were subject to secondary prevention by colonoscopy (CC) and general cancer awareness according to the ICG-HNPCC/Bethesda/Mallorca-group guidelines. None had had cancer before or at first planned prospective CC. First cancers diagnosed and death were scored as events. Annual incidence rates in 5-years cohorts from 25 to 70 years were used to calculate cumulative risk for cancer by age.

Results: 884 males and 1028 females LS were observed for 7739 years in MLH1 mutation carriers; 3750 MSH2; 1561 MSH6 and 374 PMS2. 327 cases had 348 first cancers at follow-up. Cumulative percentage risks at age 40 and 70 years and 10 years crude survival were:

Cancer	MLH1	MSH2	MSH6	PMS2	Surv 95 (CI)
Any cancer	22	75–20	79–2	53–0	37–86 (82–90)
Colorectal (CRC)	18.50	13.47	1.20	0.88	88 (82–93)
Endometrium	5.36	3.55	1.46	0.23	99 (90–100)
Ovary	2.96	6.17	0.00	0.88	88 (60–97)
All upper gastrointestinal	1.19	0.50	0.20	0.90	52 (30–70)
All urinary tract	0.2	0.2	0.9	0.0	73 (43–89)

Conclusions: Judged by survival when CRC, endometrial or ovarian cancer was diagnosed the results were good. It is noteworthy that these data do not support the belief that screening colonoscopy prevents the occurrence of colorectal cancer in Lynch syndrome: MLH1 and MSH2 carriers had high incidence of CRC despite screening CC. The different genes had different penetrance and expression when mutated, questioning the uniform screening recommendations advocated all. The results may not be used for predictions in LS patients having had one or more previous cancers. Most LS patients will now survive first cancers: there is limited information on what their future may be, to which end our Prospective Lynch Syndrome Database is designed to provide prospectively observed empirical information. The study is open for centers with similar series to join.

Keywords: Colonoscopy · Cancer incidence · Survival

14 Time between colonoscopies, colorectal cancer incidence and death in MLH1 mutation carriers

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Purpose: We report incident colorectal cancer (CRC) risk in MLH1 mutation carriers subjected to colonoscopy (CC). The Finnish applied a 3-year CC interval compared to 2 years or less in most other centers. This allowed us to examine the hypothesis that less than 3 years between CCs would be associated with lower incidence of CRC and death.

Methods: All cases were demonstrated MLH1 mutation carriers, were subject to secondary prevention by CC and general cancer awareness according to the ICG-HNPCC/Bethesda/Mallorca-group guidelines and had had no cancer before or at first planned prospective CC. First cancers diagnosed and death were scored as events. Annual incidence rates in 5-year cohorts from 25 to 70 years were used to calculate cumulative risk for cancer by age. Series were split into Finnish (Fin) and all other centers combined (Oth), and cancers scored as CRC or all other cancers (XCRC), the latter group included to consider penetrance of cancers not prevented by CC. Cumulative incidences by age and K-M crude survival for CRC were compared.

Results: The Fin series included 4640 observation years, the Oth 3099 observation years.

Series Cumulative incidence 10-years survival (95 % CI)

	40 years (%)	50 years (%)	60 years (%)	70 years (%)	
Fin_CRC	14	26	35	41	92 % (81–97 %)
Oth_CRC	23	38	52	63	91 % (79–96 %)
Fin_XCRC	4	18	33	37	
Oth_XCRC	8	23	37	62	

Observation years after 60 years were limited and results should be interpreted with caution.

Conclusions: The hypothesis that less than 3 years between CCs would be associated with lower incidence of CRC and death was not confirmed. The Finnish population has 80 % lower overall incidence rate of CRC than the other countries (35.0 versus mean 43.6 for the others) [1]. The observed difference was, however, larger: $41/63 = 65\%$. The Finnish LS-team has through three decades organized both predictive testing and national colonoscopic surveillance for their large cohort of carriers. This might have increased the compliance and motivation for both carriers and endoscopists contributing to the results [2].

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15 Prospective cancer risks and survival in MMR mutation carriers having survived first cancers

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Purpose: Estimates of the penetrance of Lynch syndrome (LS) vary widely. Most are retrospective and invalidated by ascertainment biases. Most first cancers in LS are now cured but there is limited information on risk for second or later cancers in survivors. This

collaborative effort seeks to produce information on subsequent prospectively observed incident cancers in LS mutation carriers who survive first cancers.

Methodology: The study design is an open observational trial where all cases were subject to secondary prevention by colonoscopy (CC) and general cancer awareness according to the ICG-HNPCC/Bethesda/Mallorca-group guidelines. All had had cancer before or at first planned prospective CC. First cancer diagnosed and death were scored as events. Relapse from first cancer was not scored as an event. Risk for new primaries and risk for death when new primary was diagnosed were calculated by the Kaplan–Meier algorithm.

Results: 503 male and 656 female LS patients were observed for 3879 years in MLH1 mutation carriers; 2159 MSH2; 875 MSH6 and 168 PMS2. 292 cases had cancers at follow-up. PMS2 carriers had one prospective cancer, no deaths and therefore were not included in further calculations. 10 year survival without a new cancer diagnosed (95 % CI) was: MLH1 68 % (63–73 %); MSH2 67 % (60–73 %); MSH6 87 % (79–92 %) ($p = 0.01$ for difference between groups). 10 years survival after prospectively detected further cancers was: MLH1 86 % (82–89 %); MSH2 92 % (88–95 %); MSH6 96 % (91–99 %) ($p = 0.003$ for difference between groups). Many deaths in the MLH1 carriers were associated with extracolonic cancers known to have serious prognosis.

Conclusions: LS patients continue to develop new cancers after cure of first cancers including extracolonic cancers with a serious prognosis [1]. MLH1 and MSH2 mutation carriers had the highest risk for new cancers and those in MLH1 carriers had the worst prognosis. MSH6 mutation carriers had a lower risk for new cancers and if they occurred the prognosis was better.

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Keywords: Colonoscopy · Risk for second cancer · Survival

16 Extracolonic cancer in Lynch syndrome

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Introduction: Lynch syndrome is a multi-tumor syndrome, which confers an increased risk of colorectal cancer as well as endometrial cancer, cancer of the small bowel, ovaries, upper urinary tract, hepatobiliary tract and brain tumors.

Methods: We used the Danish national hereditary nonpolyposis colorectal cancer (HNPCC) register to calculate the incidence rates and estimate the cumulative risk of other types of extracolonic cancer including kidney cancer, sarcomas, breast cancer, bladder cancer, and prostate cancer. We collected 133 tumors (28 prostate cancers, 18 kidney cancers, 20 breast cancers, 53 bladder cancers and 14 sarcomas) among 1349 mutation carriers and 1886 first-degree relatives and investigated the mismatch repair (MMR) protein expression and microsatellite instability (MSI) status.

Results: We found loss of MMR protein expression in 44–80 % of the tumor subtypes and 44–50 % of the tumors were MSI. The mean

ages at diagnosis were 43–62 years including a mean age at onset of 58 years for prostate cancer. We show that MMR gene mutations carriers have a significantly increased risk of getting bladder cancer and a non-significant higher risk for kidney cancer and prostate cancer. Among the different MMR genes, MSH2 gene mutation carriers had the significantly highest cumulative risk compared to MLH1, PMS2 and MSH6 carriers.

Conclusion: We present evidence that sarcomas, prostate cancer, bladder cancer, kidney cancer and breast cancer are associated with germline MMR gene mutations and suggest that these tumors should be included in the Lynch syndrome tumor spectrum.

Keywords: Extracolonic cancer · Cumulative incidence · Lynch syndrome

18 Predicting outcome in colonoscopic high-risk surveillance

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Objective: Surveillance with colonoscopy in risk-groups for colorectal cancer needs to be based on adequate selection of individuals to examine and a well-devised timing. To stratify the risk of finding neoplasia at colonoscopy a cohort with increased familial risk of colorectal cancer was studied.

Design: Based on family history 1203 individuals with an at least twofold increased risk of colorectal cancer were offered regular colonoscopies. The impact of different variables in the family history was assessed by logistic regression for the prevalence of adenomas and advanced adenomas. Findings at the first colonoscopy were assessed regarding the association with risk of future lesions.

Results: The prevalence of advanced lesions, when controlling for age, was associated with the number of first-degree relatives with colorectal cancer, with an age below 50 in the youngest family-member with colorectal cancer, but not with gender. Family history had a low impact on the prevalence of simple adenomas. The risk of future advanced lesions was only associated with the prevalence of advanced lesions at the screening colonoscopy, whereas a finding of subsequent adenomas was associated with advanced lesions, adenomas and hyperplastic polyps.

Conclusion: Adenomas and advanced lesions were not associated with the same risk factors. In this study the most important risk factors for advanced lesions, including cancer, were the number of first-degree relatives and a young family member with colorectal cancer. Findings of simple adenomas and hyperplastic polyps did not seem to be associated with subsequent advanced lesions.

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Keywords: Colorectal · Familial · Surveillance

19 The effect of genotype and parent of origin on cancer risk and age of diagnosis in PMS2 mutation carriers

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Purpose: Lynch syndrome (LS), a genetically inherited disorder with an increased risk of primarily colorectal cancer (CRC) and endometrial cancer (EC), can be caused by mutations in the PMS2 gene. Variability in cancer prevalence and age of diagnosis has been reported in LS patients. We aimed to test if genotype and/or parent of origin effects (POE) could explain part of this variability.

Methodology: Genotypes and clinical data of 381 European PMS2 mutation carriers were available for analysis. Mutation carriers with loss of RNA expression (group 1) were compared to mutation carriers with retention of RNA expression (group 2). Mutation carriers with a paternally inherited mutation were also compared to those with a maternally inherited mutation. *T* test and Cox regression tests [estimating hazard ratios (HR)] were performed to compare age of cancer diagnosis.

Results: The mean age of CRC diagnosis was 51.1 years (CI 48.2–54.1) in group 1 and 60.0 years (CI 52.5–67.5) in group 2 ($p = 0.035$). No significant differences in mean age of diagnosis were found for EC (mean difference: -5.2 in group 2 compared to group 1, CI: -13.2 to 2.9). Compared to mutation carriers with retained RNA expression, mutation carriers with loss of RNA expression showed slightly higher but non-significant HRs for both CRC (HR: 1.31, $p = 0.38$) and EC (HR: 1.22, $p = 0.72$) in Cox regression analysis. A trend was seen towards females having a lower CRC risk when inheriting the mutation from their father than when they inherited the mutation from their mother. However, this difference was not statistically significant.

Conclusion: A lower mean age at CRC diagnosis and a non-significant higher CRC risk was identified in the group of mutation carriers that shows loss of RNA expression. No significant evidence of a POE was found. Further unravelling and understanding of genotype and other modifying risk factors might potentially allow individual risk stratification and rational surveillance programs in the future.

Financial acknowledgment: Financial support was granted from the Dutch Cancer Society.

Keywords: PMS2 · Genotype · Phenotype

20 Your InSIGHT membership

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Keywords: InSiGHT · Council · Membership

21 Screening of at risk patients for Lynch syndrome at a Brazilian reference cancer center: the experience of the Barretos Cancer Hospital

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Purpose: Lynch Syndrome (LS) is characterized by an inherited predisposition to cancer that affects young patients and is caused by germline mutations in DNA repair genes, mainly MLH1, MSH2 and MSH6. Individuals with LS present vital cumulative risk of 60–80 % for developing colorectal cancer (CRC). Realizing the importance of identifying individuals at risk for LS and its inclusion in the prevention/reduction of cancer risk programs, the Cancer Hospital of Barretos, offers, linked to the Department of Oncogenetics (DO), the genetic testing for hereditary CRC. The work aims to identify probands and family members at-risk and include in personalized programs for prevention/treatment of cancer.

Methodology: All patients referred by DO undergo genetic testing, which occurs in two steps: (1) universal screening, performed by immunohistochemistry (IHC) for repair proteins (mlh1/msh2/msh6 and pms2) and analysis of microsatellite instability (MSI). In addition the presence of the mutation BRAF p.V600E is performed to eliminate the possibility of a sporadic cancer. All patients with IHC and/or MSI altered and without BRAF mutations, are subjected to the second step of the test, which consists in the bi-directional Sanger sequencing of the gene (s) whose protein is absent on the IHQ. The presence of rearrangements is investigated through Multiplex Ligation-dependent Probe Amplification. All potentially deleterious changes detected are confirmed by a second DNA extraction followed by PCR amplification and bi-directional sequencing.

Results: Until now, 333 people from 235 families were referred for genetic testing. All probands had personal/family history of CRC and/or endometrial cancer (met Amsterdam and/or Bethesda criteria). Regarding the universal screening, 60 % (141/235) of patients

presented microsatellite stability and a normal IHC. In 5.1 % (12/235) the test could not be performed due to the absence of tumor cells or poor quality of the material. Ten cases (4.25 %) showed disagreement between MSI and IHC. Among the discordant cases, all patients with normal IHC and the presence of MSI were mutated, while from those patients with altered IHC and with MSS, three were carriers of germline mutations. In fourteen patients (6 %), the BRAF V600E mutation was identified, and, from those, 10 were MSI-high and 4 MSS. From those patients with IHC and MSI altered (57/235) that had a genetic test performed, 52.6 % (30/57) had a deleterious mutation or variant of unknown clinical significance in one of three genes tested (40 % in MLH1, 50 % in MSH2 and 10 % in MSH6). To date 98 relatives were tested, of which 45 are mutated and have been referred for prevention/reduction of cancer programs.

Conclusion: Given the high cost of molecular testing, screening by MSI and IHC is highly predictive of genetic alterations. Detection of germline mutations enables better characterization and clinical management of patients with colorectal cancer and screening of family members at risk, enabling an increase in the rate of early detection of cancer, decrease morbidity and mortality associated with the disease, improving prognosis and expectation life of these patients.

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Keywords: Oncogenetics · Lynch syndrome · Prevention

23 Polyposis families—evolution of tracing and follow up over 90 years

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This poster will describe the history of tracing people at risk of inheriting polyposis and the methods of follow up for those affected used by the staff of our Polyposis Registry.

The Polyposis Registry at our institution was started in 1924 as a research project. By the 1950s the autosomal dominant nature of the syndromes had been identified and surgical management proved to be successful. At this point the “call up” programme was put into place.

From the 1950s into the 1970s the pathologist and his surgical colleagues traced those at risk by writing to patients to ask about their relatives. Once they had enough information it is known that they sometimes made unannounced visits to try to persuade people to agree to attend hospital to be examined!

By the mid-1970s specially designed card and family monitoring charts were used to keep track of those who had and those who had not been examined, as well as their disease status. All family pedigrees were drawn by hand on 20 × 12.5 cm cards.

Computerisation, based on the card system, was implemented in the 1980s and re-developed at the end of the 1990s. The poster will include examples of the letters sent in the 1950s, the record card system, the family monitoring charts used until computerisation and data base screens in current use to illustrate the evolution of this process.

Keywords: Polyposis · Tracing relatives · Surveillance follow up

24 Risk modifying factors in patients with PMS2 or MUTYH mutations

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Purpose: The clinical phenotype of MLH1, MSH2, MSH6 and APC has been thoroughly described. There are, however, still many outstanding questions concerning the clinical phenotype of Lynch Syndrome patients with a PMS2 mutation and, to a lesser extent, MUTYH associated polyposis (MAP). Mutations in the PMS2 gene display a lower penetrance compared to MLH1 and MSH2 for colon and other cancers, and a wide interfamilial variance in clinical phenotype [1]. It is therefore likely that external factors or genetic modifiers are involved in the PMS2 phenotype. Similarly, in MAP patients a high variance in clinical phenotype results in some patients showing hundreds of polyps (adenomas), while others develop colorectal cancer (CRC) in the absence of adenomas. Moreover, the age of cancer diagnosis is highly variable in these patients.

Methodology: To assess whether lifestyle factors influence colon cancer risk and polyp count, lifestyle questionnaires were sent to 193 PMS2 (response rate: 77 %) and 77 MUTYH (response rate 75 %) mutation carriers. A preliminary analysis was performed in IBM SPSS Statistics. Furthermore, the effect of 27 risk modifying single nucleotide polymorphisms (SNPs) from Genome Wide Association Studies (GWAS) in CRC patients was analyzed in a pilot cohort of 154 MAP patients. Genotyping was performed using a KASPar assay.

Results: A trend towards a higher CRC risk with increasing number of pack years for PMS2 mutation carriers was found (17.4 and 10.9 years for those with or without CRC respectively). For MAP patients we identified similar results, namely 18.4 and 13.2 pack years respectively. For two SNPs (Rs3802842 and Rs16892766), a significant pair wise effect of 7 years on mean age of CRC diagnosis was identified between patients with zero and more than one risk allele ($p = 0.042$).

Conclusion: Lifestyle factors such as smoking seem to influence CRC risk in PMS2 and MUTYH mutation carriers, although results are preliminary at this moment. Two SNPs, previously described as associated with lower mean age of CRC development in Lynch Syndrome, were found to have an effect on MAP patients. Data on PMS2 patients and results of a larger cohort of over 300 MAP patients is expected to follow within 2–3 months' time. New data on modifiers may facilitate the identification of high risk PMS2 and MUTYH mutation carriers and help provide these carriers with tailored colon surveillance, thereby lowering their risk for CRC.

Financial acknowledgment: Financial support was granted from the Dutch Cancer Society.

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Keywords: PMS2 · Variance · Modifiers

31 Activated systemic dendritic cell phenotype in familial adenomatous Polyposis (FAP)—Does APC mutation affect the antigen presenting cells of the innate immune system?

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Purpose: Dendritic cells (DCs), potent antigen presenting cells, play an essential role in acquiring tumour antigens and initiating anti-tumour cytotoxic T-lymphocyte reactions [1]. In cancer, systemic DCs are dysfunctional and tumours “escape” immune surveillance, most likely due to tumour-derived factors [2]. Systemic DCs in colorectal cancer (CRC) have increased expression of activation, migration and gut homing markers compared with those in age and sex-matched healthy controls [3]. However, systemic effects of APC mutation on DC phenotype in the absence of CRC had not been investigated. Our study aimed to identify differences in systemic DC phenotype in individuals with FAP, prior to development of CRC.

Methodology: Ficoll-separated peripheral blood mononuclear cells (PBMC) were obtained, from individuals with FAP with identified APC germline mutation prior to colectomy (n = 15) and age, sex-matched healthy controls. Resected colonic specimens had no high-grade dysplasia, cancer or evidence of acute inflammation. DCs were identified within PBMC as HLA-DR positive and negative for lineage cocktail (CD3-CD14-CD16-CD19-CD34-CD56-), using flow cytometry. DCs were further classified as myeloid (mDC; CD11c+) and putative plasmacytoid (pDC; CD11c-). Expression of activation and maturation markers (CD40, CD80, CD83 and CD86), lymph node migration marker (CCR7), gut homing marker (β 7) and skin homing marker (CLA) on DCs was determined.

Results: CD40 expression on all DCs was increased in FAP compared with control (FAP: 33 %, control: 16 %, $p = 0.003$). This increase was evident in both subpopulations of DCs; mDCs (FAP: 36 %, control: 18 %, $p = 0.0058$) and pDCs (FAP: 30 %, control: 14 %, $p = 0.0059$). There were no differences in CD80 CD83, CD86, CCR7, β 7 and CLA expression in FAP compared with control.

Conclusion: Activation of systemic DCs in FAP, without advanced neoplastic or inflammatory changes in the colon, suggests a possible role of APC mutation in initiating this effect. Our results suggest that individuals with FAP may have subtle inherent changes in systemic DC phenotype and function prior to development of CRC. However, further studies are required to understand the exact mechanism and effect of APC mutation on DC function.

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Keywords: Dendritic cell · Immunology · Cancer

32 Dendritic cells in the distal colonic mucosa display more tolerogenic phenotype compared with the proximal mucosa in familial adenomatous polyposis (FAP)

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Purpose: Mucosal dendritic cells (DCs) are potent antigen presenting cells, which maintain the balance between immune tolerance to commensal bacteria and immunogenicity against pathogens and tumours in the colon. In health, proximal colonic DCs are more immunogenic and distal colonic DCs more tolerogenic, which is most likely influenced by the changing microbiota load in the colon [1]. However, possible differences in immune profile between these compartments in the colon of individuals with FAP have not been characterized. We therefore determined the phenotypic differences between proximal and distal mucosal DCs in FAP—to identify any similar trends to healthy colon or any differences due to presence of adenomas.

Methodology: Paired proximal and distal mucosal samples, free of macroscopic adenomas, were obtained from colonic specimens after prophylactic colectomy in individuals with FAP (n = 9). The polyp count and distribution were assessed. DCs within cells released by collagenase digestion were identified as viable immune cells expressing high HLA-DR and low lineage cocktail (CD3-CD14-CD16-CD19-CD34-CD56-). Expression of CD40, CD83, CD86 (activation and maturation markers), ILT3 (a marker for immature DC), CCR7 (a lymph node migration marker) and β 7 (gut-homing marker) on DCs were determined by flow cytometry.

Results: In all colonic specimen, there were higher polyp counts in the distal colon compared with the proximal colon. There was no evidence of high-grade dysplasia or cancer in any specimen. Proximal mucosal DCs in FAP expressed higher CD40 (proximal: 34 %, distal: 13 %, $p = 0.0436$), CD83 (proximal 64 %, distal 20 %, $p = 0.0002$) and CD86 (proximal 76 %, distal: 42 %, $p = 0.0313$) compared with paired distal mucosal DCs. In distal mucosal DCs, there was higher expression of ILT3 (distal: 56.37 %, proximal: 22.09 %, $p = 0.0025$) and β 7 (distal: 64.10 %, proximal 16.19 %, $p = 0.0062$). There was no difference in expression of CCR7.

Conclusion: The distal colon contends with a higher bacterial load than the proximal colon. In FAP, proximal mucosal DCs displayed a more activated and mature phenotype compared with distal mucosal DCs and maybe promoting immunogenicity. By contrast, mucosal DCs in the distal colon were immature and more gut homing, which may promote tolerance to the microbiota [2]. Despite the presence of hundreds of polyps in the distal colon in individuals with FAP, mucosal DCs remained immature. Our results show similar immunological trends to those seen in healthy controls, which are influenced by changes in gut microbiota load along the colon. However, further studies are required to determine how immune tolerance can influence polyp development and progression to CRC, and whether the higher polyp number in the distal colon is related to the lower immune activity at that site.

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Keywords: Dendritic cell · Immunology · Cancer

33 Mucosal dendritic cells from proximal colon are activated but more immature in colon cancer, than in FAP

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Purpose: Mucosal dendritic cells (DCs) are potent antigen presenting cells, which can initiate cytotoxic T-lymphocyte immunogenicity against tumours. Previous studies on colorectal cancer have demonstrated ineffective immune response against malignant tumours, likely due to tumour-derived factors [1]. However, the mechanism of how tumour-derived factors affect various mucosal immune cells is unclear. Therefore, to understand the effect of malignant tumours on surrounding 'background' mucosal immunology, we compared phenotypic differences in mucosal DCs between individuals with FAP (as a model of pre-malignant stage) and CRC. Previous work from our laboratory demonstrated there were immunological differences between proximal and distal colon [2]. Therefore, comparison was made between the proximal colons of FAP and CRC.

Methodology: Mucosal specimens were obtained from the proximal colon of individuals with FAP, but not cancer (n = 9) and individuals with proximal colon cancer (n = 11), immediately after surgical resection. Samples were macroscopically devoid of polyps or cancer and representative of background mucosa. Following collagenase digestion, released DCs were identified by flow cytometry as viable immune cells expressing high HLA-DR and dim lineage cocktail; (CD3-CD14-CD16-CD19-CD34-CD56-). Expression of activation (CD40), co-stimulatory (CD86), immature DC (ILT3), lymph node migration (CCR7) and gut-homing (β 7) markers on mucosal DC was determined.

Results: Mucosal DC in proximal colon cancer expressed increased CD40 (cancer: 67 %, FAP: 34 %, $p = 0.002$), ILT3 (cancer: 63 %, FAP: 22 %, $p = 0.0036$) and β 7 (cancer: 43 %, FAP: 16 %, $p = 0.0383$) compared to mucosal DC from proximal colon in FAP. There were no differences in expression of CD86 or CCR7 between mucosal DCs in CRC and FAP.

Conclusion: Background mucosal DCs from proximal colon cancer were more activated, but maintained immature marker expression compared with those from individuals with FAP. Activated but immature DCs may induce anergic T cell responses and promote tolerance to tumour antigens, ineffective DC function in acquiring and presenting tumour antigens [3]. Our results demonstrate phenotypic differences in mucosal DCs between pre-malignant and malignant stage of colorectal cancer. However, further studies are required to determine the mechanisms producing such differences in phenotype and whether they affect mucosal DC function and tumour progression.

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Keywords: Dendritic cell · Immunology · Cancer

35 Clinical and molecular characterization of an argentinean cohort with colorectal cancer staff of Hospital Italiano de Buenos Aires Gastroenterology Department Member of PROCANHE (Programa de Cancer Hereditario)

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Purpose: Argentina Is Among The Countries With High Incidence Rates Of Colorectal Cancer (Crc) [1]. In this context, our objective is to characterize clinical, epidemiological and molecular data from a cohort of patients with CRC.

Methods: 155 prospectively recruited consecutive patients with CRC were characterized according to demographic data, risk factors and pathological data. Microsatellite instability (MSI) was identified using the NCI recommended panel. Immunohistochemical (IHC) staining for MLH1, MSH2, MSH6 and PMS2 was performed. Molecular typing BRAF and germline mutation analysis of blood samples was performed for MSH2, MSH6 and MLH1 genes.

Results: According to the clinical classification, sporadic forms, family, hereditary were 89.7, 7.1 and 3.2 %, respectively; the mean age of onset 65.7 years (SD 14.4) and 55 % male. 12.3 % had a family history of CRC with 3.2 % of Amsterdam criteria. 18 % of cases presented high MSI, of which 44.4 % were deficient in some protein expression by IHC. 9 BRAF mutations in 16 of the cases lacking MLH1 expression were found. Of the 17 individuals sequenced, 2 mutations were detected.

Conclusion: The frequency according to the clinical classification was similar to that described in other countries [2]. The incidence of MSI-H was higher than that reported in other regions [3] may be due to exposure to different factors methylation or genetic characteristics of the population included. In familial cases, the genetic study identified two cases suggestive familial cancer type x and Lynch cancer

type. This supports the position reference centers on the universal use of molecular characterization regardless of the clinical form [4].

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Keywords: Argentina · Colorectal cancer · Microsatellite instability

36 Linkage analysis in familial colon- and rectal cancer

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In Sweden, round 6000 new colorectal cancer cases are reported yearly. Up to 35 % of the colorectal cancers are said to be due to hereditary factors and many families segregate the disease as a seemingly monogenic trait. Familial polyposis and Lynch syndrome are two syndromes where the predisposing genes are known. However, only for a minority of families with colorectal cancer the predisposing genes or genetic loci are identified. In some families the segregation could possibly be contingent to the location of the tumour. Dividing the colorectal families into subgroups of colon and rectal patients could be an achievable way to find new loci. Therefore, we performed a genome wide linkage analysis in 32 colon and 56 rectal cancer families. The families were ascertained from the department of clinical genetics at the Karolinska University Hospital in Stockholm, Sweden and were considered negative for Familial Polyposis and Lynch syndrome. In total 475 subjects were genotyped using single nucleotide polymorphism array chips. Parametric- and non-parametric linkage analyses were computed using MERLIN. 88 families were analysed as two subgroups; 56 rectal- and 32 colon cancer families corresponding to 306 and 169 patients respectively. No significant LOD or HLOD score, above three, was observed. Interestingly, suggestive linkage with results close to three could be demonstrated. A HLOD = 2.55 was observed at locus 18p11.2 (rs872906) for the rectal cancer families. For the colon cancer families, HLOD = 2.49 on locus 6p21.1-p12.1 (rs722269) was observed. Our linkage study indicates that there might be disease causing genes involved in colon- and rectal cancer in these regions. Further studies are ongoing and exome sequencing data are at present being analysed in colon- and rectal cancer patients in families contributing to the HLODs in those regions as well as in an extended material.

37 Genetic features of Lynch syndrome in the Israeli population

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Diagnosis of Lynch Syndrome may be complex. Knowledge of mutation spectrum and founder mutations in specific populations facilitates the diagnostic process.

Aim: To describe genetic features of LS in the Israeli population and report novel and founder mutations.

Methods: Patients were studied at high risk clinics. Diagnostics followed a multi-step process, including tumor testing, gene analysis and testing for founder mutations. LS was defined by positive mutation testing.

Results: We diagnosed LS in 242 subjects from 113 families coming from different ethnicities. We identified 54 different mutations; 13 of them are novel. Sixty seven (59 %) families had mutations in MSH2, 20 (18 %) in MSH6, 19 (17 %) in MLH1 and 7 (6 %) in PMS2; 27 % of the MSH2 mutations were large deletions. Seven founder mutations were detected in 61/113 (54 %) families. Constitutional mismatch repair deficiency (CMMR-D) was identified in 5 families.

Conclusions: Gene distribution in the Israeli population is unique, with relatively high incidence of mutations in MSH2 and MSH6. The mutation spectrum is wide; however, 54 % of cases are caused by 1 of 7 founder mutations. CMMR-D occurs in the context of founder mutations and consanguinity. These features should guide the diagnostic process, risk estimation, and genetic counseling.

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Keywords: Lynch · Israel · BRCA

38 Ureteric complications of intra-abdominal desmoids

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Purpose: Desmoid tumours (DTs) occur in 10–25 % of familial adenomatous polyposis (FAP) patients, usually arising intra-abdominally (IA) or in the abdominal wall. Although benign, 10 %

grow relentlessly leading to bowel and ureteric complications. Little has been reported on ureteric complications occurring in association with IA desmoids in FAP. The aim of this study was to review occurrence of ureteric complications arising from IA-DT compression and their management.

Methodology: All patients with IA-DTs were identified from a prospectively maintained national registry database from 1950 to 2014. Case notes were analysed; data collected included DT medical and surgical management, location of the APC mutation, the occurrence of ureteric complications and their management. Our current protocol for assessment of the urinary tract in IA-DTs is to perform radiological imaging, usually with an ultrasound scan, every 6–12 months.

Results: A total of 153 FAP patients with IA-DTs were identified. 38/153 (25 %) had ureteric involvement. In 36/38 this was identified through radiological imaging, typically within 1-year of DT diagnosis (IQR 0–4 years). The other 2 were discovered at the time of surgery for the IA-DT. Comparing this group to those without ureteric complications, significantly more had APC mutations 3' of codon 1399 (42 vs 24 %, $p = 0.04$) and required more medical (90 vs 48 %, $p < 0.01$) and surgical interventions (58 vs 37 %, $p = 0.02$) for their DT. 30/38 (79 %) had a urological intervention; 24 had placement of a stent, 3 had a nephrostomy (1 bilaterally) and 3 had surgery with re-implantation of a ureter. Five people lost the function of a kidney due to their IA-DT.

Conclusion: Ureteric obstruction from IA-DTs is a significant problem in FAP patients that can occur at any stage. Clinicians managing these patients should regularly survey the renal tract, as early detection and intervention may prevent irreversible injury.

Keywords: Desmoid · FAP · Ureter

39 Duodenal disease in MAP

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Purpose: MUTYH-associated polyposis (MAP), like familial adenomatous polyposis (FAP), predisposes to colorectal and duodenal adenoma formation. However, duodenal polyposis is thought to be seen less frequently in MAP than in FAP, although data regarding MAP duodenal polyposis is sparse. Most centres adopt the same upper gastrointestinal (UGI) surveillance protocol for both polyposis syndromes. The aim of this study was to assess the incidence, extent and progression of duodenal adenomas in MAP at two European institutions with polyposis registries and evaluate their current surveillance protocols for MAP.

Methodology: This was a two-centre cohort study from the UK and the Netherlands. All genetically confirmed MAP cases with UGI surveillance at each centre were identified from prospectively maintained registry databases. Case notes, endoscopy and histology reports were analysed. Outcomes recorded included; the occurrence of duodenal adenomas, age of adenoma onset, time interval to advancing Spigelman stage, polyp distribution and endoscopic intervention performed.

Results: 92 MAP patients were identified and 31 (34 %) developed duodenal adenomas, with a median follow-up of 6 years (range 0–16). Median age at adenoma detection was 50 years (range 32–77). The median time to adenoma development in those with a normal baseline oesophagogastroduodenoscopy was 6 years (range 3.5–16) and occurred at a median age of 52 years (range 42–77). In the adenoma

group, 84 % were Spigelman stage I or II, 93 % had only 1–4 lesions and 67 % measured less than 5 mm in size. Most were tubular adenomas (77 %) with mild dysplasia (90 %). No high-grade dysplasia developed in this cohort. 18/31 patients had a further OGD after polyps were found. Of these, 5 progressed over 5 years (range 2–8) by one Spigelman stage. 7 'down-staged' following polypectomy/biopsy and 6 were unchanged. Interventions included; 8 polypectomies and 2 duodenectomies at a median age of 61 years (range 38–70 years). Only 2 patients had a histologically confirmed ampullary adenoma. One duodenal and one ampullary cancer were diagnosed at first OGD, aged 63 and 83, respectively.

Conclusion: Duodenal polyposis is seen much less frequently in MAP than FAP patients and this study supports that finding. This study has also shown that MAP duodenal adenomas develop at a later age, are fewer in number, are usually small and progress slowly with little histological ampullary involvement. Given this and the lack of intervention before 38 years of age, it may be feasible to consider commencing UGI surveillance at a later age of 35 years (currently 25 years). Progression in MAP duodenal polyposis mostly seems to relate to an increase in lesion size and/or villous change and not to polyp multiplicity. This raises the question of whether the Spigelman staging system is appropriate in this cohort of patients, to determine cancer risk and endoscopic surveillance interval.

40 Comparisons of long versus short polyA repeat markers for the detection of microsatellite instability in endometrial carcinomas

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Introduction: Endometrial cancer is the second most common type of Lynch syndrome tumor in females and often displays an attenuated microsatellite instability (MSI) phenotype in comparison to colon cancers using current markers. In an effort to improve detection of MSI in endometrial cancers we have evaluated a new set of long polyA repeat markers. The stability of microsatellite repeats is exponentially related to the number of tandem repeats, therefore, repeats consisting of long (40–60 bp) polyA tracts should exhibit increased MSI sensitivity over shorter (21–27 bp) polyA markers currently in wide use. The purpose of this study is to compare the performance of long versus short polyA repeat markers to determine if they are better suited for detection of MSI in endometrial cancers.

Materials and Methods: Formalin fixed, paraffin embedded endometrial cancer tissue sections were tested for MSI using a new panel of long polyA repeats and an existing panel of short polyA repeats (Promega MSI Analysis System). Loss of mismatch repair expression was determined by immunohistochemistry for MSH6, MLH1, MSH2 and PMS2 proteins. The two study populations consisted of: 100 endometrial cancers from patients without age restriction who did not have a known family history of cancer; and a second group of 100 endometrial cancers from patients 50 years old or less.

Results: The percent of MSI-H endometrial cancers observed in the first group was higher using the long polyA repeats compared to the short polyA repeats (32 % v 17 %). Larger allelic shifts observed with the long polyA repeats (12.8 bp v 4.5 bp) made it easier to interpret results. In the second group, the percent of MSI-H tumors was also higher with the long polyA repeats (10 % v 5 %) and the

allelic shifts were larger (6.9 bp v 1.9 bp). Concordance between MSI-H cases determined using the long polyA repeat panel and IHC was 78 and 83 % for the two study groups.

Conclusion: Our results comparing the outcome of MSI testing using long versus short microsatellite repeats indicate that long polyA tract markers may indeed be more sensitive than the presently used marker panels. This outcome is in agreement with previously observed MSI testing results in colorectal polyps using a similar set of long polyA markers. A combination of higher MSI frequency and a lower number of equivocal calls due to the larger size of the deletions, resulted in easier and more robust MSI scoring with the long polyA tract markers.

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Keywords: MSI · Endometrial cancer · Lynch syndrome

42 Genetic test declining, colonoscopy and high cancer risk perception in Lynch syndrome families

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Purpose: About half of people from mutation-carrying families do not undergo genetic counselling and/or testing to identify their mutation status and risk of colorectal cancer (CRC). We studied perceived CRC risk and qualitative analysis of reasons for declining in this group.

Patients and Methods: We studied 26 participants (mean age 43.1 years, 14 women) in the Australasian Colorectal Cancer Family Registry who were relatives of mismatch repair gene mutation carriers; who had not been diagnosed with any cancer at the time of recruitment and who had declined an invitation to attend genetic counselling and/or testing at the time of interview. Bounded estimates of perceived CRC risk over the next 10 years, understanding of genetic testing and CRC risk, reasons for declining testing and self-reported colonoscopy screening were elicited during a face-to-face semi-structured interviews [1].

Results: A sub group of decliners (31 %) unconditionally rejected genetic testing compared to conditional decliners who would consider genetic testing in the future. Mean perceived 10-year risk of CRC was 54 % [95 % CI 37, 71] in unconditional decliners, compared with the mean perceived 10-year risk of CRC of 20 % [95 % CI 5, 36] in people who conditionally decline genetic testing. This difference remained after adjusting for potential confounding factors (age, gender and reported screening colonoscopy).

Clinical implications: The unconditional decliner group perceive themselves to be at 3.26 times higher risk than conditional decliners, yet are not more likely to receive appropriate screening colonoscopy. Knowledge of personal CRC risk, feelings about one's personal CRC risk, and comparative measures of personal CRC risk have been found to address different aspects of health screening intention [2]. Thus, as

this potentially high-risk and under-served group may resist clinical risk messages, General Practitioners could increase appropriate colonoscopy screening by implementing surveillance appropriate to mutation carriers, in the absence of genetic testing.

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Keywords: Colonoscopy · Genetic testing · Lynch syndrome

43 A 10 mb inversion of chromosome 2p which causes Lynch syndrome is prevalent in Europe, USA, and Australia, but is not tested for routinely

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Purpose: Mutation testing for Lynch syndrome (LS) is typically by sequencing and Multiplex ligation-dependent probe amplification (MLPA). We found a Welsh LS family (with relatives in Australia) in which their tumours showed MSI and loss of MSH2, but no mutation could be found. However, cytogenetic analysis found an inversion of 2p [46,XX,inv(2)(p21.1p22.2)] which appeared identical to that described previously [1]. So, we have investigated other genealogically linked families in Wales, Scotland and England.

Methods: Cytogenetic analysis was by conventional G-banding. Sanger sequencing was performed on MSH2, as was MLPA using the MRC-Holland kit P003-C1. Breakpoint PCR used published primers [1]. Control DNA was provided by Leiden.

Results: All UK families proved to have the previously observed inversion, as shown by the unique novel insertion of CACATAT at the 5' breakpoint [1]. Cytogenetic review in Wales highlighted that the inversion was unlikely to have been detected without targeted analysis of 2p. Similarly, the other laboratories reported that without targeted analysis the inversion was often only detected by the checker, not the primary analyst Cytogenetic sensitivity is thus around 50 %. It is also notable that array Comparative Genomic Hybridisation did not detect this inversion either. However, breakpoint PCR detects the inversion with 100 % efficiency [1].

Conclusion: This inversion of 2p impacting MMR gene/protein function is present in the UK, mainland Europe, USA and Australia. The prevalence is likely to be underestimated as it is missed by routine sequencing and dosage analysis (and it is also unlikely to be

detected by Next Generation Sequencing). Cytogenetic abnormalities in general may be an under-ascertained cause of LS and other cancer genetic syndromes, and it reminds us that all testing modalities have finite sensitivity. Further genealogical work is underway to determine how the UK families are related to those in mainland Europe. MRC-Holland (<http://www.mlpa.com/>) are modifying their MMR gene kit (P003), so it should become clearer what the population distribution is.

Please report all MMR gene variants and mutations to <http://insight-group.org/variants/database/>!

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Keywords: Lynch · MSH2 · Inversion

45 Metachronous colorectal cancer in general national cohorte from 1943–2012 and its relevance as indicator of hereditary colorectal cancer

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Purpose: Hereditary colorectal cancer increases the risk of metachronous colorectal cancer (mCRC) [1]. To our knowledge it is unknown which proportion of patients with mCRC are affected due to hereditary disposition, and whether this proportion has changed since colonoscopic surveillance is offered to individuals with hereditary risk of CRC and patients cured of sporadic CRC to reduce the risk of mCRC. Register based studies are very favourable in Denmark. Since 1968 all Danes have a unique Central Population Registry number (CPR number), which allows for patient tracking throughout the country. The Danish National Cancer Register was established in 1943 and has a completeness of 98 % [2]. The national Danish HNPCC-register was established in 1991 and contains families with HNPCC and hereditary moderate risk for CRC. CRC diagnosed before the age of 50 is sufficient to diagnose at least moderate hereditary risk of CRC according to Danish guidelines. The CPR number makes it possible to identify patients in the National Cancer Register, who are also registered in the HNPCC-register.

Aim: To estimate the proportion of patients with mCRC who belong to a family with hereditary risk of CRC, and analyse possible change over time.

Methodology: All CRC cases diagnosed between 1943 and 2012 were collected from The Danish National Cancer Register. We excluded patients with no time at risk for mCRC because of death or end of study within 1 year after first CRC and CRCs with known histology other than adenocarcinoma. Hereditary risk of CRC was defined as individuals classified with hereditary risk of CRC in the HNPCC-register or being diagnosed with CRC before the age of 50 years. The total number of mCRC and hereof the proportion of mCRC due to hereditary disposition was calculated in three time periods (1943–1990, 1991–1998, 1999–2012). Statistical analyses were performed in SAS.

Results: 195429 CRC cases were collected from The Danish National Cancer Register. Excluded were 1817 (0.9 %) CRCs not being

adenocarcinomas and 83081 (42.5 %) cases not in risk of mCRC. Included were 110531 CRC cases in 107301 patients. 2486 patients (2.3 %) had 2573 mCRCs. The mean proportion of hereditary mCRC was 20.4 % (95 % CI 16.7–24.1) with no change over the three time periods. The true proportion is higher as some hereditary mCRCs were not assessed as hereditary—because the patient died before 1968 (did not get a CPR number and therefore could not be traced in this study) or because the patient had an undiagnosed hereditary disposition for CRC.

Conclusion: mCRC is an indicator of hereditary CRC, as at least 20 % of patients with mCRC have a hereditary disposition, and referral for genetic counseling should be considered. We did not show a decrease in the proportion of hereditary mCRCs over time. This is possibly due to an under diagnosis of hereditary CRC in the first time periods in which HNPCC register was not established and many patients could not be identified due to lack of CPR numbers (before 1968).

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Keywords: Metachronous colorectal cancer · National cohorte · Indicator of HNPCC

46 Validation of a digital questionnaire for identifying people at risk of familial and hereditary colorectal cancer

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Purpose: Surveillance colonoscopies are recommended for patients with familial colorectal cancer (FCC) or Lynch syndrome (LS), in order to reduce morbidity and mortality from colorectal cancer (CRC). These patients often go unrecognized by physicians. We developed and validated a self-administered digital questionnaire to document familial cancer history, in order to facilitate the detection of persons with a familial or hereditary CRC risk.

Methodology: The development of the questionnaire was based on nationwide criteria for referral to genetic specialists due to a LS suspicion, as well as criteria for surveillance colonoscopies because of an increased risk of FCC. Validation was performed at a private colonoscopy center in patients scheduled for colonoscopy. Performance of the questionnaire was assessed by comparing referrals based on questionnaire data against referral decisions based on full pedigree data. In a second validation phase, referrals based on questionnaire data were compared with referrals based on data collected in a telephone interview. We calculated inter-observer agreement in referral decisions.

Results: In the first validation phase 9 of 50 patients had a suspicion of LS and 1 fulfilled criteria of FCC, according pedigree data. All patients qualifying for referral were also detected through the questionnaire, except for one patient with suspected LS. One patient who did not qualify for referral based on the pedigree did have a referral indication based on the questionnaire. This results in a sensitivity of 90 % (95 % CI: 55–98 %) at a specificity of 98 % (95 % CI: 87–100 %) in identifying persons qualifying for referral. In the second validation phase 8 of 100 patients had a LS suspicion and 2 had FCC. After the telephone verification, it became clear that 3 LS suspected patients did not qualify. Referral advice for FCC did not change after verification. In this phase sensitivity was 100 % (95 % CI: 63–100 %) at a specificity of 97 % (95 % CI: 91–99 %). In both validation phases an inter-observer agreement of 100 % in referral decisions was achieved.

Conclusion: The digital questionnaire has a high sensitivity and specificity in identifying persons qualifying for referral because of suspected LS or FCC. Familial risk assessment showed a very good inter-observer agreement. The questionnaire may result in better treatment and surveillance recommendations for persons at increased CRC risk.

Keywords: Questionnaire · Family history · Lynch syndrome

47 Characterization of germline mutations on MLH1, MSH2 and MSH6 genes in at-risk patients of Lynch syndrome at the Barretos Cancer Hospital

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Purpose: Lynch Syndrome (LS) is one of the most common cancer susceptibility syndromes. Approximately 3 % of colon cancer is caused by germline mutations in DNA mismatch repair (MMR) genes, mostly MLH1 and MSH2. The identification of a pathogenic mutation confirms the diagnosis in the patient and enables predictive testing for family members. In this context, 3 years ago the Barretos Cancer Hospital implemented a screening approach to identify patients that would benefit from genetic counseling and molecular testing. Therefore, this study aims to identify and characterize the deleterious genetic changes in MLH1, MSH2 and MSH6 genes in these families.

Methodology: Clinical criteria (Amsterdam criteria II and Revised Bethesda) were used to assist in diagnosis of LS. The first screening step was to evaluate genes expression and microsatellite instability (MSI) in tumor tissue, linked to V600E mutation analysis in the BRAF oncogene. All patients with IHC altered and/or presence of MSI and without BRAF mutation undergo to the second step of the test. Genomic DNA was extracted from peripheral blood and genes with altered expression in IHC were amplified by PCR and sequenced by the Sanger method. Furthermore, gene rearrangements were verified by Multiplex Ligation-dependent Probe Amplification technique. All potentially deleterious changes detected were confirmed by a second PCR reaction followed by bi-directional sequencing. The identified genetic alterations were classified into known deleterious mutations and variants of unknown clinical significance, according to the specific database.

Results: Pathogenic mutations were found in 49 % (28/57) of individuals (families) with abnormal MSI and IHC test. Of these, 24 % (14/28) were found in MSH2 gene, 19 % (11/28) in MLH1 gene and 5 % (3/28) in MSH6 gene. In addition, two variants (p.Leu676Pro in MLH1 gene and p.Gly322Asp in MSH2 gene) with unknown clinical significance were identified. The most frequent mutations found were frameshift (36 %), followed by nonsense (33 %) and missense mutations (10 %). Furthermore, one family was diagnosed with an indel mutation (c.1853delAinsTTCTT) in the MLH1 gene and four families diagnosed with rearrangements (highlight for deletion of exons 17–19 in MLH1 gene). The mean age at diagnosis was 43 years and the primary tumors more frequently associated were colon (57 %), rectum (14 %), endometrium (7 %), prostate (7 %), stomach (7 %) and uterus (7 %). To date, 98 relatives were tested, of which 45 were mutated and were referred for prevention/reduction of cancer programs.

Conclusion: The results show that half of all patients with cancer family history who sought treatment at the institution presented germline mutations in MMR genes. The identification of a pathogenic mutation is important because confirms the diagnosis and enables predictive testing for family members. Despite the high cost, the molecular genetic diagnosis of LS offers an opportunity for intensive targeted clinical surveillance of healthy carriers, which has been proven to reduce significantly cancer morbidity and mortality.

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Keywords: Lynch syndrome · Genetic · Mutation

48 Experiences and attitudes towards directly approaching individuals at high risk of hereditary cancer

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Purpose: Since 1997 Danish legislation has allowed a national HNPCC register to approach members from high risk families directly. Today a total of 2112 potential risk individuals have received a letter with information about familial risk and the possibility of prevention. Prior to sending out the letter, probands were asked to inform their relatives about it’s coming. The purpose of the study was to examine risk individuals’ experiences and attitudes towards using direct approach as a way of sharing relevant medical and genetic information.

Methods: From the register we identified all individuals receiving a letter since 1997. A pilot study was performed including 12 individuals from families with known mutations, who were informed within the last 2 years, representing both men and women, younger and older and positive and negative mutation test. A letter with an invitation to a telephone interview was sent and eight agreed to participate. Each interview lasted approximately 30 min. The interviews were taped and transcribed verbatim and analyzed using content analysis. The results formed the basis for development of a

questionnaire that will be sent out to the a more than 1000 individuals receiving the letter from 2005 to 2014.

Results: Of the 2112 individuals receiving a letter from the HNPCC register, 47 % were women. 65 % were risk persons from families with an identified mutation in an MMR-gene, and 35 % were risk persons from families with no identified mutation who fulfilled Amsterdam I or II criteria. Results from the pilot study showed that overall the informant expressed a positive attitude towards a direct approach and considered the information provided in the letter important and relevant. Most of the informants had been informed beforehand that the letter would come. Some knew about the high risk due to their family history and were glad to get an explanation for the many cancer incidences in the family. Being informed about the letter by a close relative was preferable. If they did not feel emotional related to the relative or the relative were distant with very little contact, they preferred receiving the information directly from the Health care system. They all preferred getting a letter, even unprepared of its coming, to not getting the information at all. A few informant described surprise and chock when first receiving the letter, but none expressed they would rather have been without the information.

Conclusion: The results suggest that directly approaching with information is acceptable and in some cases preferable. However the study is a small pilot study based on few informants. The results of the study has formed the base of a larger cohort study, which will provide a broader picture to which extend these attitudes and experiences are representative in a larger sample. Results from this questionnaire will also be presented at the InSight Conference.

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Keywords: HNPCC register · Directly approaching · Patient experiences

49 Cancer risks in family members of CMMR-D patients

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Purpose: Biallelic germline mutations in the mismatch repair (MMR) genes cause a recessive form of childhood cancer that has been referred to as Constitutional Mismatch Repair Deficiency (CMMR-D) syndrome. Family members of CMMR-D patients are at risk of being a heterozygous carrier of a mutation in a MMR gene and thus for having Lynch syndrome (LS). The cancer risks for these family members have not yet been analyzed. It is expected that their cancer risk will be different than cancer risks reported before for LS families that were ascertained because of cancer in the family. CMMR-D families havenot been ascertained because of cancer in the family, but because the index patient has a distinct phenotype.

Methodology: Data collection of families with a CMMR-D index patient has started in 2014. The aim is to collect at least 50 families. Once all data is collected a competing risks analysis will be performed to calculate cancer risks. For family members of whom the carrier status is unknown, the probability of carriership will be computed based on the distance to obligate carriers and phenotypes in the family.

Results: Thus far we have collected data on 697 PMS2, 148 MSH6, 21 MSH2 and 16 MLH1 family members of CMMR-D-patients in 40 families. These family members include 186 proven mutation carriers. Preliminary analysis using Kaplan–Meier shows that cumulative risks for colorectal cancer (CRC) at age 70 are 12 % for PMS2 and 14 % for MSH6. The MSH2 patient and MLH1 groups are not large enough to allow analysis at this moment.

Conclusion: Preliminary results suggest that CRC risk is substantially lower in family members of CMMR-D patients than previously reported in LS patients. These results might implicate that colon screening for these CMMR-D family members and possibly, also MMR mutation carriers detected through population based screening should be advised less intensive colon screening.

Keywords: PMS2 · MUTYH · Cancer risk

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51 Genetic variants in mismatch repair genes in an Argentinian population of colorectal cancer

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Purpose: Lynch syndrome, which is characterized by defective DNA mismatch repair, is responsible for 3 % of colorectal cancer cases [1]. The aim of this study is to describe variants in the common colorectal cancer susceptibility genes, MLH1, MSH2, MSH6, and PMS2 seen in an Argentinian population of colorectal cancer patients.

Methods: A total of 34 families were selected from the Hereditary Colorectal Cancer Registry of the Hospital Italiano de Buenos Aires. 17 families met Amsterdam I criteria, 9 families met Amsterdam II criteria, and 8 families met Bethesda guidelines. Germline mutation analysis of MLH1, MSH2, MSH6, and PMS2 was performed on DNA samples from 43 patients.

Results: A total of 51 variants were described in the 34 families, including variants that affect function (pathogenic) and variants that do not affect function (neutral). 13 of the variants identified have been previously reported as pathogenic in the InSiGHT database, the Universal Mutation Database, or in the literature. Two previously unreported variants that affect function were identified: c.3646+29-32delCTAT in MSH6 and c.588+5G>T in MLH1.

Conclusion: Genetic sequencing in this population yielded a low number of pathogenic variants, however it allowed for the description of two previously unreported pathogenic variants.

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Keywords: Patients variants · Lynch · Argentina

52 Serrated polyps and mucus: a prospective study

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Introduction: Serrated colorectal polyps are potentially precancerous lesions, involved in both sporadic and syndromic colorectal cancer. Sessile serrated adenomas/polyps (SSA/P), can be difficult to recognize endoscopically, but are sometimes covered by tenacious mucus causing a characteristic appearance that is an important clue to their presence. There are no prospective data on the sensitivity and specificity of mucus as an indicator of SSA/P. We have performed a prospective evaluation of a series of polyps to provide this critical information.

Methods: All polyps removed at colonoscopy by a single endoscopist from September 2013 to May 2014 were included. Patients with a known polyposis syndrome and polyps that had previously been biopsied were excluded. Polyps were described by location, size and shape, and a comment was made about the presence of mucus. These descriptors were then compared with final histology.

Results: There were 591 polyps, 123 (20.8 %) with mucus. The most common histology was tubular adenoma (340), 7.9 % of which were coated with mucus. Next most common histology was hyperplastic polyp (100, 24.0 % mucus), followed by SSA/P (87, 71.3 % mucus), normal mucosa (47, 14.8 % mucus, and tubulovillous adenoma (17, 17.6 % mucus). The results of an overall classification function analysis show that the sensitivity for mucus for SSA/P was 71.3 %, specificity was 87.9 % and overall accuracy was 85 %. Positive predictive value was 50.4 % and negative predictive value was 94.7 %. The presence of mucus is more accurate in predicting the histology of right sided polyps (positive predictive value 53.9 %, accuracy 86.1 %) than left sided (positive predictive value 33.3 %, accuracy 80.9 %).

Conclusion: The presence of mucus on a polyp favors a diagnosis of SSA/P but is not conclusive. Endoscopists must be alert to other features of serrated polyps such as subtle changes in crypt pattern and mucosal vasculature.

Keywords: Serrated polyps · Endoscopy · Diagnosis

53 Characterization of patients with colorectal cancer at young ages

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Introduction: Lynch syndrome (LS) is mainly caused by germline mutations in genes of the DNA damage MMR (mismatch repair system) system repair. Individuals with LS have a cumulative cancer risk of 60–80 % for colorectal cancer (CRC) and, in addition, have an increased risk for extracolonic tumors (cancer of endometrium, ovary, stomach, urinary tract, bile duct). Although a family history of cancer and age at diagnosis are strong indicators of a predisposition to LS, numerous publications emphasize the importance of molecular and histopathological findings in this identification.

Objective: To perform a histopathological and molecular characterization of patients with CRC diagnosed before 50 years old, treated at a cancer reference hospital located in the rural area of Sao Paulo state (Barretos Cancer Hospital) in the period between 2006 and 2010.

Methods: Observational cohort study with retrospective data collection, based on clinical chart review.

Results: 473 patients were included, 239 (50.5 %) were male and 234 patients (49.5 %) were female. From these 473, 226 (47.8 %) are alive without disease, 102 (21.6 %) are alive with disease, 19 (4.0 %) alive with unknown disease status. 125 patients died, and in 98 (20.7 %) of them the cause of death was cancer. The average age at diagnosis was 41 years (SD: 6.83, median 43 years). Regarding the family history 242 (51.2 %) had positive family history, with 27.7 % reporting presence of at least one first degree relative affected by cancer. The most frequent primary tumor sites were: distal (splenic flexure, left colon—descending, and sigmoid) (33.4 %, 158 cases), rectum (32.6 %, 154 cases) and 75 cases (15.9 %) with involvement of the proximal side (right colon—ascending, hepatic flexure and transverse). Nineteen patients (4 %) were found with more than one primary tumor and the most frequent sites (other than CRC) were

ovary (1 %) and breast (0.4 %). The most common histologic types were tubular adenocarcinoma (276 cases, 58.4 %), mucinous (28 patients, 5.9 %), low-grade tubulovillous adenoma (13 patients, 2.7 %), with neuroendocrine differentiation (10 patients, 2.1 %) and high grade tubulovillous adenoma (8 cases, 1.7 %). About the degree of differentiation, 313 (66.2 %) were moderately differentiated, 47 (9.2 %) well differentiated, 35 (7.4 %) poorly differentiated and 3 (0.6 %) undifferentiated. Regarding the general classification of tumors (TNM), the majority of patients had stage T3 (48.6 %), N0 (46.7 %) and M0 (68.3 %). Molecular investigations are ongoing. To date, analysis of microsatellite instability (MSI), BRAF and immunohistochemistry for mismatch repair genes hMLH1, hMSH2, hMSH6 hPMS2 were performed in 52 out of 473 patients included. **Conclusion:** A wide characterization of patients potentially at-risk for hereditary cancer is important as it creates the opportunity of personalize follow up and cancer care according to the cancer risk identified, as well as give the opportunity for early treatment and intensified prevention.

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Keywords: Cancer colorectal · Lynch syndrome · Microsatellite instability

54 Clinical features of young patients with colorectal cancer

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Introduction: Lynch syndrome is a rare hereditary cancer predisposition syndrome, with an autosomal dominant inheritance, mainly caused by germline mutations in the tumor suppressor genes MLH1, MSH2, MSH6 and PMS2. Cumulative risk for colorectal cancer (CRC) in patients with Lynch syndrome ranges from 60 to 80 %. Additionally, there is an increased risk of extracolonic tumors (cancer of the endometrium, ovary, stomach, urinary and biliary tract).

Objective: Perform a detailed clinical and surgical characterization of patients with CRC diagnosed before the age of 50 years, treated at the Barretos Cancer Hospital (BCH), and to create a database of clinical-surgical information.

Methods: Observational cohort study with retrospective data collection based on clinical chart review of patients diagnosed (<50 years) in the period of 2006–2010.

Results: 473 patients were included. Of these, 345 (72.9 %) did not have any treatment before admission at BCH. Regarding neoadjuvant therapy performed by patients treated exclusively at BCH, 131 (27.7 %) underwent neoadjuvant chemotherapy and 119 (25.2 %) underwent neoadjuvant radiotherapy. The most common surgical procedure was resectosigmoidectomy (161 cases, 34 %), most of them in a curative basis (59 cases, 12.5 %). From the total of patients that had surgeries at BCH, 11 (2.3 %) died after surgery. 302 patients (63.8 %) were submitted to adjuvant therapy, with 300 (63.4 %) receiving chemotherapy and 30 (6.3 %) receiving radiotherapy. About the histological types, the most common was tubular adenocarcinoma (276 cases, 58.4 %), followed by mucinous (5.9 %), low-grade tubulovillous adenoma (13 cases, 2.7 %), 10 (2.1 %) with neuroendocrine differentiation and 8 (1.7 %) high-grade tubulovillous adenoma. Most tumors were moderately differentiated (66.2 %) had stage T3 (48.6 %), N0 (46.7 %) and M0 (68.3 %). In addition, 287 (60.7 %) and 128 (27.1 %) had pathological and radial tumor-free margins respectively. Besides, 127 (26.8 %) had also tumor-free distal margin. 255 patients (53.9 %) did not suffered recurrence, while 211 (44.6 %) relapsed, mainly in the liver (35 cases, 7.4 %). In relation to environmental risk factors BMI was measured and 191 patients (40.4 %) were considered of normal weight, 111 (23.5 %) were overweight, 51 (10.8 %) were obese and 25 (5.3 %) malnourished. Regarding the current status of these patients, 73 % are alive (47.8 % alive without disease).

Conclusion: The surgical and clinical characterization of patients is essential for a better understanding of those patients, as well as to identify the presence of factors suggestive of an inherited predisposition to cancer.

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Keywords: Cancer colorectal · Lynch syndrome · Clinical features

55 Evidence of influence of aspirin on mucosal immune status and on the carcinogenic effects of obesity support the need for the dose non-inferiority study, CAPP3

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The CAPP2 RCT demonstrated a significant reduction in cancers in Lynch syndrome. The original protocol anticipated a 10 year follow up which will be reached for the last recruit in 2015. By 2013 there had been 45 primary colon cancers among the 434 randomised to placebo compared to 25 among the 427 who took aspirin for an average of 2 years. CaPP3 will test three aspirin doses in a blinded randomised non-inferiority trial which commenced in 2014. This is needed to determine whether the 600 mg daily dose used in CAPP2 was more effective than the 100 mg dose used as an anti-platelet dose in those at risk of cardiovascular disease. It can be argued that very low dose aspirin might influence the efficiency of apoptosis while the higher 600 mg dose has an additional anti-inflammatory effect. Secondary data analysis and biobank analysis from CAPP2 provides additional insights. We hypothesised that aspirin may reduce the risk of cancer in LS patients by altering the tissue-infiltrating immune milieu. Dual immunofluorescence staining for CD8 and FoxP3 was performed on normal mucosal biopsies. Amongst all patients assigned to the aspirin intervention group the infiltrating Treg densities in the post-intervention biopsies (mean 24.9 cells/mm², CI = 19.49–30.21) were significantly higher than in the pre-intervention biopsies (mean 20.1 cells/mm², CI = 14.38–25.72, $p = 0.037$, Wilcoxon signed ranked). In contrast, there was no difference between the Treg densities observed in the pre- and post-intervention biopsies in the placebo group (Mean densities: Pre-intervention, 21.27 cells/mm², CI = 15.49–27.05. Post-intervention, 19.02 cells/mm², CI = 14.25–23.80. $p = 0.291$, Wilcoxon signed rank). When this difference was compared between the aspirin and the placebo groups the change was significantly greater in the aspirin group (Mean changes: Aspirin, +4.8 cells/mm², CI = 0.59–9.01; Placebo, –2.3 cells/mm², CI = –5.88 to 1.40, $p = 0.017$, Mann–Whitney U). In contrast, no changes in total T-lymphocyte density were seen between the aspirin and placebo groups (Mean changes in density: Aspirin, +2.24 cells/mm², CI = –73.53 to 78.01; Placebo, –15.37 cells/mm²,

CI = –66.05 to –35.32. $p = 0.620$, Mann–Whitney U). These data indicates an aspirin induced modulation of the immune status of normal mucosa in Lynch Syndrome. Obesity is associated with an increased cancer risk thought to involve a chronic inflammatory effect. In CAPP2, for obese participants, CRC risk was 2.41 (95 % CI: 1.22–4.85) times greater than for the reference group (underweight and normal weight participants) and CRC risk increased by 7 % for each 1 kg m² increase in BMI. The excess CRC risk associated with obesity was confined to those randomized to the aspirin placebo group (adjusted HR: 2.75; 95 % CI: 1.12–6.79, $p = 0.03$).

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Keywords: Aspirin · t reg cells · Obesity

56 Adrenal tumors in patients with familial adenomatous polyposis: a Dutch cohort study

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Purpose: The lifetime prevalence of adrenal tumors in patients with familial adenomatous polyposis (FAP) reported in literature is 7–13 %, compared to 5 % in the general population. The clinical relevance of these tumors is unknown and long-term information on the clinical course is limited. In addition, no information is available on the occurrence of adrenal tumors in the related syndromes attenuated familial adenomatous polyposis (AFAP) and MUTYH associated polyposis (MAP). This study aims to increase the current knowledge on adrenal tumors in patients with FAP, AFAP and MAP. **Methodology:** This is a retrospective single center cohort study performed at the Academic Medical Center. Medical files, imaging reports (CT, PET, MRI) and laboratory results of all registered patients with FAP, AFAP and MAP were analyzed for data on adrenal tumors. Patients were not routinely screened for adrenal tumors. Treatment decisions were based on imaging characteristics and laboratory results.

Results: 16 of 194 patients with FAP, 1 of 32 with AFAP and 0 of 29 with MAP were diagnosed with an adrenal tumor. Of these 17 patients, 9 were female and mean age at time of diagnosis was 49.3 (SD 15.6) years. In 10 patients the tumors were hormonally inactive, in 6 information on hormonal activity was not (yet) reported and 1 FAP patient had hypercortisolism, for which adrenalectomy was performed. In 2 other FAP patients adrenalectomy was performed: 1 with multiple malignancies and radiologic signs of adrenal metastasis. The other was operated on due to the size (4.3 cm), irregular aspect and calcifications on CT. Pathology showed an adenoma in 2 of these 3 patients and was inconclusive on adenoma or hyperplasia in 1. One FAP patient was recently referred for surgery due to a large tumor size (5.8 cm), but not operated due to comorbidities. The non-resected tumors were followed-up by imaging in 9 patients having a median tumor size of 2.0 (IQR 1.4–3.0) cm at baseline and a median follow-up of 2.5(IQR 1.9–6.5) years. Two tumors remained stable, 4 progressed, 3 decreased in size and 3 patients developed a contralateral tumor. Data will be completed in due time as several patients have recently been referred for further analysis.

Conclusion: Our findings confirm that adrenal tumors seem to occur more frequently in FAP compared to the general population. Patients were not screened routinely and most tumors were co-incidental findings, appeared non-functional and, in line with previous studies, were benign upon resection. One tumor was detected in an AFAP patient, none in MAP. Despite the relatively mild character of the tumors in our cohort, we believe that the clinical relevance, risk factors and follow-up need to be further assessed before screening recommendations can be made.

Keywords: Adrenal tumors · Familial adenomatous polyposis · Follow-up

57 The forgotten GI cancers in FAP

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Purpose: Small bowel (SB) and gastric polyps are recognised features of familial adenomatous polyposis (FAP) but their importance is unknown. Not all groups advocate surveillance and evidence is sparse. This study reports FAP associated SB and gastric cancer occurrence at a single institution between 1950 and 2014.

Methodology: All FAP patients developing SB (ileal/jejunal) or gastric cancer were identified from a prospectively maintained registry database. The primary outcome measure was the occurrence of SB or gastric adenocarcinoma. Secondary outcomes included; age at diagnosis, presenting symptoms, Spigelman stage, tumour stage and survival.

Results: Details of 1330 FAP patients held on our database between 1950 and 2014 were reviewed. Six patients (0.45 %) developed SB adenocarcinoma (median age 53 years) and 8 (0.60 %) gastric adenocarcinoma (median age 52 years). Four of the SB tumours were jejunal and 2 ileal. Most SB tumour patients presented with anaemia, 3 with stomal bleeding but only 2 with obstructive symptoms, despite all tumours staged as T3 or T4. 4/6 died within 18 months of diagnosis. 6/8 gastric cancers occurred in patients under regular oesophagogastroduodenoscopy surveillance, all had extensive cystic gland polyps and 4 were anaemic. Two lesions were located in the cardia/fundus, 3 in the body and 2 in the antrum. Six patients died and 4 of these were stage T1/T2 tumours, the remaining 2 were T3/T4. 1 patient is terminally ill and the other lost to follow-up. Four people had chemotherapy only, 3 underwent a partial or total gastrectomy and 1 had no treatment. Interestingly, 5/8 gastric cancer patients had a history of desmoid tumour occurrence; a further 2 had a significant family history of desmoid. The majority (79 %) of SB and gastric cancer cases had Spigelman stage III/IV duodenal disease.

Conclusion: SB and gastric cancers are rarely seen in association with FAP. They may present with subtle signs, such as anaemia, and are usually associated with advanced stage at diagnosis and poor prognosis. There may be a possible association between gastric cancer and desmoid tumour occurrence. A SB or modified gastric cancer surveillance programme cannot be recommended currently but specialist investigations (such as capsule endoscopy or endoscopic ultrasound) should be considered for those with unexplained anaemia, especially when associated with significant duodenal or gastric polyposis.

Keywords: FAP · Gastric tumours · Jejunal/ileal tumours

58 MicroRNA expression associated with desmoid tumours in FAP

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Purpose: Desmoid tumours are rare non-metastasising, myofibroblast tumours. They occur frequently, often intra-abdominally, in familial adenomatous polyposis (FAP). Although most are not associated with significant complications, 10 % grow aggressively and are a leading cause death in FAP. Identifying aggressive desmoids early may influence the timing of intervention. MicroRNAs (miRNA) are short non-coding RNA molecules that regulate post-transcriptional gene expression and can influence tumour development. MiRNAs exported into the circulation can act as accurate non-invasive biomarkers of disease. The aim of this study was to investigate miRNA expression in serum in FAP-associated desmoids. Development of a miRNA marker may aid in early identification and offer possible future treatment targets for these rare tumours.

Methodology: RNA was extracted from sera obtained from 24 individuals with FAP (12 with desmoid and 12 without). Those without desmoid were confirmed through negative CT scan findings and were at least 3 years after prophylactic colectomy. The study had full ethical approval and participants were consented for a single blood test. Serum miRNA expression was compared using a panel of 370 different miRNAs. MiRNAs with significant differences in expression between the 2 groups were identified. Serum miRNAs of interest were then selected and expression in desmoid tumour tissue assessed using in situ hybridisation.

Results: Comparing sera miRNA expression between desmoid formers and non-formers identified 19 differentially expressed miRNAs. In particular, miR-34a had significantly increased expression in desmoid formers compared to non-formers ($p = 0.0046$). This miRNA has been associated with other fibrotic conditions and is a regulator of the wnt signalling pathway that is also controlled by the APC gene. To validate this finding expression of miR-34a was assessed in formalin-fixed paraffin embedded tissue sections from 4 desmoid tumours using in situ hybridisation. All desmoid tumours expressed miR-21 (a control probe) and all expressed miR-34a. This would support the findings from the sera array.

Conclusion: This study successfully extracted miRNAs from FAP participants' sera, with and without desmoid. We have identified a potential miRNA marker for desmoids in FAP and confirmed its expression in desmoid patient sera and tumour. These results will now require further validation.

Keywords: Desmoid · MicroRNA · Biomarker

59 “Glowing In The Dark”: Do some FAP patients with desmoid disease receive too many CT Scans?

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Introduction: Patients with FAP-related intra-abdominal desmoid disease undergo multiple CT scans of their abdomen, pelvis, and

sometimes chest. These are indicated to monitor the response to treatment and to assess the possibility of recurrent or new tumors. CT is generally preferred to MRI because of the better quality and more easily interpretable images. However there is concern about the cumulative dose of radiation associated with multiple CT scans. We performed this retrospective study to assess the radiation exposure of FAP desmoid patients related to CT scans.

Methods: Patients with FAP-related abdominal desmoid disease who were managed in our department were accessed and the number of abdominal/pelvic and chest CTs from the time of desmoid diagnosis to the last imaging follow-up was totalled. When available, the radiation dose was obtained and when not available, an average dose was applied. Patient demographics and the worst stage of the desmoid disease were noted. In this study we report the 22 patients with more than 13 CT scans over the time of follow-up.

Results: There were 22 patients, 7 men and 15 women, with a mean age at desmoid diagnosis of 28.4 ± 1.0 years. The worst Stage was Stage II in three patients, Stage III in 7 and Stage IV in 12. Overall mean follow-up was 111 months (median 107, range 34–215). The mean total number of abdominal/pelvic CT scans was 28 (median 31, range 14–42) and chest CT scans was 4.8 (median 3.5, range 0–18). The mean frequency of scans was every 4.5 months (median 3.9, range every 1 month to every 12 months). The mean cumulative dose of radiation associated with abdominal and pelvic CTs was 491 mGy*cm, equivalent to 491 mSv or 0.491 Sv. The highest dose was in a patient with a stage IV desmoid receiving 42 CT scans over 10.5 years for a total of 574 mSv (0.57 Sv). One Sv is associated with a 0.055 % chance of cancer. The additional dose associated with Chest CT was a mean of 265 mSv (0.26 Sv). No patient developed a cancer that could be attributed to radiation from the CT scans.

Conclusion: In patients with advanced abdominal desmoid disease the total dose of radiation accumulated over a mean of 9 years follow up is significant in terms of predisposing to cancer. This should encourage the use of alternative forms of imaging such as MRI, or at least a less frequent use of CT scans.

Keywords: Desmoids · CT scans · Radiation

60 A fading threat? Does the severity of FAP-associated desmoid tumors decline with age?

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Introduction: Desmoid disease affects 30 % of patients with familial adenomatous polyposis, and in half of the affected patients there are tumors. A desmoid staging system is useful for measuring the severity of the tumor by symptoms, size and growth rate. We have noticed that the severity of intra-abdominal desmoid tumors seems to wane as patients age. We performed a study to see if this was true.

Methods: We accessed patients with intra-abdominal desmoid tumors from our familial adenomatous polyposis database. We included patients with an intra-abdominal tumor who had been followed by us for at least 10 years. We excluded any patient who had undergone complete or partial resection of an intra-abdominal tumor. We applied the desmoid tumor staging system as described by Church et al. in 2005. [1] Stages III and IV were combined and labeled as “severe desmoid”.

Results: There were 34 Females and 16 Males. Mean follow-up was 18.6 years. Of the 50 patients, 18 (36 %) had severe desmoids at their initial staging. 24 (48 %) had severe desmoids as their worst stage and 8 (16 %) had severe desmoids at their final stage. An analysis of desmoid stage by 5 year age periods in patients shows a noticeable trend to milder disease with older age. 29 % of patients in their

twenties had advanced tumors. In patients in their thirties the proportion was 22.5 % and in patients in their forties it was 21.7 %. Only 15 % of patients fifty and over had severe tumors.

Conclusion: Desmoid tumors do become less severe over time, probably as a result of treatment and the effects of aging on cell growth in the context of a germline APC mutation. This gives hope to affected patients and their caregivers.

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Keywords: Desmoids · staging · Age

61 Analysis of germline MSH6 mutations in Brazilian patients with Lynch syndrome

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Lynch syndrome (LS) is the most common hereditary colorectal cancer syndrome, caused by germline mutations in one of the major genes involved in mismatch repair (MMR): MLH1, MSH2, MSH6 and PMS2. Clinically, the identification of families with suspected LS by the Amsterdam and modified Bethesda criteria. LS is characterized by early onset (~45 years) colorectal cancer, as well as high risk for extra-colonic tumors, including endometrial, ovarian, gastric, small bowel, pancreas, hepatobiliary and brain tumors. MMR Mutation carriers have a lifetime risk of up to 90 % of developing at least one of the tumors of the spectrum. MLH1 and MSH2 are the most commonly affected genes, and its mutations cause the classical phenotype. Mutations in MSH6 and PMS2 are more frequently associated with an atypical phenotype. Approximately 18 % of the LS families worldwide harbor MSH6 mutations, and in these families late onset of tumors, endometrial cancer and/or low degree microsatellite instability in the tumor are common features [1]. The aim of this study is to describe germline MSH6 alterations (entire coding region, intron–exon boundaries and screening for gene rearrangements) in a cohort of Brazilian patients with criteria for LS. Sixty-five patients (37 with Bethesda and 28 with Amsterdam criteria) were recruited after informed consent. MSH6 genotyping was performed in DNA obtained from peripheral blood by Sanger sequencing and results were analyzed using the CLC Main WorkBench V6.1.1 software. Screening for rearrangements was done by Multiplex Ligation-Dependent Probe Amplification (MLPA) using the SALSA P072-C1 MSH6 kit (MRC-Holland). A total of twenty-six MSH6 sequence alterations were identified including six small deletions in intronic regions and twenty single nucleotide variation, twelve in coding regions (seven synonymous and five non synonymous). Only one of these variants (c.719G>A/p.Arg240Gln) has not been previously described in any of the the databases researched (HGMD, NCBI and LOVD), but in silico analyses indicate that it is a non pathogenic alteration. No rearrangements were detected in this series. The prevalence of MSH6 seems to vary significantly among different populations. In addition to population differences, the fact that criteria used were insensitive for detecting MSH6 mutations must be considered [2]. Despite absence of deleterious mutations, previous immunohistochemical analysis

showed that the MSH6 protein was absent in tumor tissue of 18 patients (46.15 %), indicating that other genes or other processes, besides gene mutations may be altering MSH6 protein expression. Exemplifying this, additional testing showed that between the 18 patients with loss of expression of MSH6, seven (38.9 %) had deleterious mutations (two rearrangements and five point mutations) in MSH2 gene.

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62 Association between microsatellite instability testing and immunohistochemistry of mismatch repair proteins in Japanese colorectal cancer patients gastroenterologist, endoscopist

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Purpose: Immunohistochemistry (IHC) for mismatch repair (MMR) proteins has been increasingly used in screening for Lynch syndrome (LS) patients. IHC is not just a cost-effective alternative to the microsatellite instability (MSI) test, but it could also offer additional information to suggest the mutated MMR genes. This study aimed to examine feasibility of Lynch syndrome screening using IHC and to evaluate the concordance between the results of IHC and MSI tests. **Methodology:** Between November 2010 and January 2014, colorectal cancer (CRC) patients who met the revised Bethesda criteria were enrolled in the present study. After obtaining written informed consent, both MSI test using the standard Bethesda panel (BAT25, BAT26, D2S123, D5S346, and D17S250) and IHC for four MMR proteins (MLH1, MSH2, PMS2, and MSH6) were performed using formalin-fixed paraffin-embedded sections of primary CRC tissues. Patients with tumors showing MSI-high (MSI-H) status and/or any loss of expression of MMR proteins were referred to genetic counseling, where germline mutation testing was performed with written informed consent for genetic testing.

Results: A total of 165 CRC patients were enrolled (89 men, 76 women). 33 cases were MSI-H; among these, 28 cases showed any loss of MMR protein(s), including MLH1/PMS2 (11 cases), MSH2/MSH6 (11 cases), MSH6 (4 cases), and PMS2 (2 cases). The remaining 5 cases with MSI-H showed no abnormalities in IHC. 132 patients were MSI-L or MSS in MSI test, and none of these cases showed abnormalities in IHC. Overall, the results of MSI and IHC tests were consistent in 160 cases (97 %). Among the 28 cases with

MSI-H status and/or abnormal IHC results, 22 cases underwent genetic testing for LS. 12 patients were found to have pathogenic mutations, including 2 cases for MLH1; 7 for MSH2; 1 for MSH6; and 2 for PMS2; whereas no pathogenic mutation was identified in 10 cases. The germline mutation statuses were always consistent with the results of IHC. Three of five CRC cases with MSI-H and normal IHC had germline testing for MLH1, MSH2, and MSH6, but no mutations were identified.

Conclusion: The present study confirmed the high concordance between MSI and IHC together with types of the MMR gene mutations herein estimated in IHC. IHC is an appropriate diagnostic modality to screen germline mutations of MMR genes for LS.

Keywords: Lynch syndrome · Immunohistochemistry · Microsatellite instability

64 Treatment strategy of multiple duodenal polyposis associated with familial adenomatous polyposis using spigelman classification

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Background/purpose: Duodenal cancer is high ranked cause of death among familial adenomatous polyposis (FAP) patients. Since up to 36 % of FAP patients with Spigelman classification (SC) stage IV duodenal polyposis (DP) are known to develop invasive duodenal cancer, close endoscopic surveillance or consideration of surgical treatment is mandatory. Therefore, SC has been advocated for evaluating DP. We herein show the cumulative incidence of SC stage IV DP at our institution and review our experience of pancreas preserving total duodenectomy (PpTD) for SC stage IV DP patients.

Patient and methods: We reviewed our FAP patients all of whom were periodically followed by upper gastrointestinal endoscopy. Stages are classified according to the sum of scores given based on polyp number, size, histology, and severity of dysplasia. SC stage IV patients and PpTD was carried out. Total of seven patients were investigated. PpTD was performed with distal gastrectomy. Reconstruction was in Billroth I fashion. Clinicopathological factors, SC and surgical outcomes were retrospectively reviewed.

Results: In our institution, fifty eight FAP patients have upper gastrointestinal surveillance every several years. 43.1 % of the patients were found to have some kind of duodenal polyposis (SC stage II, III and IV). Among them, 13.8 % of them were stage IV. The cumulative incidence of duodenal polyposis was 7.7 % at the age of 30, 40 % at 40, 75 % at 50 and 92 % at 60, respectively. The cumulative incidence of stage IV patients was 2 % at the age of 30, 5 % at 40, 10 % at 50, 25.9 % at 60 and 30.1 % at 70, respectively. PpTD was carried out for these patients. The median age was 52 (range 30–68). There were three males and four females. We did not experience mortality or major postoperative complications. Histopathological findings demonstrated mucosal carcinoma with multiple adenomas in three patients, multiple adenomas only in four patients.

Discussion: It has been reported that SC stage IV duodenal polyposis are candidates for endoscopic treatment and down stage may be achieved. However, follow-up data shows that the stage often return to IV again within 2 years. We must admit that endoscopic approach has its limitation. Therefore, minimally invasive, solid

and safe procedure should be implemented for prophylactic DP removal. There are some papers reporting PpTD in low malignant diseases occurring at duodenum but we are first to describe PpTD with distal gastrectomy. No patient was diagnosed as postoperative diabetes mellitus.

Conclusions: Ratio of SC stage IV patients gradually increase in the time course. Less invasive procedure such as PpTD can be an option for SC stage IV patients for complete cure of DP. Our technique of PpTD with distal gastrectomy seems safe and sufficient procedure.

Keywords: Pancreas preserving total duodenectomy · Spigelman classification · Familial adenomatous polyposis

65 Lynch syndrome in 3D

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Introduction: Hereditary non-polyposis colorectal cancer (HNPCC) has always been defined by family history, antedating the discovery of the genetic mechanism behind its major component, Lynch syndrome. Now, tumor testing and germline testing are used to classify patients with a pattern of inherited colorectal cancer in the family, producing several subgroups and fostering confusion. We wanted to clarify hereditary colorectal cancer by identifying and defining the different groups that fall under the banner of HNPCC, using three dimensions (family history, tumor testing, and germline testing) of classification. We applied these definitions to patients and families enrolled in our registry.

Methods: Family history (Amsterdam I or II Criteria vs not Amsterdam Criteria) was used to define patients and families with HNPCC. Tumor testing (MSI-H [microsatellite instability-high] vs MSS [microsatellite stable]; IHC [immunohistochemistry] loss of expression [LOE] vs normal IHC) and germline testing (mutation carriers versus no mutation) were then performed to subclassify patients and families with HNPCC. The permutations of these classifications are applied to our registry.

Results: There are 585 Families (1271 individuals) in our Non Polyposis Registry. An Amsterdam compliant family history was present in 245 families (41.8 %) of which 108 families carried a germline mismatch repair gene mutation. FCC Type X (Amsterdam positive family and microsatellite stable tumor) was found in 26 (10.6 %) families; Likely-Lynch (Amsterdam Compliant family, MSI/LOE tumor, negative germline mutation testing) was found in 5 families (2 %), and 106 (43.2 %) of families are HNPCC (Amsterdam compliant family). There are 152 Lynch syndrome families. 54 Lynch syndrome families are 3D (Amsterdam positive family history, MSI-H/LOE tumor, and Germline MMR gene mutation), 72 Lynch syndrome families are 2D (having either a Family history or MSI-H/LOE tumor, plus a Germline MMR gene mutation), and 26 Lynch syndrome families are 1D (only having a germline MMR gene mutation). 108 of the Lynch syndrome families met Amsterdam criteria (71 %), Mismatch repair deficient tumors were found in 72 (47.3 %).

Conclusion: Classification of HNPCC and Lynch syndrome is confusing. Sorting families in three dimensions can clarify the confusion and may direct further testing and, ultimately, surveillance.

Keywords: Lynch syndrome · Diagnosis · HNPCC

66 Molecular characterization of Brazilian patients suspected for Lynch syndrome

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Purpose: Lynch syndrome (LS), former known as Hereditary Non Polyposis Colorectal cancer (HNPCC), accounts for 3–5 % of all colorectal cancers (CRC) and is inherited in an autosomal dominant fashion. This syndrome is characterized by early CRC onset, high incidence of tumors in the ascending colon, excess of synchronous and metachronous tumors, and accelerated adenoma-carcinoma transition (2–3 years). Nowadays, LS is regarded of patients who carry deleterious germline mutations in one of the five Mismatch Repair genes (MMR), such as MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6), post-meiotic segregation increased 1 (PMS1) or post-meiotic segregation increased 2 (PMS2). **Methodology:** In order to characterize Brazilian patients suspect for LS, we assessed 116 suspected Lynch syndrome patients to determine the frequency of germline point mutations in MLH1, MSH2, MSH6, PMS1 and PMS2 by capillary sequencing. We also assessed chromosomal deletions/duplications in MLH1 and MSH2 and MSH6 through MLPA, generating a complete characterization of MMR genes.

Results: The analysis of the five MMR genes revealed 46 carriers of pathogenic mutations, including 25 in MSH2 (54 %), 16 in MLH1 (35 %), four in MSH6 (9 %) and one in PMS2 (2 %) gene. In our analysis we found 22 novel alterations, including 10 pathogenic mutations and other 12 novel VUS described for the first time. Mutations in the three “minor” MMR genes (MSH6, PMS1 and PMS2) account for only 4.5 % (5/116) of all Brazilian Lynch syndrome patients. In addition, our analysis revealed that MSH2 gene is more frequent in our population and the frequency of MSH6, PMS2 and PMS1 mutations is lower than the expected for other populations.

Conclusion: The identification of families carrying pathogenic mutation is of high importance since the test can be offered to relatives and help to guide more cost effective cancer screening protocols.

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Keywords: Lynch syndrome · Mutation · Colorectal cancer

67 Searching for high penetrance genes in familial colorectal cancer type X through whole-exome sequencing

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Purpose: Lynch syndrome represents 3–5 % of all cases of colorectal cancer (CRC) and is an autosomal-dominant inherited cancer predisposition syndrome caused by germline mutations in mismatch repair genes (MMR). Currently, the clinical diagnosis of Lynch syndrome is based on family history, according to the Amsterdam criteria I and II. In addition, the clinical suspicion is accomplished through the Bethesda guideline, which basically includes CRC diagnosis under the age of 50 year and positive microsatellite instability as an indicative for MMR germline mutation screening. However, a significant portion of families who meet the Amsterdam criteria and Bethesda guideline show no pathogenic mutations in Mismatch repair genes, suggesting that they carry pathogenic mutation in novel, yet to be discovery, colorectal predisposing genes. Currently, these families have been reported with “Familial colorectal cancer type X” that can reach up to 50 % of Amsterdam Criteria families. Thus, the present study aims to determine novel susceptibility genes with autosomal dominant pattern which is thought to be typical of Familial Colorectal Cancer Type X.

Methodology: It was proposed a family-based sequencing of one family that fulfilled the Amsterdam criteria but showed no mutation in the mismatch repair genes—MLH1, MSH2, MSH6, PMS2 and PMS1 genes (assessed by sequencing of complete coding region by Sanger method and MLPA for MLH1, MSH2 and MSH6). For that, we used whole-exome sequencing approaches in the SOLiD 5500 platform. The experimental design comprised sequencing of 3 affected and 2 unaffected individuals in order to facilitate the identification of candidate genes.

Results: Two novel candidate genes segregating with the disease were selected, including a deletion in WDR27 gene and a silent mutation near to the splice site of exon 13 of c-kit gene. Both genetic variants found were not reported in population-based SNPs databases (1000 Genome, NHLBI GO-ESP and dbSNP databases) and were not detected in ~250 healthy Brazilian controls. The clinical significance of these alterations remains to be evaluated.

Conclusion: The discovery of novel genes may help clarify the etiology of Familial Colorectal Cancer Type X as well as help to determine specific screening protocols in order to improve management and prevention of patients at high risk.

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Keywords: Familial cancer · Sequencing · Colorectal cancer

68 Adenomas in Lynch syndrome: the perfect storm of colorectal carcinogenesis

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Introduction: Lynch Syndrome Is The Result Of A Germline Mutation That Inactivates Mismatch Repair And Is Characterized by multiple colorectal cancers occurring at an early age. The cancers arise from pre-existing adenomas, but the adenoma-carcinoma sequence can be extremely rapid. We analyzed biologic factors that may

reflect the enhanced malignant potential of adenomas in patients with Lynch syndrome.

Methods: Patients with a germline mutation in a DNA mismatch repair gene were accessed from the Cologene™ database. We excluded patients without any adenomas on colonoscopy and those who had undergone total colectomy and ileorectal anastomosis. Adenomas were characterized by size, shape, histology and location. They were compared with adenomas from a series of average risk screening patients.

Results: There were 81 Lynch syndrome patients who had 407 colonoscopies (average 5 per patient); 220 exams (54 %) were normal or yielded only hyperplastic polyps. In 187 colonoscopies (46 %), 293 adenomas were found. 37 patients had one adenoma, 29 had from 2 to 5, 5 had between 6 and 9, and 10 patients had 10 or more adenomas (maximum synchronous adenomas 22). 114 (38.9 %) of the adenomas were “high risk” by size (≥ 10 mm) or histology (>25 % villous architecture, high grade dysplasia). There were 51 tubular adenomas >10 mm, 58 tubulovillous adenomas and 5 villous adenomas and 24 (8.2 %) adenomas had high-grade dysplasia. There were 378 average risk screening patients who had 715 adenomas. Lynch syndrome patients had significantly more adenomas per patient (3.6 vs 1.9), were more often women (64.2 vs 36.2 %, $p < 0.001$), had more advanced adenomas (38.9 vs 14.1 %, $p < 0.001$), more flat or depressed adenomas (17.3 vs 4.9 %, $p = 0.03$), more right sided adenomas (83 vs 70 %, $p = 0.05$), and more adenomas with high grade dysplasia (8.2 vs 1.1 %, $p < 0.001$). In addition more Lynch patients had multiple adenomas (54.3 vs 21.3 %, $p = 0.023$), and were the only group to have >9 synchronous adenomas.

Conclusion: Adenomas in Lynch Syndrome represent the “perfect storm” of colorectal carcinogenesis. They are advanced histologically, multiple, small, proximal, and easily missed. Colonoscopists beware.

Keywords: Adenomas · Lynch syndrome · Colonoscopy

69 Balancing uncertainty in Lynch syndrome: managing VUS's

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Introduction: A variant of unknown significance is a mutation that is not known to be associated with deleterious alteration of gene expression. Universal tumor testing and the advent of Hereditary Cancer Panels for germline testing will increase the number of mismatch repair gene (MMR) mutation carriers and also the number of patients with MMR gene variant of unknown significance (VUS). Interpretation of the VUS mutation and development of recommendations for patients and families with a VUS can be challenging. In this study we aimed to determine the incidence of cancer and pattern of inheritance in patients with a VUS.

Methods: A single institution Cologene database was queried for families and patients with a variant of unknown significance (VUS) in a MMR gene. The type of cancer, clinical diagnostic criteria (i.e. Amsterdam), and VUS genotype were documented.

Results: There were 63 VUS carriers in 22 families, 16 of which fulfilled Amsterdam criteria. These 63 carriers had 188 cancers. 8 families had a VUS in MLH1; 6 were Amsterdam compliant and there were 24 individuals carrying the VUS. 19 (79 %) of the VUS carriers had 111 cancers including 94 colorectal, 8 endometrial, 2

pancreatobiliary, 5 in the duodenum/small bowel, 1 ovarian and 1 transitional cell. 6 families had a VUS in MSH2; 5 were Amsterdam compliant and there were 21 individuals carrying the VUS. 12 (57 %) of the VUS carriers had 52 cancers including 37 colorectal, 5 endometrial, 2 pancreatobiliary, 1 in the duodenum/small bowel, 4 gastric, 1 glioblastoma, 1 ovarian and 1 transitional cell. 5 families had a VUS in MSH6; 4 were Amsterdam compliant and there were 14 individuals carrying the VUS. 8 (57 %) of the VUS carriers had 22 cancers including 15 colorectal, 4 endometrial, 2 pancreatobiliary, and 1 gastric. 3 families had a VUS in PMS2; 1 was Amsterdam compliant and there were 4 individuals carrying the VUS. 3 of the VUS carriers had 10 cancers including 7 colorectal, 2 endometrial and 1 ovarian.

Conclusions: The combination of an MMR gene VUS and an Amsterdam positive family history is associated with a high cancer risk in the family. Although the VUS cannot be used to triage relatives into surveillance versus non-surveillance, the families should be surveyed as if they had Lynch syndrome. Our data reflects the real need for an assay to better define the clinical significance of a VUS in a mismatch repair gene.

Keywords: Lynch syndrome · Variant of uncertain significance · Surveillance

70 Does weight or BMI predict the presence of small bowel intussusception in children and adolescents with Peutz Jeghers syndrome?

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Purpose: Small bowel intussusception (SBI) is the most common and serious complication of Peutz Jeghers syndrome (PJS) in children and adolescents, historically resulting in laparotomy rate of 70 % of affected individuals by age 18 years [1]. Endoscopic surveillance strategies are in place to identify small bowel polyps before they cause SBI. Our hypothesis was that children and adolescents with clinically apparent or occult SBI might be identifiable from their body habitus or weight loss. Growth in PJS patients has not been published to date.

Methodology: Weight and height were consistently collected from children and adolescents (age 0–16 years) attending paediatric outpatient clinic from 2000 to 2014 for elective investigations, or when presenting with an episode of SBI. Measurements were obtained from affected individuals who had SBI necessitating surgery, and those who had not developed this complication (controls). Weight standard deviation score (SDS), weight for height (Wt:Ht) and Body mass Index (BMI) were compared in those with and without SBI, and these values analysed.

Results: Data were analysed from a cohort of 37 children with PJS. Auxology data was available in 28/37 patients. 8/28 had documented SBI, 1 of these 8 had 2 separate episodes of SBI. Thus 9 episodes of SBI were included, along with data on 84 clinical reviews on patients without SBI at the time (control data). The median values for weight z score (SDS), Wt:Ht & BMI were compared in the control series who did not have SBI (n = 84) versus those who had SBI (n = 9). Weight median z score in the control series was not significantly different in the SBI group (0.07 vs -0.04 respectively). In addition, comparing control vs SBI there was no significant difference in Wt:Ht ratio (0.21 vs -0.4 respectively) nor BMI (16 vs 17 kg/m² respectively). Weight SDS showed bimodal distribution in both those with and without SBI, with nearly equal distribution around the mean whether or not patients

had SBI. No patients had weight z score > -2 SDS below the mean. Those patients with a weight SDS below the mean had no discernible increased risk of SBI. Sequential growth data were available in 7/8 patients with SBI with a median increase in BMI of 0.48 kg/m² post SBI surgical repair, and of the 5/7 with increase in BMI, the median increase was 1.1 kg/m². Insufficient data were available to identify a drop in weight or BMI pre SBI.

Conclusion: The data suggest that there is no single auxological predictor of impending SBI within the paediatric PJS population. With complete data on 8 children with SBI, weight SDS, nor BMI could not reliably predict SBI. To improve the quality of this study, complete data are being collected prospectively on all PJS patient encounters to seek a change in weight z score in those who subsequently have SBI. On these data, the prudent clinician cannot allow the weight nor habitus of a child with PJS to predict or exclude the possibility of occult SBI.

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Keywords: Peutz Jeghers syndrome · Intussusception · Growth

71 Mutations in DNA polymerase genes (POLD1 & POLE) in individuals having early-onset colorectal cancer and/or multiple adenomas

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Background: Recent studies have found that germ-line mutations in the DNA polymerase genes POLD1 and POLE confer high risk for multiple colorectal adenomas and carcinoma (CRC) as well as for endometrial cancer. The clinical phenotype of POLD1 and POLE mutation carriers is variable and is still not fully recognized.

Objectives: To assess whether germ-line mutations in POLD1 and POLE genes are responsible for early-onset microsatellite stable (MSS) colorectal cancer and multiple colorectal adenomas in a cohort of Israeli subjects and to describe the genotype-phenotype correlation.

Methods: This is an ongoing prospective case control study. To date, the study group includes 42 Israeli Jews having early-onset (less than 50 years old) MSS CRC and/or multiple (>10) colorectal adenomas and normal APC and MUTYH genetic evaluation. The control group includes healthy individuals age >50 years old having normal colonoscopy and no family history of colorectal or endometrial cancer. Germ-line DNA was analyzed for a panel of 13 mutations using the nano-fluidic 48.48CS dynamic array chip. Mutations analyzed were: POLE gene (p.D275V, p.P286R, p.S297F, p.V411L, p.L424V, p.R446Q, p.S459F, p.E277G), POLD1 gene (p.R311C, p.P327L, p.S478N, p.V759I, p.R195X).

Results: We report here our preliminary results as we have detected a substantial number of mutation carriers. 6 out of 42 (14.3 %) subjects tested were found to carry one of the DNA polymerase panel mutations. 38 of the 42 study subjects had multiple colorectal adenomas and 5 were found as mutation carriers: four carried the same POLD1 mutation: p.V759I and one carried the POLE mutation: p.E277G. Additionally, 4 of those 5 mutation carriers had early-onset MSS CRC as well as having multiple polyps. The other 4 of the 42 study subjects had early-onset MSS CRC without multiple polyps and one was found

to carry a novel POLD1 mutation: p.R195X. The clinical phenotype of mutation carriers included colorectal adenomas, advanced adenomas, few hyperplastic polyps and left-sided CRC. Age of polyp and CRC onset was <50 years. The POLE mutation: p.E277G was associated with a unique clinical phenotype of ‘café au-lait’ spots and glioblastoma multiforme. Family history of mutation carriers included diverse spectrum of cancers including lung, lymphoma, skin, and gum in addition to CRC.

Conclusions: Germ-line mutations in the DNA polymerase genes: POLD1 and POLE are relatively frequent in individuals having MSS early-onset CRCs and/or multiple adenomas, when no mutation is detected in the APC and MUTYH genes. The clinical phenotype of mutation carriers is variable and includes mixed polyposis phenotype, early-onset MSS CRC (mainly left-sided) and family history of cancer.

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72 “Second Class” Lynch? How important are germline MSH6 and PMS2 mutations

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Introduction: In 2013 the National Comprehensive Cancer Network (NCCN) amended Lynch syndrome surveillance guidelines to begin colonoscopy screening at age 30–35 years for MSH6 mutations and 35–40 years for PMS2 mutations (cf 20–25 years for MLH1 and MSH2). We were alarmed at this change and wondered how many cancers might be missed were it to be generally adopted. We analyzed our families with MSH6 and PMS2 mutations to see if delaying the onset of colonoscopy would miss any CRC in our patients.

Methods: Lynch syndrome patients were classified by germline mutation and sorted by cancer diagnosis and age of onset. Patients without a documented age of cancer were excluded (n = 84).

Results: There are 156 Lynch syndrome families (one family excluded because the family does not have colorectal or endometrial cancers) with 834 colon, rectal, or endometrial cancers in 700 individuals. The average age of onset of cancer in MLH1 mutation carriers was 48.3 years (range 20–90), while that for MLH1 VUS was 46.0 years (range 22–95), for MSH2 was 49.3 (range 16–86), MSH2 VUS 52.2 years (range 21–92), MSH6 55.7 years (range 27–80), MSH6 VUS 56.2 (35–90), PMS2 43.7 (range 23–75), PMS2 VUS 47.6 (range 40–64). 283 MLH1 mutation carriers had colorectal cancer, 1 under age 20 and 25 (8.9 %) between age 21 and 30. 81 MLH1 VUS carriers had cancer, 10 between ages 21 and 30. 263 MSH2 mutation carriers had colorectal cancer, 2 at age 20 or younger

and 17 (6.5 %) from age 21 to 30. 33 MSH2 VUS carriers had colorectal cancer, 2 under age 30. 20 MSH6 mutation carriers had colorectal cancer, 2 under age 30. 9 PMS2 mutation carriers had colorectal cancer, 2 under age 30 and 1 under age 35. Delaying the start of colonoscopy screening to age 35 would have missed 2 cancers (15 %) in MSH6 carriers and 3 (22 %) cancers in PMS2 carriers. In 2014 the NCCN amended the screening recommendations for MSH6 and PMS2 to Colonoscopy at age 25–30. Using these guidelines would have missed 2 cancers in 1 PMS2 mutation carrier.

Conclusion: The changes in 2013 NCCN guidelines were inappropriate. Although guidelines are not a “catch all”, our hope is to prevent cancer by providing individualized care to all patients affected by Lynch syndrome.

Keywords: Lynch syndrome · Screening · Guidelines

73 Operating on children with FAP: where the surgery is done matter

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Background: Most patients with familial adenomatous polyposis (FAP) are operated in their late teens or older but occasionally the severity of polyposis or the presence of symptoms demands that surgery is done earlier. In such cases correct decision making and good technique are especially important. To determine the impact of colectomy or proctocolectomy in the very young we reviewed cases where surgery was performed at age 14 or younger.

Methods: Patients having surgery at age 14 or younger were identified from our polyposis database. Age at diagnosis, colorectal phenotype, indication for surgery, presence of cancer, age at colectomy, type of surgery, perioperative morbidity, length of stay, and long-term outcomes were abstracted from the database and chart review. Patients were stratified according to where the surgery was performed.

Results: There were 84 patients, 44 females and 40 males. 57 (68 %) had their colectomy at the Cleveland Clinic (CCF) (56 by colorectal surgery one by pediatric surgery), and 27 elsewhere. Mean age at FAP diagnosis was similar between CCF patients and outside patients (10.6 vs 10.3 years). Age at surgery was also similar (12.7 vs 11.6). Current age reflects a difference in length of follow-up (29 vs 43 years). 74 % of CCF patients underwent colectomy and IRA, and 26 % had an ileal pouch anal anastomosis (IPAA). Proportions operated outside were similar (63 % IRA and 33 % IPAA respectively). There were significant differences in number of laparoscopic index surgeries (30/57 vs 4/27), the number of subsequent surgeries (17/57 vs 19/27, $p = 0.002$) and in length of stay for index surgery (6.8 vs 12.5 days, $p < 0.05$) Subsequent surgeries included lysis of adhesions, proctectomy, ventral herniorrhaphy, and ileostomy. Desmoids developed in 7/57 (12 %) of Cleveland Clinic patients and 10/27 (37 %) of outside surgeries ($p = 0.018$).

Conclusion: Children having colorectal surgery for FAP at the specialist center tended to have colectomy and IRA, the majority laparoscopic. The benefits of this conservative choice of procedure are shown in fewer reoperations and fewer desmoid tumors.

Keywords: FAP · Children · Surgery

74 A studded rectum: an important phenotypic clue to the presence of MYH associated polyposis

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Introduction: MYH associated polyposis is a recessively inherited syndrome of colorectal cancer predisposition due to biallelic germline mutations in the base excision repair gene MYH. Its clinical presentation varies but is typically with young age of onset colorectal cancer or oligo-adenomatous polyposis. Clinically, MYH associated polyposis overlaps with attenuated familial adenomatous polyposis, sporadic oligopolyposis, serrated polyposis and Lynch syndrome. We have noticed that patients with MYH associated polyposis may present with rectums that are studded with hyperplastic polyps. We report this as a possible unique phenotypic feature of the syndrome. **Methods:** Patients undergoing colonoscopic management of oligopolyposis were evaluated. Over the course of a year colonoscopies were prospectively graded for the presence of a studded rectum.

Results: There were 20 patients being managed endoscopically with oligo-adenomatous polyposis. Eleven had biallelic germline mutations of MYH and of these 6 had rectal studding. These six were all women and two were sisters. Mean age at last endoscopy was 63.4 years. A sample of rectal polyps was biopsied and in each case these were hyperplastic polyps. Four patients with biallelic MYH mutations had no studding: one woman and three men. The only difference in colorectal phenotype was a higher total number of polyps removed in the unstudded group (mean 58 vs 84). The studding was independent of genotype. There were 2 patients with a germline APC mutation, 3 patients without any germline mutation and 4 patients who were not tested. One of the untested patients had rectal studding.

Conclusion: Rectal studding may be a helpful sign of MYH associated polyposis and raises questions about the biology of abnormal base excision repair.

Keywords: MYH polyposis · Proctoscopy · Hyperplastic polyps

75 A case of polyposis coli due to low APC somatic mosaicism

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Purpose: To present a patient with familial adenomatous polyposis (FAP) caused by adenomatous polyposis coli (APC) somatic mosaicism. Case description: A 21 year old female presented to the hospital with rectal bleeding and abdominal pain. The patient underwent a colonoscopy and esophagogastroduodenoscopy, which revealed extensive polyposis of the recto-sigmoid junction and distal sigmoid, extensive polyposis of the proximal right colon and cecum and scattered polyps in the left and transverse colon. The rectum was essentially spared aside from two small pedunculated polyps. The stomach and duodenum, including the papilla, were normal. In

preparation for recto-sigmoid sparing surgery, more than sixty polyps were removed from the recto-sigmoid junction and distal sigmoid. The patient had no extra-colonic signs of FAP. Her maternal grandmother was diagnosed with colon cancer at age seventy-six, but there was no other family history of polyps or colon cancer. **Methodology:** Next-generation sequencing (NGS) analysis was performed using the ColoSeq™ panel1 on DNA extracted from both peripheral blood lymphocytes and colonic polyps.

Results: Molecular analysis detected the p.E1408X deleterious mutation in the APC gene in 12 of 276 (4 %) reads of the DNA in the peripheral blood and in 30 % of the DNA from colonic polyps.

Conclusions: Somatic APC mosaicism has previously been reported to cause polyposis syndrome in a few cases^{2, 3}, but has been underestimated as a cause of polyposis coli. In this patient, 4 % APC mosaicism of the peripheral blood lead to florid polyposis. This case should reinforce the need for NGS analysis in all patients with a personal history of polyposis, no family history of colon polyps/cancer, and no identified germline mutation by traditional less sensitive approaches.

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Keywords: APC · Mosaicism · NGS

76 Identifying Lynch syndrome using universal colorectal cancer screening: implications of patient age

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Background: Although various criteria have been suggested to guide tumor testing as screening for Lynch syndrome in colorectal cancer (CRC), none are sufficiently sensitive to identify all cases. Universal screening provides near perfect sensitivity, but lacks specificity particularly with advancing patient age. The goal of this study was to report on the yield of identifying Lynch syndrome by universal screening, stratified by age.

Methodology: Universal screening of colorectal cancers for Lynch syndrome has been routinely performed at our institution since April 2009. Screening consisted of microsatellite instability and/or immunohistochemistry (IHC) for expression of the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2. Tumors with high microsatellite instability (MSI-H) and loss of MLH1 underwent testing for BRAF mutation and/or MLH1 methylation. Patients with molecular suggestion of Lynch syndrome were referred for genetic counseling and testing. Patient demographics, family history, tumor characteristics, and genetic test results were prospectively collected in a dedicated database and analyzed.

Results: 882 patients were included. 122 (13.8 %) were mismatch repair deficient (dMMR; i.e. MSI-H or lacked MMR IHC expression). 34 patients (3.9 %) had suspected or confirmed Lynch syndrome

based on tumor and genetic testing. With advancing age, a higher percentage of tumors were mismatch repair deficient, but a smaller percentage of the MMRd tumors were Lynch syndrome. The number Lynch syndrome patients and the number of (dMMR) cases per age grouping was as follows: age <50 years: Lynch/12 dMMR (92 %); age 50–59: 9 Lynch/15 dMMR (60 %); age 60–69: 8 Lynch/23 dMMR (35 %); age 70–79: 4 Lynch/33 dMMR (12 %); age 80–89: 2 Lynch/32 dMMR (6 %); age >90 years: 0 Lynch/7 dMMR (0 %). 19 % of Lynch syndrome patients were older than age 70.

Conclusions: Universal screening of colorectal cancers identifies patients with Lynch syndrome, even in patients with advanced age that likely would not have been diagnosed based on age cutoff criteria. This has significant implications for them and their descendant generations. Advanced age should not preclude universal testing. Cost-effectiveness analysis for testing at advanced age is in progress.

Keywords: Lynch syndrome · Universal screening · MSI-H

77 Surgical management Of MYH-associated polyposis: is more better?

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Background: MYH-associated polyposis (MAP) is a recessively inherited predisposition to colorectal malignancy. Despite increasing knowledge about the genetics and the phenotypes of MAP, the natural history and the surgical management of colorectal cancer in MAP has not been well-defined This study reports on the incidence, surgical management, and natural history of colorectal cancer after surgery in MAP patients from a single institution.

Methods: A single institution hereditary colorectal cancer database was queried for patients with genetically confirmed MAP and colorectal cancer. Treatment approaches, subsequent colonoscopic findings, and survival were recorded and analyzed.

Results: Forty-eight families with MAP were reviewed. Twenty-four patients from 24 families had MAP and colorectal cancer. There were 11 males and 13 females. The mean age at cancer diagnosis was 48 years. Seven patients had synchronous colorectal cancers; total of 39 cancers in the 24 patients. Nineteen cancers were located in the right colon, 9 in the left colon, and 11 in the rectum. Seven patients were treated by segmental colectomy, 7 patients underwent total colectomy and ileorectal anastomosis, and 9 patients underwent restorative proctocolectomy. The median follow-up was 60 months. For patients treated by total colectomy and ileorectal anastomosis, none developed subsequent rectal cancer. On endoscopic follow-up and intervention, 24 rectal adenomas were removed from 9 patients over a total of 703 surveillance months. For the five patients who were treated by segmental colectomy, one developed a metachronous colon cancer. During the follow-up surveillance, 391 metachronous adenomas were removed over a total of 1125 surveillance months.

Conclusions: MAP is associated with a significant synchronous and metachronous colorectal neoplasia. Colectomy and ileorectal anastomosis is the recommended treatment of colon cancer in this syndrome. Regardless of treatment, meticulous surveillance is required for any remaining colon or rectum.

Keywords: MYH-associated polyposis · Surgery · Colon cancer

78 Can oral rehydration therapy correct the metabolic disturbances and improve quality of life after colectomy?

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Purpose: We have demonstrated metabolic disturbances and poor quality of life post-colectomy in patients with Familial Adenomatous Polyposis (FAP). We aimed to evaluate the efficacy of oral rehydration therapy (ORT) in restoring water and electrolyte balance post-colectomy.

Methodology: A blinded placebo-controlled randomised cross-over trial was undertaken. Thirty patients with demonstrated hyperaldosteronism from on-going observational study were recruited. Patients were randomised to receive either placebo or ORT first in a cross-over trial with an intervening washout period. Patients attended clinical investigation day (CID) three times. CID: Fasting urine and blood samples were collected to measure sodium loss, hydration status and renin-angiotensin-aldosterone system (RAAS) activation. Quality of life (QoL) was assessed using SF-36 and FACIT-F questionnaires. Full ethics approval was obtained and the trial has been registered (ISRCTN76735966).

Results: Observational study 70 patients who had undergone colectomy were recruited. 34 patients (49 %) demonstrated fasting hyperaldosteronism (>250 pmol/L) leading to higher urinary losses of potassium ($p = 0.03$) and creatinine ($p = 0.01$). Cross-over RCT Biochemistry results Data acquired so far in 16 patients ($n = 48$ CIDs) have demonstrated fasting plasma aldosterone concentration post-ORT to be significantly lower compared to baseline [189.25 (7.24) vs 536.25 (12.56) pmol/L; $p = 0.05$]. QoL results SF-36–Post-ORT, patients reported an improvement in six of the eight dimensions of health (role physical, bodily pain, vitality, general health, social functioning and role emotional) with an overall improvement in both composite scores (physical and mental component summary scores) FACIT-F–Post-ORT, patients reported higher scores on four of the five scales (physical well-being, social well-being, emotional well-being and fatigue scale) with higher total scores.

Conclusion: Metabolic disturbances are common after colectomy leading to a poor quality of life. ORT forms a safe and effective intervention to correct the metabolic disturbances post-colectomy resulting in a positive impact on quality of life.

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Keywords: Oral rehydration · Metabolic disturbances · Colectomy

79 Clinical features of breast cancer arising in patients with Peutz-Jeghers syndrome

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Purpose: It is now widely accepted that patients with Peutz-Jeghers syndrome (PJS) are at an increased risk of developing a number of cancers including breast cancer. Currently, a number of surveillance protocols are used with little evidence to support them. We evaluated breast cancer in patients with PJS at our institution with respect to demographics and tumour type to help rationalise surveillance.

Methodology: A retrospective review of a prospectively maintained database at a tertiary referral centre was carried out. 136 patients (M: F; 55: 81) with PJS from 92 families were identified. A detailed review of patients' medical records, database records, family files, genetic, histopathology and radiology reports was undertaken.

Results: Sixteen breast cancers occurred in 13 patients (16%; all female). A further three patients developed benign breast pathology (1 phyllodes tumour; 2 fibroadenoma). The median age when the patients were first seen for PJS was 20 years (range 2–65). Median follow-up period was 32 years (range 11–57). STK11 mutation was detected in 60 of 92 families and in 10 of the 13 patients diagnosed with breast cancer. Median age at the diagnosis of breast cancer was 41 years (range 31–67) with a median follow up from diagnosis of 2 years (range 1–21). Four patients were diagnosed with breast cancer at an age less than 35 years. Three patients died of metastatic breast cancer at 1, 2 and 8 years aged 53, 33 and 43 years respectively following the diagnosis of breast cancer. Six patients had a positive family history for breast cancer. Detailed histopathology report was available for 13 of the 16 cancers and the histology was ductal carcinoma in situ (DCIS) in 12 patients. Two patients had recurrent breast cancer involving the previously affected breast at intervals of 4 and 7 years respectively. Five cancers were detected through mammogram and three others were detected on MRI.

Conclusion: Breast cancer was common in our PJS cohort and occurred at a young age. The median age at diagnosis was 41 years. In the majority, the histology was DCIS. Given the rarity of PJS, a multi-centre study focussing on age of onset and histological type is required to provide an evidence base for future surveillance.

Keywords: Peutz-Jegher's syndrome · Breast cancer · Surveillance

80 Expanding the mutation spectrum and phenotype of polymerase proofreading-associated polyposis: novel and previously reported POLE variants in an Italian series

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Background: Constitutional mutations of the POLE and POLD1 genes, coding for DNA polymerase ϵ e δ subunits, respectively, have been recently identified in patients with multiple colonic adenomas and colorectal cancer (CRC). So far, only few families with this autosomal dominant inherited cancer predisposition, named Polymerase Proofreading-Associated Polyposis (PPAP) have been reported (1, 2), and the phenotype and prevalence of the condition are not well defined. We therefore investigated a clinic-based series for mutations in the POLE and POLD1 exonuclease proofreading domains to verify their frequency and associated clinical characteristics.

Patients and methods: A total of 52 patients with multiple colorectal adenomas (>10) and/or early onset CRC and/or familial CRC were

investigated. These had previously been tested for mismatch repair deficiency by MSI and/or immunohistochemistry, and, when appropriate, for APC and MUTYH mutations, with negative results. The coding region of the exonuclease domains of POLE and POLD1 (aa 278–471 e 304–517, respectively) was directly sequenced from genomic DNA isolated from blood leukocytes. In-silico analyses were performed using Polyphen2, SIFT, Mutation Taster, ClustalOmega, Phyre2, and Chimera 1.6.2.

Results: POLE mutations were identified in 2/52 patients. In one patient with two metachronous tumors (colorectal cancer at 42 years, followed by adenocarcinoma of the ileum at 57 years) and a positive family history of CRC, the following two POLE variants were identified: c.1175A>G (p.Asp392Gly) and c.1274A>G (p.Lys425Arg). Both are previously unreported. Bioinformatic analyses are concordant in predicting a pathogenetic effect for p.Lys425Arg, while interpretations of p.Asp392Gly are discordant. In another family with an autosomal dominant phenotype of Turcot syndrome (multiple polyps associated with gliomas) and cutaneous manifestations (multiple pilomatricomas), the proband was found to be heterozygous for the c.1270C>G (p.L424V) POLE variant in exon 13. This variant has already been reported in patients with PPAP (1, 2).

Conclusions: Our results confirm the role of polymerase proofreading domain sequence variants in predisposition to colorectal cancer and expand the phenotype and mutation spectrum of PPAP.

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Keywords: Polyposis · polymerase · Turcot syndrome

81 MLH1 mutation type and frequency in colorectal carcinomas demonstrating solitary loss of PMS2 protein expression

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Purpose: Immunohistochemistry for mismatch repair (MMR) proteins MLH1, PMS2, MSH2 and MSH6 is used to screen for Lynch syndrome in patients with colorectal carcinoma. The pattern of loss of expression is usually indicative of the underlying genetic defect. Loss of PMS2 with normal MLH1 expression in tumour cells suggests a germline mutation in PMS2; however no deleterious mutation is found in a significant proportion of these cases and therefore no diagnosis of Lynch syndrome can be made [1]. We hypothesized that a germline mutation in MLH1 will explain a clinically relevant proportion of these cases with solitary loss of PMS2 expression.

Methodology: Patients with colorectal carcinoma were selected from the Colon Cancer Family Registry based on the presence of a

microsatellite instability phenotype, solitary loss of PMS2 expression and absence of germline PMS2 mutation by long-range PCR and MLPA (multiplex ligation-dependant PCR amplification). Germline MLH1 mutation testing was performed by Sanger sequencing and MLPA.

Results: There were 76 colorectal carcinomas showing a solitary loss of PMS2 expression but retained expression of MLH1 protein. A germline mutation in PMS2 was identified in 59 cases (78 %). From the 17 cases with no mutation detected, blood-derived DNA for MLH1 testing was available for 14 patients. A deleterious MLH1 mutation was identified in 4 patients (c.113A>G p.Asn38Ser; c.230G>A p.Cys77Tyr; c.199G>A p.Gly67Arg; c.350C>T p.Thr117Met) and an unclassified variant was identified in 3 patients (c.299G>C p.Arg100Pro; c.187G>C p.Asp63His; c.1607C>T p.Pro536Leu). All MLH1 variants were missense suggesting that the MLH1 protein may retain its antigenicity, accounting for the immunohistochemical results.

Conclusion: A missense mutation in MLH1 may explain up to half of colorectal carcinoma cases with solitary PMS2 loss of expression for which no PMS2 mutation has been found, establishing the diagnosis of Lynch syndrome. Patients with colorectal carcinoma showing loss of PMS2 and normal MLH1 expression should be screened for MLH1 mutation if no PMS2 mutation has been identified.

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Keywords: MLH1 mutations · PMS2 protein loss · Lynch syndrome

83 High rate of familial cancer in a population-based consecutive cohort of ovarian cancer patients

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Objective: To determine the rate of familial cancer in a population-based series of epithelial ovarian cancer cases in Newfoundland. Background: The Canadian province of Newfoundland and Labrador has a very high rate of familial colorectal cancer (1) and well-defined founder mutations in MSH2 (2). The contribution of inherited causes in ovarian cancer has never been studied in this population.

Methods: All cases of invasive epithelial ovarian cancer diagnosed in the province from 1999 to 2007 (n = 220) were included. Information regarding diagnoses, surgeries, chemotherapy, pathology and outcome were collected. All patients or next of kin were approached to provide family history of all diagnoses of cancer in immediate or extended pedigrees. When relevant, cancer diagnoses in relatives

were confirmed. Each pedigree was reviewed and a risk assessment for either Lynch syndrome or Hereditary Breast Ovarian Syndrome (HBOC) was assigned. The rate of families meeting Amsterdam and/or Bethesda criteria for Lynch Syndrome or High/Intermediate HBOC Score was the primary outcome.

Results: Of 220 cases, 145 family members (proxies) and 75 living probands were offered participation in a family history study. Detailed clinical information was available for 98 % cases. 124 (56 %) had serous cell types and 61(28 %) were poorly differentiated. 134 probands and proxies agreed to participate in the project. 102 pedigrees were collected. Participants were younger than non-participants (56.6 vs 62.3 years, $p = 0.001$). There was no difference in stage at presentation, cell type or grade between participants and non-participants. Probands were more likely to agree to participate than Proxies (57 vs 41 %, $p = 0.02$). Thirty eight (40 %) pedigrees were classified as low risk for any inherited cancer predisposition. Hereditary Breast Ovarian Cancer: Eleven of ninety five (11.6 %) families met high risk criteria for inherited HBOC. Only 4 of these were from previously known BRCA families. An additional 2 known families met intermediate risk criteria. Overall the study group identified 6 known mutations and an additional 4 families in whom partial BRCA testing had been done and another 3 high risk HBOC families where no testing has yet been offered. Overall 44/95(46.3 %) pedigrees contained a family member with breast cancer. Lynch Syndrome: Five (5.3 %) families met Amsterdam criteria, of whom 2 were previously know MMR mutation carriers. 2 have had no previous genetic testing. One had negative IHC on concurrent endometrial/ovarian cancer. Twenty seven (28.4 %) families met Bethesda Criteria. There was no difference in age at diagnosis in those who did or did not meet Bethesda Criteria. Endometrioid cell type was more common (22 vs 14 %) in Bethesda + families. Forty seven (49.5 %) pedigrees contained a family member with colon cancer.

Conclusion: In this population, 28 % of cases have a pedigree meeting Bethesda criteria for Lynch Syndrome and 14 % have a pedigree suggestive of high or intermediate risk of HBOC. Identification of ovarian cancer in this population serves as a highly useful method of finding families at high risk for Hereditary Breast Ovarian Cancer or Lynch Syndrome. Ovarian cancer cases in Newfoundland represent a population in need of urgent clinical and research testing.

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Keywords: Ovarian cancer · Lynch syndrome · BRCA

84 Hereditary cancer national survey in Argentina

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Purpose: National Cancer Institute of Argentina applied a national survey of Human and Molecular Hereditary Cancer Resources. Main objectives were to identify, quantify and categorize all the cancer genetic counseling clinics and laboratories performing molecular testing. Secondary objectives were to detect areas with inadequate

access to genetic counseling and availability of complete molecular testing for the most frequent syndromes.

Methodology: From November 2011 to September 2013, public and private institutions within the country were reached. Initial contact was made by phone. Then a questionnaire was sent by email to every institution that confirmed to provide genetic counseling or molecular testing. Institutions were identified from different databases that cover clinical genetics services, tertiary level hospitals, laboratories with molecular biology output, cancer research groups and key informants. Questionnaire included information regarding contact details, academic degree and training of professional in charge of the genetic counseling session, name of the syndrome and genes analyzed, molecular techniques available, type of tissue needed and time to results.

Results: From 199 institutions surveyed, 47 genetic counseling clinics and 29 laboratories were included. Two-thirds of the institutions are private and one-third public. All services are concentrated in 11 out of 24 provincial jurisdictions. Although in 100 % of the public institutions genetic counseling is provided by specialized professionals, 22 % of private institutions do not have specialized professionals in charge of the counseling session. A total of 15 Syndromes, 25 different genes and 2 non-syndromic entities can be tested in Argentina. Next generation sequencing is available for most frequent syndromes (colorectal and breast). Lynch Syndrome, Familial Adenomatous Polyposis, MUTYH associated Polyposis, Peutz Jeghers, Hereditary Diffuse Gastric Cancer and Breast/Ovarian Hereditary Cancer can all be comprehensively tested, but only in private laboratories. Conclusion: Cancer risk assessment and genetic testing in Argentina are very heterogeneous considering the geographic distribution and wide variability of services provided. The most frequent hereditary cancer syndromes can be comprehensively tested with high quality techniques. This national survey is a very important resource to delineate public health policies, aimed at increasing the number of genetic counseling clinics, reaching consensuated management strategies, and improving accessibility to molecular testing. This survey was the source to the recently created National Argentinian Familial Cancer Network (RACAF) that is actively working to accomplish the above-mentioned objectives.

National Cancer Institute of Argentina.

<http://www.msal.gov.ar/inc/index.php/programas/plan-nacional-de-tumores-familiares-y-hereditarios-procafa>

Keywords: Hereditary cancer survey · National program · Public health policies

85 Bi-allelic somatic mutations as a cause of tumour mismatch repair-deficiency in colorectal cancer: implications for identifying mismatch repair gene mutation carriers within population-based colorectal cancer

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Purpose: Tumour mismatch repair (MMR) deficiency, determined by immunohistochemical (IHC) loss of MMR protein expression, is used diagnostically to identify individuals with Lynch syndrome. A high proportion of colorectal cancers (CRCs) that demonstrate tumour MMR-deficiency are categorised as having “suspected Lynch syndrome” due to the absence of tumour MLH1 methylation or germline MMR gene mutations after standard screening approaches [1]. The aim of this study was to identify all causes of tumour MMR-deficiency, including bi-allelic somatic mutations and more complex germline mutations, within a cohort of population-based CRC cases and determine whether a revision of the current testing and triaging strategies for the identification of MMR gene mutation carriers in the population is warranted.

Methodology: Population-based incident CRC-affected cases from Australasian Colorectal Cancer Family Registry were tested for MMR protein expression by IHC (n = 804). MMR-deficient CRCs (n = 90) were screened for germline MMR gene mutations using Sanger sequencing and MLPA of the MLH1, MSH2, MSH6, and PMS2 genes. CRC FFPE tumour tissue DNA was also screened for MMR gene somatic mutations using AmpliSeq custom capture and sequencing on the Ion Proton and for Loss of Heterozygosity (LOH). MLH1 and MSH2 gene promoter methylation and BRAF V600E somatic testing was also performed on CRC cases demonstrating tumour MMR-deficiency. Finally, germline whole genome sequencing was performed on individuals with unexplained tumour MMR-deficiency to identify putative intronic, promoter and structural variation mutations within the MMR genes or mutations in genes other than the four main MMR genes. In addition, family history of CRC and extra-colonic cancers, including fulfilment of Amsterdam criteria and Bethesda guidelines, and tumour pathology features were assessed for their positive and negative predictive value in identifying mutation carriers.

Results: Of the 90 MMR-deficient CRC cases observed, n = 40 were shown to carry a germline MMR gene mutation after the initial screen with Sanger sequencing and MLPA. MLH1 methylation was identified in n = 13 CRC-affected cases demonstrating loss of MLH1/PMS2 expression. No evidence of MSH2 gene promoter methylation was observed. Of the remaining n = 37 CRC cases with suspected Lynch syndrome, 13/37 (35 %) demonstrated double somatic mutations comprised of either two point mutations or a single point mutation and loss of heterozygosity while no bi-allelic mutations were observed in CRC tumours from 11 of the MMR gene mutation carriers tested. A single somatic point mutation or LOH event was identified in 24/37 (65 %) of CRC-suspected Lynch syndrome cases suggesting that a germline “first hit” is still to be found; whole genome sequencing is currently being performed to interrogate extended intronic and promoter sequences.

Conclusions: Bi-allelic somatic mutations are a significant cause of tumour MMR-deficiency in population-based CRC cases and, therefore, revision of the current triaging and diagnostic testing strategies used to identify individuals and their relatives with Lynch syndrome would be warranted.

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Keywords: Mismatch repair genes · Population screening · Somatic mutations

86 Genetic studies of sporadic and familial colorectal carcinoma (CRC) in Colombia

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Background and purpose: Colorectal carcinoma has a high rate of incidence and mortality [1–2]. This Research describes some molecular phenotypes in a sample of 1279 patients. Variants of the mismatch repair (MMR) genes MUTYH and APC were evaluated in addition to other genes involved in hereditary syndromes [3].

Methods: Clinical criteria were applied to differentiate the hereditary carcinoma. Immunohistochemistry (IHC) was used to analyze the expression of the hMLH1 gene and somatic mutations were evaluated in tumor DNA. Genomic libraries were created using microfluidic gene chips (Fluidigm) and the MiSeq sequencing system (Illumina) was used for sequencing. Candidate variants were selected and validated by Sanger sequencing. The microsatellite instability was analyzed using PCR-FCE [4–6]. We have developed an amplicon sequencing method that uses microfluidics PCR to simultaneously identify mutations in up to 480 amplicons. To do so, the samples are barcoded individually using indexing adapters that allows pooling of multiple amplicon libraries for up to 384 samples in a single MiSeq run. The sequence data is analyzed with a locally developed bioinformatics pipeline that uses WA, VarScan V2.0 and custom shell and Perl scripts.

Results: Analysis of 574 cases using IHC-MLH1 showed loss of expression in 7.1 % of the cases evaluated. MSI (microsatellite instability) analysis of 451 cases indicated a high value of MSI-H (22.6 %). Analysis of 159 patients with familial CRC showed 48 mutations, 18 of which showed functional implications and three were founder MSH2 mutations appearing in two and three individuals, respectively, the third c.596delTG. According to the pathology reports, the average age is 57 years, with 29 % of the cases being less than 50 years old. Locoregional metastasis was diagnosed in 75 % of the patients. The most common location of the tumor was in the rectum (41 %). The most common familial syndrome observed was the syndrome of Lynch with 85 % of cases having MSH2 mutations.

Conclusions: Analysis of sporadic CRC indicated variants in the genes APC, POLE, ARID1A, AMER1, FBXW7 and ATM. The identification of gene variants with founder effect and the mutations in genes related to familial carcinoma will allow the establishment of preventative strategies by screening for mutations in the populations at risk. Screening using immunohistochemistry of MLH1 and the determination of microsatellite instability allows the identification of patients with Lynch syndrome. We have developed a rapid, cost effective, and efficient method for screening mutations in known cancer genes. We have developed a low-cost, high-throughput pipeline, and method to screen 480 customizable amplicons (~20 genes, ~144Kbp) for up to 384 samples per run. By combining a bioinformatics pipeline to design customized screening panels with Fluidigm microfluidics PCR, Illumina MiSeq, and variant analysis pipelines. It also allows us to achieve a high depth of coverage and maintain a low cost per sample.

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Keywords: Colorectal cancer · Hereditary cancer · Molecular biology

87 Two MUTYH mutations causing MUTYH associated polyposis first described in the Arab population in Israel

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MUTYH is a base excision repair gene responsible for correcting errors in DNA. Two common MUTYH mutations (G396D & Y179C) have been identified in Caucasians, accounting for 80 % of mutations causing MUTYH associated Polyposis (MAP), an autosomal recessive disorder characterized by multiple colorectal adenomas. Homozygotes have an increased risk for colorectal cancer (CRC) and also duodenal cancer [1]. In Israel these mutations are detected mainly in the Jewish North African population [2]. Of 60 patients evaluated for polyposis in 2013 we detected 7 patients with biallelic MUTYH mutations and 2 heterozygotes. All biallelic carriers were found to have multiple adenomas and 3 had CRC. Heterozygotes presentation was variable. Recently, we detected two mutations that have not been previously described in the Arab population in Israel. 1st: 41 yo male with rectal bleeding had >30 adenomatous colon polyps. Family history: 2/6 siblings had CRC in addition to multiple adenomas at the ages of 28 and 44; a third sibling with 30 adenomas and hyperplastic polyps at 38 years; two healthy siblings with normal colonoscopies. His MUTYH sequencing analysis was positive for a p.His85Arg homozygote mutation. This mutation was suspected to be causative in a 50 years old Turkish patient with 10–100 adenomas. Segregation was performed on all family members. All family members with polyposis and/or CRC were found to be homozygous for the mutation detected in the proband while all healthy subjects, with normal colonoscopies, were either negative for the mutation or heterozygote. This strengthens the likelihood of pathogenicity of this mutation. No other cancers and no gastric or duodenal polyps have been reported in this family. 2nd: 44 yo female, had resections of renal liposarcoma (age 39) with local recurrence and re-surgery (age 41). Papillary Thyroid cancer was diagnosed at the age of 43. A routine follow-up PET CT detected a FDG avid lesion in the cecum. Colonoscopy revealed dozens of adenomatous polyps including a larger adenoma with HGD in her right colon. No family history of cancer. Due to multiple adenomas APC sequencing was performed

and was normal. Her MUTYH sequencing analysis was positive for a c.1437_1439delGGA homozygote mutation. This mutation has been described in Italians [3] with polyposis in homozygote and compound heterozygote with one of the common MUTYH mutations. Total colectomy with an IRA was performed. **Conclusions** We present two mutations in MUTYH causing MAP and CRC that have never been described in the Arab population. In the first case no additional extraintestinal cancers have been reported while the second was associated with thyroid and liposarcoma which to the best of our knowledge has never been described in association with MAP.

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Keywords: MYH polyposis · Colorectal adenoma · Colon cancer

88 Miss-rate and delay in diagnosis of serrated polyposis syndrome in a clinical cohort

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Purpose: Serrated polyposis syndrome (SPS) is a new and under recognised colorectal cancer predisposition syndrome. Previous studies reported miss-rates of SPS diagnosis varying from 40 to 82 % in patients presenting with at least 1 serrated polyp [1, 2]. Since SPS patients and their first degree relatives have an increased risk of colorectal cancer, early recognition is important. We aimed to determine the miss-rate of SPS during follow-up with more colonoscopies during which SPS could be diagnosed.

Methodology: We retrospectively identified all patients diagnosed with ≥ 1 colorectal polyp or carcinoma detected at our tertiary referral center between 1986 and July 2013 using a nation-wide pathology registry. A cumulative polyp count was scored for adenomatous and serrated polyps per patient. Size and location of serrated polyps was recorded to assess if patients fulfilled the WHO criteria for SPS. Based on the available diagnosis in the patient files, miss-rate and 95 % confidence interval (95 % CI) were calculated.

Results: We randomly assessed 4000 patients for this interim analysis of which 1587 (39.4 %) had ≥ 1 serrated polyp. Sixteen patients fulfilled the WHO criteria, 7 male and 9 female patients with a median number of 24 serrated polyps (range 15–59) and 2 adenomas (range 0–9). In four patients no prior SPS diagnosis was made, leading to a miss-rate of 25.0 % (95 % CI 3.7–46.2). Duration of follow-up varied from 2 to 16 years in these missed cases. In 3 of these patients familial colorectal cancer was diagnosed instead of SPS. These patients were under strict follow-up with surveillance intervals ranging from 1 to 6 years. The diagnosis in the other patient was probably missed because the majority of serrated polyps had been removed before the formulation of the WHO criteria for SPS in 2000 and the pathology reports were not easily available. Of the patients

diagnosed with SPS only one had a delay of 2 years before diagnosis, however, the surveillance interval (every 2 years) was adequate. A fifth patient fulfilling the SPS criteria was diagnosed with Lynch syndrome based on a MSH2 mutation, and as such was not marked as a missed case.

Conclusion: The miss-rate for diagnosis of SPS is significant, even during longer follow-up with repeated colonoscopies. Failure to recognize SPS was the result of not systematically applying the WHO criteria or the unavailability of older pathology reports to the clinician. Awareness of this colorectal cancer predisposition syndrome needs to be raised to lower the miss-rate of SPS.

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Keywords: Serrated polyposis · Awareness · Diagnosis

91 Set up of an in vitro mismatch repair assay in a diagnostic laboratory

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A significant proportion of DNA mismatch repair (MMR) variants identified in suspected Lynch syndrome patients are missense. They are classified as variants of unknown significance (VUS) precluding diagnosis. One key step to sort out uncertainty is to determine whether the variants result in non-functional proteins. The in vitro MMR assay is used to assess the mismatch repair, likely the most important function of a MMR protein. However, robustness of the assay, critical for its routine use in the clinical setting, requires technical specialization and accurate reagent preparation. Also, standardized protocols are lacking.

Purpose: The aim of the present work was to set up the in vitro MMR assay for the functional characterization of VUS in MLH1 and PMS2 genes meeting quality control standards.

Methodology: Reference materials and standardized operative procedures (SOP) for HEK293T cells transfection, whole cell protein extraction, nuclear extraction, mismatched plasmid substrate generation, repair buffer, and MMR assay were provided by Dr. Plotz and optimized in the laboratory.

Results: Monitoring of cell lysis for nuclear extraction was assessed by trypan blue staining and enrichment for nuclear extract proteins by western blot. Average protein concentration in nuclear extracts was 4.7 $\mu\text{g}/\mu\text{l}$. Transfection efficiency was up to 60 % and protein concentration of whole cell extracts was about 10 $\mu\text{g}/\mu\text{l}$. Use of HPLC-purified oligomers and verification of complete digestion improved the quality of the mismatched plasmid. Control plasmids were used in each experiment. Assay performance was preliminarily validated with MLH1 D41H VUS which showed a decreased activity (23 ± 6 % of the wildtype level) with minimal intraexperimental variability supporting its pathogenicity.

Conclusion: High quality reagents and optimized protocols are critical to standardization of the in vitro MMR repair assay allowing the obtention of robust and clinically interpretable results.

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Keywords: MMR assay · Variant of unknown significance; MLH1

92 MLH1 constitutional epimutations: complex methylation patterns and structural alterations in MLH1 locus

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MLH1 constitutional epimutations as cause of Lynch syndrome can be found in 6–10 % of patients presenting methylated MLH1-tumors. Little is known about the mechanistic basis of these epimutations and consequently, its inheritance pattern.

Purpose: We aimed at exploring the presence of structural alterations close to MLH1 locus as putative inducers of hypermethylation of the region and MLH1 silencing.

Methodology: Six Spanish MLH1 epimutation carriers were included in this study. The presence of structural alterations and methylation profiles in blood DNA were evaluated using two customized arrays with 15 K probes (Agilent Technologies) surrounding the locus of interest (region chr3:36334841-37792337). Agilent Genomic Workbench was used for the analysis. Methylated DNA was precipitated using anti-5-methylcytosine antibody (Eurogentec). Bioinformatic analysis was done with R 2.15.12 and Bioconductor packages Limma, Ringo and Biomart. Data was normalized using the method “Nimblegen” in Ringo, and immunoprecipitated enriched regions were determined.

Results: Deletions in the analyzed region were found in 3 of the 6 epimutation carriers (deletion sizes ranging 0.5–20 Kb). None of them had been described as CNV. Methylation analysis yielded dependable results in 5 of the 6 patients. Widespread hypermethylation of the whole region (1.46 Mb) including the MLH1 promoter was observed in a carrier of two deletions with size of 15 and 19 Kb (average of fold change log: 1.5) and two cases without detected deletions. Conversely, two cases harboring deletions of 0.5 and 20 Kb, respectively, did not display a wide hypermethylation profile.

Conclusion: Our results point to a complex pattern of structural and methylation patterns in constitutional MLH1 epimutations. Further studies are needed to confirm the molecular nature of the observed aberrations and to assess the causal relationship between structural alterations surrounding MLH1 and constitutional epimutations.

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Keywords: Epimutation · MLH1 · Methylation

93 The presence of the c.3956delc mutation in the APC gene is a genetic marker of familial adenomatous polyposis in patients from Northern Brazil

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Purpose: Familial adenomatous polyposis (FAP) is a hereditary cancer predisposition syndrome with autosomal dominant inheritance caused by germline mutations, mainly in the APC gene (Rossi et al., 1998; Half et al., 2009; Hosogi et al., 2009). In the north and northeast regions of Brazil, gastrointestinal tumors are the second most frequent type of cancer among men and the third most frequent among women (Silva, 2012). The aim of this study was to characterize APC gene mutations, correlate them with patient phenotypes, and evaluate genomic alterations in individuals diagnosed with FAP in northern Brazil.

Methodology: A total of 15 individuals diagnosed with FAP from 5 different families of northern Brazil were analyzed in this study. The proband of each family was sequenced to identify germline mutations using the Ion Torrent platform, while the remaining individuals were assessed for mutation detection using the amplification refractory mutation system. The aCGH technique was performed to quantify genomic alterations.

Results: All 15 patients exhibited germline mutations in the APC gene, and all mutations were detected in exon 15 of the gene. The c.3956delC mutation in the APC gene was present in all patients. Quantitative genomic alterations were detected in several genes in the patients analyzed.

Conclusion: The presence of the c.3956delC mutation in all studied families suggests that this mutation was introduced in the population of the State of Pará through ancestor immigration, i.e., a de novo mutation that arose in one member belonging to this State (Suzuki, 1992). Regardless of its origin, the c.3956delC mutation is a strong candidate biomarker of this hereditary cancer syndrome in families of northern Brazil.

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Keywords: FAP · APC · Familial adenomatous polyposis

94 Detection of APC germline mosaicism by next-generation sequencing in an FAP patient

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Familial adenomatous polyposis of the colon (FAP) is characterized by multiple polyps in the intestine and extra-colonic manifestations. Most of FAP cases are caused by a germline mutation in the tumor suppressor gene APC, but some cases of adenomatous polyposis are result from germline mutations in MUTYH, POLD1, or POLE. The rate of mutation detection depends on the methods used for genetic testing and the genes analyzed in the patients. Although sequence analysis of APC by the Sanger method is routinely performed for the genetic testing, there remain cases whose mutations are not detected by the analysis. In our clinic, we encountered a male, 41 years of age, who suffered from multiple polyps in his large intestine. He earlier visited a hospital because of occult blood in his fecal test. Colonoscopy detected polyps with the number of less than 100 and subsequent histological examination of the polyps diagnosed adenomatosis. Since he had no family history of polyposis or colorectal cancer, he was suspected to be a de novo case of FAP or a patient of MUTYH-associated polyposis (MAP). Direct sequencing of APC was performed by the conventional Sanger method using DNA extracted from his lymphocytes to examine the 5'-half of the coding region where most of the APC mutations occur. However, no pathogenic mutations were detected. Since next-generation sequencing has enabled us to analyze the comprehensive human genome, improving the chance of identifying disease causative variants, we tested the efficacy of next-generation sequencing in his genetic test. We carried out whole-genome sequencing of the DNA, and identified eight variants in the APC gene. Among the eight, we detected a nonsense variant (c.3175G>T p.E1059X) in 6 of 50 reads (12 %). We re-sequenced the region by the Sanger method, and found a very low peak of mutant allele. Additional deep sequencing determined the mutation in 453 of 3726 reads (12.2 %) in peripheral blood. Interestingly, the mutation was observed in 3774 of 83,679 reads (4.5 %) in hair follicles, and 2099 of 69,169 (3.0 %) and 4860 of 66,557 (7.3 %) in buccal mucosa. In addition, we found different frequencies of the mutation in non-tumorous colonic (9.2, 3.4, 12.3, 5.8, and 9.0 %) mucosa. Our data implied that genetic analysis by next-generation sequencing is an effective strategy to identify genetic mosaicism in FAP.

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Keywords: FAP · Mosaicism · NGS

95 Spectrum of cancer phenotypes in Asian Lynch syndrome families

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Purpose: Not much is known about cancer phenotypes in Asian Lynch syndrome families. A few studies have reported a higher prevalence of gastric and hepatobiliary cancers[1], but a lower prevalence of colorectal cancers, with less synchronous or metachronous and proximal colorectal cancers [2,3] in the Asian Lynch syndrome families compared with Caucasian Lynch syndrome families. Disease phenotype distribution is important to be known for cancer screening guidelines for Asian Lynch syndrome families which may be different from the current cancer screening guidelines that are based on phenotypes occurred in Caucasian families.

Methodology: We studied cancer phenotypes in a cohort of the 1054 members from 41 Asian (Chinese, Japanese, Korean, or another South East Asian ethnicity) compared with 37,519 members from 720 Caucasian Lynch syndrome families in which at least one member had a MMR gene mutation. These families are recruited from the Colon Cancer Family Registry (USA, Canada, Australia, and New Zealand) and the Familial Cancer Centre at the Royal Melbourne Hospital (Australia) between 1997 and 2011. We calculated the proportion of individuals with each cancer phenotype out of the total individuals in families in Asian and Caucasian Lynch syndrome families. We estimated odds ratios (ORs) to compare the cancer phenotypes in the Asia Lynch syndrome families with Caucasian Lynch syndrome families, adjusting for sex, gender and ascertainment.

Results: The proportions for each cancer phenotype in the Asian and Caucasian Lynch syndrome families were as follow: colorectal (8.92 vs 7.87 %), followed by endometrial (4.02 vs 3.84 %), ovary (1.34 vs 1.24 %), gastric (1.04 vs 0.98 %), brain (0.66 vs 0.53 %), pancreas (0.47 vs 0.43 %), renal pelvis/ureter (0.38 vs 0.74 %), biliary (0.28 vs 0.09 %), small intestine (0.19 vs 0.22 %) and gall bladder (0.09 vs 0.09 %). Except for colorectal (OR 1.43, 95 % CI 1.14–1.81, $P = 0.002$) and biliary cancer (OR 4.29, 95 % CI 1.28–14.3, $P = 0.018$), there was no evidence for a difference in other cancer phenotypes (including endometrial, gastric, brain, ovary, small intestine, renal pelvis/ureter and gallbladder) between Asian and Caucasian families. There was also no evidence for a difference of synchronous/metachronous and proximal colorectal cancers between Asian and Caucasian families.

Conclusion: There is an increase risk of developing colorectal and biliary cancers in the Asian Lynch syndrome families compared with Caucasian Lynch syndrome families. Further larger studies are required to confirm this finding and to generate cancer penetrance for proper clinical management in Asian Lynch syndrome families.

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Keywords: Lynch syndrome · Asian · Phenotype

96 A phase 3 placebo-controlled trial of celecoxib in pediatric subjects with familial adenomatous polyposis

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Purpose: Chemoprevention is shown to prevent colorectal polyps in adults with familial adenomatous polyposis (FAP). The effect of chemoprevention on colorectal polyps in children with FAP is not well studied. This double blind, 18 center, 13 country, 5 year study evaluated the efficacy and safety of celecoxib versus placebo in the prevention of colorectal polyps in pediatric subjects with FAP.

Methods: Subjects aged 10–17 years, with a diagnosis of FAP (based on genotype and or phenotype) and less than 20 polyps >2 mm in size which were completely excised on baseline colonoscopy were eligible. Patients were excluded if they had 20 or more colorectal polyps >2 mm in size on baseline colonoscopy. Subjects were randomized in a 1:1 ratio to celecoxib 16 mg/kg/d or placebo. Subjects had yearly visits, with colonoscopies at each visit. The primary end point was the time-to-treatment failure, defined as the time from randomization to the earliest occurrence of ≥20 polyps (>2 mm in size) at any colonoscopy during the study or diagnosis of colorectal cancer.

Results: The first patient was randomized in 2006. The study was terminated in October 2013, by the sponsor, at the Data Monitoring Committee recommendation, due to lower than expected rate of end points. 106 subjects (55 celecoxib/51 placebo) with a mean age of 12.6 and 12.2 years respectively, were randomized. Treatment duration was 23 months in the celecoxib arm and 25.5 months in the placebo arm. 13 % subjects in the celecoxib group and 26 % in the placebo group developed 20 or more polyps >2 mm in size. Among them, the median time to disease progression was 2.1 years in the celecoxib group and 1.1 years in the placebo group, respectively. None of the subjects developed colorectal malignancy. All causality treatment-emergent adverse events (TEAE) was similar between the treatment groups: 40 (76 %) subjects in the celecoxib group and 35 (73 %) subjects in the placebo group. Treatment-related TEAEs was also similar: 18 (34 %) subjects in the celecoxib group and 15 (31 %) subjects in the placebo group. The most common AEs (occurring in more than 10 % of subjects in a group) were abdominal discomfort, abdominal pain, diarrhea, nausea, vomiting, fatigue, seasonal allergy, influenza, nasopharyngitis, upper respiratory tract infection, pain in extremity, headache, cough, and oropharyngeal pain.

Conclusions: Pediatric FAP patients randomized to celecoxib were observed to have half the event rate and a delay in progression of

adenomas compared to patients in the placebo arm after a median of 2 years of treatment. Due to the premature termination of the study, the impact of the results cannot be certain. High dose treatment with celecoxib in children with FAP was generally well tolerated and safe.

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Keywords: FAP · Chemoprevention · Pediatrics

97 Validation of Lynch syndrome prediction models in Asian populations

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Purpose: At least seven prediction models exist that estimate probability of carrying a germline mutation in a MMR gene: PREMM1,2,6, MMRpredict, MMRpro, Leiden, Myriad Genetics Prevalence table, Amsterdam-Plus (AmP) and Amsterdam-Alternative (AmA). These models were derived using European/Caucasian populations. Their utility for Asian populations is not known as MMR mutation carriers may have a different prevalence and cancer risks, and their performance have not been evaluated in Asian populations where family size may be restricted[1]. Patient characteristics: We studied members of 34 Lynch syndrome families (65 individuals with MMR mutation) and 67 Non-Lynch syndrome families (829 individuals). These families (Chinese, Japanese, Korean, or other South East Asian ethnicity) were recruited from the Colon Cancer Family Registry (USA, Canada, Australia, and New Zealand) and the Familial Cancer Centre between 1997 and 2011.

Methods: Each mutation and non-mutation carrier was subjected to two clinical scenarios for each model: full family history of cancer; or restricted family history simulated under a one-child policy where pedigree was truncated to exclude all siblings (including the sibling of the parents) and excluding all children except the eldest child in each successive generation. We evaluated the sensitivity and specificity for all models at cut-offs of 5, 10, 20 and 30 %. Precision was compared by ROC curve analysis using Area under the Curve (AUC)[2]. Results: In the full family history scenario, at 5 % cut-off, AmA had the highest sensitivity with 98.7 % (CI 92.8–100), with the remaining models ranged from 78.7 (Leiden) to 96 % (AmP). Myriad, AmP and AmA had the lowest specificity with 12.8 % (CI 7.5–20), 36.8 % (CI 33.4–40.2) and 13.1 % (CI 10.8–15.6) respectively. The AUC value for individual models ranged from 75.8 (Myriad) to 93.5 % (PREMM1,2,6), with no statistical evidence for differences in the models.

When comparing each model in both scenarios, there is a trend towards a reduction in the value of AUC, sensitivity and specificity in the one-child policy scenario. The AUCs for full family and one-child policy scenario are PREMM1,2,6 (93.5 vs 82.9), MMRpredict (88.3 vs 83.2), MMRpro (95.3 vs 90.3), Leiden (89.1 vs 73), Myriad (75.8 vs 74), AmP (90.8 vs 77.2) and AmA (88.2 vs 79.1). The sensitivity of the models in the one-child policy scenario ranged from 46.8 (Leiden) to 98.7 % (AmA). There is an overlap of the 95 % CI in these tested parameters.

Conclusion: (a) We have no evidence that these models will perform poorly in Asian families. (b) These models may under-estimate the probability of carrying a mutation in families of restricted size (reduced sensitivity) and therefore alternative methods for assessing who should be tested for mutations need to be developed other than family history.

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Keywords: Lynch syndrome · Asian · Screening

98 Short-term risk of colorectal cancer for Lynch syndrome: a meta-analysis

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Purpose: For carriers of germline mutations in DNA mismatch repair (MMR) genes, the most relevant statistic for cancer prevention is colorectal cancer (Lynch syndrome) risk, particularly in the short-term as it is relevant for decisions of screening modality and frequency.

Methodology: We conducted a meta-analysis of all independent published Lynch syndrome studies that estimated colorectal cancer risks for various age- and sex-categories of the mutation carriers. We estimated: 5-year colorectal cancer risk over different age groups, separately for male and female mutation carriers; and number needed to screen to prevent one death (based on current estimates of the mortality reduction due to colonoscopy for Lynch syndrome); and number of expected serious complications due to colonoscopy (based on current estimates of frequency of death, perforations, bleeding and postpolypectomy syndrome following colonoscopy).

Results: We pooled estimates from analyses of 1114 Lynch syndrome families from five studies that comprised a total of 508 MLH1 and 606 MSH2 mutation carriers (there were insufficient studies to include MSH6 or PMS2 mutation carriers in this analysis). We estimated that, on average, 1 in 71 male and 1 in 102 female MLH1 or MSH2 mutation carriers aged in their 20s will be diagnosed with colorectal cancer in the next 5 years. These colorectal cancer risks increase with age and peak when the carriers are aged in their 50s (1 in 7 males and 1 in 12 females), and then decrease with age (1 in 13 males and 1 in 19 females when aged in their 70s). We estimate that annual screening by colonoscopy for 5 years of 16 males or 25 females when aged in their 50s would prevent one death from colorectal cancer while resulting in almost no serious complications. In comparison, annual screening by colonoscopy for 5 years would be needed for 115 males or 217 females when aged in their 20s to prevent one death while resulting in approximately one serious complication.

Conclusion: These are the most precise age- and sex-specific risks available of colorectal cancer for Lynch syndrome. Current guidelines for most countries recommend screening colonoscopy every 1–2 years for MLH1 or MSH2 mutation carriers, starting when they are aged in their 20s. Our findings support this regimen from age

30 years; however, it might not be justifiable for carriers aged in their 20s.

99 Impact of anastomotic anatomical configuration on postoperative leak rates in patients undergoing prophylactic surgery for familial adenomatous polyposis and MUTYH associated polyposis

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Purpose: To compare ileo-sigmoid and ileorectal anastomoses in patients with adenomatous polyposis and a low rectal polyp burden, on a background of regular postoperative endoluminal surveillance. Ileo-sigmoid anastomosis represents a deviation from the current standard whereby the inferior mesenteric artery pedicle is preserved with intracorporeal dissection being undertaken to the distal sigmoid. Thereafter, extracorporeal anastomosis is performed using a linear TLC 75 mm and a transverse TA 90 mm stapler, in a side of ileum to a side of sigmoid configuration. This is in contrast to the intracorporeal methods, which utilise a curved circular stapler (CDH) per rectum to achieve an anastomosis between the ileum and rectal remnant in one of four orientations (End ileum-to-End rectum (ETE), End ileum-to-Side (anterior) rectum (ETS), Side Ileum-to-Side (anterior) rectum (STS), Side ileum-to-End rectum (STE)).

Methodology: A retrospective case review of patients with FAP or MAP who underwent colectomy was performed. Patient and peri-operative characteristics, and postoperative leak rates were collated. Chi square test was employed to ascertain differences in the patient population with respect to postoperative anastomotic leakage. P values less than 0.05 were taken to indicate significance.

Results: A total of 110 patients, who underwent surgery between Jan 2006 and Nov 2014, were available for analysis. There were 41(37.3 %) 10–20 year olds, 28(25.5 %) 20–40 year olds and 41(37.3 %) older than 40 years, with an overall median age of 28 years. There were 45(40.9 %) males. Patients' overall fitness for surgery was represented by ASA grades: ASA1 = 52(47.3 %); ASA2 = 49(44.5 %); ASA3 = 2(1.8 %). 100 patients (90.9 %) underwent laparoscopic-assisted surgery. A single patient received a prophylactic defunctioning ileostomy. The remainder underwent primary intestinal continuity restoration. There were five anastomotic configurations [ETE (n = 47, 42.7 %), ETS (n = 7, 6.4 %), STS (n = 4, 3.6 %), STE (n = 42, 38.2 %) and extracorporeal ileosigmoid (n = 10, 9.1 %)]. The overall leak rate was 13/110 (11.8 %): [Intracorporeal: ETE: 7/47, 14.9 %—1 radiologically drained leak and 6 reoperated leaks; ETS: 0/7, 0 %; STS: 0/5, 0 %; STE: 6/42, 14.3 %—6 reoperated leaks; Extracorporeal: 0/9, 0 %]. There were significantly more postoperative leaks in the youngest and oldest age groups [(10–20 years: 7.3 % (3/38); 21–40 years: 0 % (0/28) and >41 years: 24.4 % (10/31), $p = 0.026$]. Other parameters such as gender, ASA status and operative access did not significantly impact upon anastomotic leakage risk.

Conclusion: There were no significant differences in leak rates following ileosigmoid or ileorectal anastomoses in this select group of patients undergoing prophylactic colectomy. Larger patient series are required to ascertain the utility of ileosigmoid anastomosis in this setting. If indeed there are lower anastomotic leak rates with ileosigmoid anastomoses, the regular luminal surveillance by specialist endoscopists may allow for patients to receive a potentially less morbid operative procedure with the proviso that the regular screening will allow for prompt detection of polyps in the remaining colorectum.

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Keywords: Prophylactic surgery · Anastomotic configuration · Postoperative leak

100 Aspirin modifies immune cell infiltration of colonic mucosa in Lynch syndrome: a possible mechanism for cancer prevention

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Purpose: The CAPP2 study showed that 600 mg aspirin daily reduced the risk of colorectal cancer (CRC) in Lynch Syndrome (LS) patients [1]. The mechanism by which this occurs is unknown. LS cancers are particularly immunogenic. Aspirin may reduce the risk of cancer in LS patients by altering the immune milieu of the colonic mucosa. We aimed to determine the density of Foxp3-positive T-regulatory cells (Treg) and CD3-positive T-cells in the normal colonic mucosa of LS patients enrolled in the CAPP2 study. We then aimed to assess any links between this and aspirin use. We also aimed to assess whether the immune infiltrate density within the colon remained stable over time in order to help determine whether any observed differences reflected treatment effects.

Methodology: Serial sections (pre- and post-intervention) from normal colonic biopsies of LS patients treated with 600 mg aspirin daily or placebo were immunohistochemically stained for Foxp3 and CD3. For a selection of patients two post-intervention biopsies from different time points were stained. The level of infiltration was determined by manually counting stained cells. The observer was blinded to treatment group. A selection of biopsies were recounted by a second independent observer.

Results: Within the aspirin intervention group the infiltrating Treg densities in the post-intervention biopsies (mean 24.9 Tregs/mm²) were significantly higher than in the pre-intervention biopsies (mean 20.1 Tregs/mm², $p = 0.033$). The change in Treg density from pre- to post intervention in the aspirin group (mean +4.8 Tregs/mm²) was significantly greater than the change in the placebo group (mean –2.3 Tregs/mm²; $p = 0.016$). Total T-lymphocyte levels appeared to be unaffected by aspirin. For patients with two analysed post-intervention biopsies taken at different time points, the infiltrate densities measured in the pairs of biopsies correlated significantly with each other for both Tregs ($p = 0.003$) and total T-cells ($p \leq 0.001$). No difference in counts was seen between the two observers.

Conclusions: Our results provide the first evidence that aspirin use increases the density of infiltrating Tregs in the colonic mucosa of LS patients. Given that aspirin use has been shown to decrease the risk of CRC in LS patients, this may represent a novel mechanism of aspirin's cancer preventative effects. The correlation of the post-intervention infiltrate densities suggests that the immune infiltrate of the

colonic mucosa remains stable over time. This is the first time that this has been demonstrated. It also helps to confirm that the changes seen were caused by aspirin treatment rather than natural variation.

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101 Utility of single nucleotide polymorphisms to guide risk appropriate colorectal cancer screening

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Purpose: Single nucleotide polymorphisms (SNPs)—common genetic variants—have been identified that are associated with colorectal cancer risk. The effect of each SNP on colorectal cancer risk is small, but in combination the association may be sufficient to identify proportions of the population who are at sufficient risk of colorectal cancer to justify more intensive screening.

Method: We conducted a literature review to identify all SNPs that have been confirmed (in independent samples) to be associated with colorectal cancer risk for people of European descent. For each SNP we extracted the allele frequency of the 'risk' allele for colorectal cancer and the odds ratio per risk allele. Using PLINK we simulated a population of one million people of which 5 % developed colorectal cancer by age 70 years (equal to the age-specific cumulative risk for the Australian population). The distribution of SNP risk alleles in the simulated population was selected to match risk allele frequencies and per allele odds ratios of the known colorectal cancer associations. We assumed a simplistic model of risk where the association with colorectal cancer for each SNP was independent and additive (on a log scale) across SNPs. Using this simulated data, we estimated the 5-year risks of colorectal cancer by the number of risk alleles of these SNPs by age, sex and family history.

Results: We identified 39 SNPs that were independently associated with colorectal cancer. Average risk allele frequency was 0.42 (range 0.07–0.90). Average odds ratio per risk allele was 1.14 (range 1.06–1.53). There was a high degree of overlap for the number of risk alleles between colorectal cancer affected and unaffected people (colorectal cancer affected had median 34 risk alleles, range 15–53; unaffecteds had median 32 risk alleles, range 14–51). The odds ratio per allele for colorectal cancer was 1.8 for people in the highest decile (top 10 %) of risk alleles, and 0.4 for people in the lowest decile (compared to the median number of risk alleles). The risk of colorectal cancer to age 70 years was 8.9 % for people in the highest decile of risk alleles compared with 1.7 % for those in lowest decile. At age 50, those who had a first-degree relative with colorectal cancer and who had the top decile of risk alleles, had a 5-year colorectal cancer risk of 2 %, which is equivalent to the risk of the average population at age 75 and approaches the risk appropriate for regular colonoscopy.

Conclusion: There is potential for use of the currently known SNPs to stratify the population into colorectal cancer risk categories—even using only the small number of SNPs reaching the standard but non-sensitive threshold for genome-wide statistical significance. Use of more sophisticated statistical analyses of SNP data could improve on

these findings to provide an avenue for risk appropriate screening for colorectal cancer.

Keywords: SNPs · Colorectal · Risk

102 Immunohistochemistry expression of DNA mismatch repair proteins in adenomas

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Summary: Background: Lynch Syndrome (LS) is the most prevalent hereditary colorectal cancer syndrome. It has an autosomal dominant mode of inheritance and it is caused by a germline mutation in one of the DNA mismatch repair genes: MSH2, MLH1, PMS2 or MSH6. Immunohistochemistry tests of the DNA mismatch repair proteins in tumor tissue is an important diagnostic tool in screening high risk patients for LS [1, 2].

Purpose: Verify immunohistochemistry expression of MSH2, MLH1, PMS2 and MSH6 proteins in adenomas detected in high risk patients for LS.

Methodology: Sixty-seven individuals that fulfill the Amsterdam, Familial Colorectal Cancer or one of the Bethesda's criteria, with colonoscopies performed on the purpose of screening or surveillance, were prospective selected for this study. Fifty-eight lesions were detected and resected or biopsied. Results: Of the 25 patients that had lesions diagnosed on their colonoscopies, six (24 %) had loss of expression of at least one of the mismatch repair proteins.

Conclusions: It is possible to perform immunohistochemistry of mismatch repair proteins in adenomas, when this is the only neoplastic tissue available for testing, in high risk individuals for LS.

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Keywords: Lynch syndrome · Immunohistochemistry · Mismatch repair

103 Impact of colonoscopy on risk of colorectal cancer for members of Lynch syndrome families

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Background: Lynch syndrome is an inherited susceptibility to colorectal cancer caused by a germline mutation in one of the DNA mismatch repair (MMR) genes, which confers a very high risk of developing the disease [1]. As a prevention strategy, current guidelines recommend 1–2 yearly colonoscopy, starting in the mid-twenties, to mutation carriers and their first-degree relatives [2–4]. Using the largest sample to date, we investigated the association between colonoscopy screening and risk of colorectal cancer for MMR gene mutation carriers, non-carriers and their untested first-degree relatives.

Methods: We conducted synthetic birth-cohort analyses of 4717 members of Lynch syndrome families participating in the Colon Cancer Family Registry (506 from Canada, 3020 from Australia and 1190 from the United States). Of these, 1986 had a pathogenic mutation in one of the MMR genes (carriers), 1583 did not have a pathogenic mutation (non-carriers) and 1148 were first-degree relatives of MMR gene mutation carriers but not genotyped for MMR gene mutations. We used weighted Cox proportional hazards regressions to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs) for associations between self-reported colonoscopy and the risk of colorectal cancer.

Results: During 35,615 person-years of observation, 831 carriers (42 %) were diagnosed with colorectal cancer. In the cohort of non-carriers, 39 (3 %) were diagnosed with colorectal cancer over 2236 person-years of observation. In the un-genotyped first-degree relative cohort, 37 (3 %) were diagnosed with colorectal cancer over 1655 person-years. A lower risk of colorectal cancer was associated with having at least one colonoscopy procedure for carriers (HR 0.12, 95 % CI 0.09–0.16), non-carriers (HR 0.23, 95 % CI 0.08–0.61) and the un-genotyped first-degree relatives (HR 0.10, 95 % CI 0.02–0.26), compared with those who did not have any colonoscopy. Of those who reported having at least one colonoscopy, the mean number of procedures undertaken during follow-up was 1.25 for carriers (HR per procedure 0.61, 95 % CI 0.54–0.68), 1.16 for non-carriers (HR per procedure 0.85, 95 % CI 0.61–1.19) and 1.18 for the un-genotyped first-degree relatives (HR per procedure 0.58, 95 % CI 0.34–0.97).

Conclusion: Our study provides additional and population-based evidence that colonoscopy is very effective in reducing CRC risk for MMR gene mutations carriers. Our results also show that colonoscopy is effective for reducing colorectal cancer risk for their non-carrier relatives as well as un-genotyped first-degree relatives.

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Keywords: Colonoscopy · Colorectal cancer risk · Lynch syndrome

104 A novel POLE variant, identified by exome sequencing, causes colorectal- and extra-colonic cancers

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Purpose: We describe a Norwegian family with accumulation of colorectal adenomas and adenocarcinomas in addition to extra-colonic cancers. Several CRC predisposing genes have previously been analysed with Sanger sequencing. However, no causative mutation was identified. Due to the striking dominant inheritance in this family, we strongly suspected a highly penetrant variant as the cause of cancer predisposition. We therefore performed exome sequencing to detect the cancer predisposing mutation in this family.

Methodology: All patient samples and clinical information was obtained with informed written consent and the study was approved by the Regional Committee for Medical and Health Research Ethics of Central Norway (approval 2012/1707). Exome capture was performed using SureSelectXT Human All Exon V5+UTRs. The libraries were sequenced on Illumina HiSeq2500 with 2 × 100 bp paired end sequencing. Exome sequencing data was aligned to the human genome (hg19, UCSC assembly, February 2009) using the Burrows-Wheeler-Aligner. PCR duplicates were removed with Picard-tools and BAM files were converted with SAMtools. Variant calling was done using GATK version 3.1. Variants were annotated with ANNOVAR and subsequent filtering was done using the filtering tool FILTUS version 0.99–9.

Results: We identified the novel POLE variant c.1373A>T (p.Tyr458Phe) as the cause of cancer predisposition in this family. POLE and POLD1 encode the catalytic and proofreading subunits of DNA polymerase ε (POLE) and δ enzyme complexes, respectively. Pathogenic germline mutations in these genes have recently been described to cause the CRC syndrome PPAP [1]. This is a highly penetrant, autosomal dominant syndrome predisposing to development of multiple adenomas and carcinomas. Tyr458 is a highly conserved residue located at the active site of POLE. Studies in microorganisms show increased mutation rate due to reduced exonuclease activity when the residue corresponding to Tyr458 is replaced with Phenylalanine. The POLE mutation segregates with disease and is associated with colorectal cancers and adenomas in addition to cancers of ovaries, small intestine and pancreas. We also observe a large phenotypic variation among the POLE mutation carriers which might be explained by modifying variants in other genes. In addition, we identified variants with potential functional effects which might explain some of the phenocopies observed in this family.

Conclusion: The POLE variant p.Tyr458Phe predisposes to colorectal adenomas and carcinomas in addition to extra colonic cancers.

Funding

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Keywords: Exome sequencing · PPAP · POLE

105 Whole exome and genome sequencing of individuals with serrated polyposis syndrome

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Purpose: Serrated Polyposis Syndrome (SPS) is a colorectal polyposis condition associated with an increased risk of developing colorectal cancer (CRC) in both the affected individual and their relatives. Currently, the underlying genetic basis of SPS is unknown. We have previously shown that genes associated with other polyposis syndromes including; SMAD4, BMPR1A, PTEN, MUTYH and GREM1 are rarely mutated in individuals with SPS[1] while a recent study has suggested a new set of genes as putatively associated with multiple sessile serrated adenomas (ATM, TELO2, RBL1, XAF1, PIF1, RNF43 and ULK4)[2]. Therefore, the aim of this study was to identify germline susceptibility variants for SPS using whole genome and whole exome sequencing (WGS/WES).

Methodology: The International Serrated Polyposis Register is a multi-institutional study of individuals with clinically defined SPS and their relatives, formed as a resource to support studies on the aetiology and clinical management of SPS. We have recruited SPS cases, meeting WHO criteria 1 or 3, from Genetics or Family Cancer Clinics within Australia, Canada, USA (predominantly Ohio) and from a single gastroenterology service at Middlemore Hospital, Auckland, New Zealand. Colonoscopy and histology records were collected to establish polyp counts. Participants provided a blood sample and data on ethnicity, lifestyle and environmental risk factors, and family history of cancer and, when possible, archival polyp/CRC tissue was collected for pathological review and molecular characterisation. To date, 406 SPS cases are enrolled (mean age 48.7 ± 14.7 years, range 18–78 years, 62 % females, mean polyp count = 44 ± 36). CRC developed in 113 cases while 25 SPS cases had a first degree relative with SPS. Whole exome capture was performed using Agilent XT SureSelect_V4 52 Mb capture while sequencing comprised of 100 bp pair-end sequencing on a HiSeq2500. Variant filtering strategies included only variants with (1) a frequency of <1 % in reference databases (1000 genomes and ESP6500), (2) were likely deleterious variants producing a non-sense/stop gain, frameshift, or splice-site and (3) were present in at least 20 % of the SPS cases tested.

Results: The findings from whole exome sequencing (n = 56) and whole genome sequencing (n = 4) revealed no likely deleterious germline coding mutations in the known-polyposis associated genes. Similarly, no likely deleterious variants in recently described CRC-associated genes OGG1 [3], GALNT12 [4], POLE, POLD1 [5], BUB1 and BUB3 [6] were observed. The genes putatively associated with multiple sessile serrated adenomas (ATM, TELO2, RBL1, XAF1, PIF1, RNF43 and ULK4) did not harbour rare likely-deleterious variants in our cohort. Our variant filtering strategies identified 138 candidate genes that had the highest burden of likely deleterious variants. The results from the validation and characterisation of these candidate genes in our extended cohort of SPS cases will be presented.

Conclusions: Mutations within previously identified polyposis- and CRC-associated genes do not underlie the vast majority of individuals with SPS. Therefore, novel candidate SPS genes remain to be identified.

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Keywords: Serrated polyposis · Germline mutations · Sequencing

106 Updating the insight database to meet the challenges of the genome sequencing era

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The InSiGHT database is a long-running locus-specific database in the field of inherited colorectal cancer (Lynch Syndrome, Familial Adenomatous Polyposis). It is a compilation of variant information from a variety of sources including laboratory submissions, published literature and other mismatch repair gene databases. Since 2011, the InSiGHT Variant Interpretation Committee has endeavoured to improve variant classifications for all variants in the database. This resulted in the classification and associated evidence displayed and

linked to each variant on the InSiGHT database, which has increased the visibility and clinical usefulness of the database [1]. An increasing number of visitors to the database is apparent in the website statistics (>30,000 "hits" per month). While achieving success in classifying previously difficult to interpret variants, the majority of missense variants remain of uncertain clinical significance (VUS). This is largely due to the lack of available evidence for remaining VUS. To address this problem, the InSiGHT database is adapting to the new technological advances taking place in sequencing and database systems. With current NGS technology, variants from more than 20 colorectal cancer associated genes are now possible to detect per sequencing run. Sharing with Human Variome Project Nodes, and new databases such as ClinVar will see more information reach InSiGHT in a systematic way. To meet the expanding challenges of the next decade, the InSiGHT database has recently upgraded to the LOVDv3 system, which will improve its capabilities and useability. We have also employed social media methodology to streamline communication with database users. However, the problem of transmitting clinical and phenotype data alongside variants remains a major challenge.

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Keywords: NGS · Variant · Database

107 Worldwide study of cancer risks for Lynch syndrome: international mismatch repair consortium (IMRC)

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Purpose: To bridge critical gaps in Lynch syndrome research, the International Mismatch Repair Consortium (IMRC) was formed in 2010. The IMRC comprises major worldwide consortiums involved in the research and/or clinical treatment of Lynch syndrome (cancer predisposition caused by inherited mutations in mismatch repair genes: MLH1, MSH2, MSH6, PMS2 and EPCAM); <http://www.sphinx.org.au/imrc>. The establishment of the IMRC was facilitated by the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) and the Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA). Currently, the IMRC has 205 members from 74 centres/clinics in Africa, Australasia, Europe, North and South America, and membership is open to anyone involved in research related to Lynch syndrome and/or the treatment of Lynch syndrome families. Accurate cancer risk estimates are needed to develop genetic counselling guidelines, and are of importance for the clinical management of mutation carriers and members within high-risk families. Risk may differ not only by age and gender and the gene that is mutated, but also by the country, and ethnicity of the carrier. The only way to thoroughly address this potential heterogeneity is to conduct comprehensive penetrance analyses on large, ethnically heterogeneous samples of persons/families segregating mutations in MMR genes.

Methodology: The IMRC will: (i) establish a combined data set of pedigree data from around the world for approximately 8800 Lynch syndrome families; (ii) estimate the age-specific cumulative risk (penetrance) of cancers at each anatomical site by sex, mismatch repair gene, type of mutation, and nationality/geographic region; and (iii) develop a personal risk tool for clinical use that provides 10-year risks of cancer based on the age, sex, mismatch repair gene, type of mutation, and nationality/geographic region.

Results: Since July 2014, IMRC investigators from 63 sites were contacted and requested to submit the MMR family data from their clinics/centres. Instructions on the preferred data format were provided, including data dictionaries for personal and family history of demographic data, cancers, MMR gene mutation status, screening, surgery and mortality. As of November 2014, 45 sites have agreed to submit their MMR data and of these, data for 690 families has been received from 7 sites (countries include France, Switzerland, Spain, Canada, the Netherlands and the US). Two sites have declined to participate because of insufficient resources to collate and send data. For many of the sites contacted, the required data for this analysis is not in electronic form and requires manual data entry at the site.

Conclusion: Collection of MMR family data from many international sites, with varying resources (many of which were not established or designed for epidemiological research) is challenging. The IMRC will be investigating ways to facilitate data collection for this project to ensure the maximum benefit is gained from this collegial and international consortium.

Keywords: Lynch syndrome · Ethnicity · Penetrance

108 Lack of mismatch repair gene germline mutation identified in a subset of colorectal cancers with microsatellite instability-high and mismatch repair deficiency: characterizing Lynch-like syndrome

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Lynch syndrome (LS) is defined by the Amsterdam criteria and/or Bethesda guidelines as highly penetrant families with colon and other associated cancers. Patients are identified through microsatellite instability (MSI) testing and DNA mismatch repair (MMR) protein immunohistochemistry (IHC). However, germline testing for mutations within the DNA MMR genes is the gold standard for diagnosing Lynch syndrome. The implementation of universal screening of all colorectal tumors ≤ 70 years old at our institution (UTSW) has provided an opportunity to characterize both Lynch and Lynch-like patients. During a 2 year period from 09-01-2011 to 09-01-2013, immunohistochemistry staining of the MMR proteins and MSI evaluation identified 45 cases with abnormal results that would classify them as Lynch syndrome according to NCCN guidelines. All the cases were BRAF/hypermethylation negative. Of the 45 cases, we identified a germline mutation in a mismatch repair gene (MLH1, MSH2, MSH6 or PMS2) in 37 cases (80 %). However, in 9 cases (20 %) an identifiable mutation was not detected despite reduced MMR protein expression. MLH1-PMS2 were decreased in 5 cases (56 %), MSH2–MSH6 were decreased in 3 cases (33 %) and one case showed decreased MLH1 protein expression (11 %); we define this as Lynch-like syndrome (LLS) and they are managed in accordance with Lynch syndrome screening guidelines. In the 9 cases defined as LLS, none of them met Amsterdam criteria and only 4 (45 %) met Bethesda criteria. None of them presented with any other type of Lynch-related cancer. Patient with LLS have an older mean age at colorectal cancer diagnosis (54.4 vs 46.6 years for LS with a MMR germline mutation). Although LS was more prevalent in white 18 (50 %) versus Hispanic

9 (25 %), LLS showed a trend to be higher in Hispanics 4 (45 %) versus white 3 (33 %). Commercial laboratories have advocated a direct-to-germline (DTG) testing approach which does not utilize tumor samples. Initial DTG testing would have missed all our cases with Lynch-like syndrome and prevention strategies to their family members may not have been implemented. By utilizing tumor testing, we were able to identify 9 additional patients with an elevated risk for colon cancer but negative germline testing. This may be due to a type of mutation that is difficult to find and/or the possibility of a genetic change on an as yet unidentified gene associated with Lynch syndrome. These patients and their families are still at elevated risk of developing colorectal cancer and should follow preventive guidelines. Effective genetic testing for LS requires both tumor and germline testing and the recognition by the clinician of Lynch-like syndrome.

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Keywords: Lynch syndrome · Genetic · Colon cancer

109 Colon pathology characteristics in Li-Fraumeni syndrome: size doesn't matter

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Background: Li-Fraumeni Syndrome (LFS) is a rare hereditary cancer syndrome associated with germline mutations in the TP53 gene. Carriers of germline mutations in the p53 gene have a markedly increased risk of cancer-related morbidity and mortality during both childhood and adulthood, and thus require appropriate and effective cancer risk management. While many tumor types can be seen in patients with LFS, four core cancers (breast, sarcoma, brain and adrenocortical carcinoma) make up about 80 % of LFS-associated tumors. The next most frequently associated cancers include leukemia, lung, colorectal, skin, gastric, and ovarian. However the characteristics of colorectal pathology has not been fully evaluated in LFS.

Aim: We investigated the frequency and characteristics of colonic polyps and colorectal cancer in LFS.

Methods: Pedigrees and medical records of 50 TP53 mutation positive patients were retrospectively reviewed from the Huntsman Cancer Institute LFS registry at the University of Utah. We identified subjects who underwent colonoscopy evaluation. The colonoscopy and pathology findings were reviewed.

Results: Among 50 TP53 mutation-positive patients, there were 20 males (40 %) and 30 females (60 %). 26 and 24 patients were older and younger than 25-years-old (52 and 48 % respectively). 31 (62 %) patients underwent colonoscopy evaluation, 26 (25–61 yo) were older and 5 (18–23 yo) were younger than 25-years-old (84 and 16 % respectively). Since these patients have more than one colonoscopy evaluation, a total of 49 procedures were reviewed. 32 (65 %) colonoscopies did not show any abnormality. A total of 50 lesions were identified in the remaining 17 (35 %) colonoscopies, the predominant lesions were tubular adenomas (TA) 38 (76 %) followed by hyperplastic polyps (HP) 6 (12 %), tubulovillous adenoma (TVA) 2 (4 %), sessile serrated adenomas (SSA) 2 (4 %) and colorectal cancers (CRC) 2 (4 %). All HPs were localized in the left colon with an average size of 2.5 mm (1–4 mm). TAs were mainly localized in the right colon 29 (76 %) versus left colon 9 (24 %) with a medium size

of 2.8 mm (2–8 mm). TVA were localized in the rectum-sigmoid (4 mm and 4.5 cm). SSA were localized in the sigmoid area (5 and 8 mm). We identified two CRC, the mean age was 22.5-years-old, both have family history (FH) of CRC and were adenocarcinoma. One CRC was localized inside a TVA and was less than 5 mm with microinvasion and the other CRC was in the sigmoid, less than 10 mm with lymph nodes metastasis. No association was seen between phenotype and type/location of the TP53 mutations.

Conclusion: Small tubular adenoma (2–3 mm) in the right colon is the most frequent lesion found in patients with LFS during screening colonoscopy. CRC in LFS presents as small lesions with invasive and metastatic capabilities. Early-onset CRC appears to be a component of LFS especially if FH of CRC is present.

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Keywords: Li-Fraumeni · Colon · Cancer

110 Long term data for chemoprevention in colorectal disease in familial adenomatous polyposis (FAP)

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Purpose: Non-steroidal anti-inflammatory drugs (NSAIDs) may be of benefit in reducing the number and size of colorectal adenomas in FAP. The exact place of these drugs in the management of patients with FAP is unclear. This is due, at least in part, to a lack of any long term data. These drugs seem to have been used most in the management of rectal polyps in patients who have undergone prophylactic colectomy. Some advocate their use to manage pouch polyps or to delay colectomy. However there are no long term data. In addition there have been reports of cancer development in patients with FAP receiving NSAIDs, which raises the question of safety of long term NSAIDs. Our aim was to assess the long term outcomes in patients from a single institution who received NSAIDs for chemoprevention.

Methodology: We retrospectively analysed data from a prospectively maintained database. Patients receiving NSAIDs were identified; those prescribed NSAIDs for the management of desmoid or duodenal disease were excluded. Patients who received NSAIDs only as part of a clinical trial, without ongoing clinical indication for therapy were also excluded. Only patients followed up at our institution were included. Data were obtained from the registry database, endoscopy reports, histology reports and medical notes.

Results: 54 patients were identified, which comprises the study cohort; a further 5 patients were offered NSAIDs but declined. 16/54 were female. There are 191 patient years follow up, median follow up 38.5 months (range 7–167). The NSAIDs used were: indomethacin 27, sulindac 9, celecoxib 8, mixed 10. Median age at initiation of NSAIDs was 36 years; median duration of therapy was 32 months. NSAIDs therapy indication was rectal disease in 45 (83 %), pouch polyps 6 (11 %) and to delay colectomy 3 (6 %). 8 patients stopped treatment due to side effects. High grade dysplasia was present in 6/54 before NSAIDs initiated, of which 3 later developed cancer (median interval 30 months). In total 4 patients developed cancer (3 rectum, 1 pouch) at a median 25.5 months after NSAIDs commenced. Where data are available (3/4), all patients who developed cancer had had a reduction in polyp burden on treatment.

Proctectomy for benign disease was performed in 2 patients after 7 and 24 months respectively of therapy. Pouch excision and colectomy

have not been performed. 47/54 have not required surgical intervention. On NSAID therapy as an adjunct to polypectomy, latest endoscopy showed static disease in 9/50 (18 %), a reduction in 28 (56 %) and increase in 13 (26 %).

Conclusions: The place of NSAIDs in the management of FAP remains unclear. Guidelines for the initiation and withdrawal of NSAIDs are required; surveillance intervals in those on “chemoprevention” need to be defined. 22/50 (44 %) showed no response to NSAIDs. NSAIDs may reduce polyp burden in some but this does not equate to a reduction in cancer risk. Patients need to be counselled of a risk of cancer development on treatment with NSAIDs.

Keywords: Chemoprevention · FAP · Colorectum

111 Functional characterization of the APC I1307K allele

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Background and Aims: The I1307K (isoleucine > lysine) APC is a missense variant identified in 6 % of Ashkenazi healthy controls which confers an increased risk ~ 1.5–1.7 of colorectal adenomas and carcinomas. Our aim was to evaluate the functional contribution of the I1307K variant to colorectal tumorigenesis.

Methods: I1307 S1028R and E1317Q variants were studied. BIAcore T100 (GE) assays were set up to study the interaction between β -catenin and APC protein. Transient and stable transfectants with I1307 S1028R, E1317Q and WT cDNA APC were established in SW480 and DLD-1 cells. The effect on β -catenin/Tcf-4 complex transcription levels was assessed. APC, MYC and AXIN2 expression levels were determined using LightCycler 480 platform in transient transfectants of SW480 cells.

Results: Biacore assay revealed that the KD (Dissociation constant) is higher for the APC I1307K and E1317Q (KD = 3.96E–06M and 4.19E–06M) than for APCwt (KD = 3.11E–06M) The KD is also higher for APC S1028R (KD = 4.34E–06M). In both cell lines, all variants significantly increased the β -catenin/Tcf-4 complex mediated transcription levels when compared with APCwt with the exception of APC E1317Q in SW480 cell line. In all transfectants APC expression levels were high irrespective of the variant transfected, ruling out dose as responsible of the observed effects. MYC expression levels were high in all transfectants although differences were only statistically significant for APC I1307K. No differences were observed for AXIN2 expression among the distinct transfectants.

Conclusion: The I1307K APC variant affect β -catenin binding and enhances transcription mediated by the β -catenin/Tcf-4 complex. These alterations suggest that the I1307K APC allele may promote carcinogenesis by a direct deregulation of the wnt pathway.

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Keywords: APC · Variant · Functional characterization

112 CIMP subgroups within a cohort of 75 patients with MSI-H and Mlh1-MMR deficiency and suspicion of Lynch syndrome: 3 exceptions of MLH1 methylation or BRAF mutation in tumors do not rule out Lynch syndrome

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Purpose: Lynch Syndrome (LS) is caused by germline mutations in genes involved in the DNA mismatch repair (MMR), and frequently indicated by high microsatellite-instability and protein loss in tumor tissue. The mutation detection rate of pathogenic mutations in our patients with MMR-defects is on average 65 %, in 17 % uncertain variants (VUS) were found, and in 18 % of the cases no germline mutation was detectable. Sporadic colorectal cancers can also show MSI-H and MLH1-deficiency due to acquired MLH1 hypermethylation (CIMP) and are frequently associated with the activating BRAF mutation p.Val600Glu. We set up a cohort of 75 colorectal cancer patients with MSI-H and MLH1-MMR deficiency in the tumor, part of them with MMR-germline mutations or VUS (variant of uncertain significance) and analyzed their tumors for MLH1 promotor methylation and BRAF mutation [1].

Methodology: In tumor DNA of 75 colorectal cancer patients with MSI-H and MLH1-deficiency we investigated MLH1 and MGMT promotor methylation by MS-MLPA analysis (methylation-specific Multiplex Ligation-dependent Probe Amplification by MRC-Holland, Kit ME011) and performed BRAF exon 15 sequencing.

Results: Of the 75 tumors 4 cases (5.3 %) showed only MLH1 promotor methylation and all displayed BRAF-WT (wildtype); 15 cases exhibited methylated MLH1 and MGMT promoters (20 %), of those, 10 had the BRAF mutation p.Val600Glu (66 %), while two of the 5 with BRAF-WT were LS-patients with an MLH1 germline mutation. Of the 15 cases with exclusively MGMT promotor methylation (20 %) only 4 carried the BRAF mutation p.Val600Glu (26.6 %). In three LS-patients with MMR-germline mutations MGMT methylation was found in their tumors, and one of those with a MSH2 mutation also showed BRAF mutation. Of the remaining 41 cases (54.6 %) with neither MLH1 nor MGMT promotor methylation the absent protein staining could not be explained, strikingly, one of those carried the BRAF mutation p.Val600Glu (2.5 %) without CIMP.

Conclusion: CIMP and LS are not mutually exclusive as demonstrated in 6.6 % (5/75) of the cases. Two unrelated MLH1 mutation carriers displayed MLH1 promotor methylation^[2,3] but no BRAF mutation in their tumors. Three LS patients with germline mutations in MLH1, MSH2 or PMS2 displayed MGMT methylation, one patient even showed a BRAF mutation p.Val600Glu. We disclosed that the presence of BRAF mutation not always indicate promotor methylation of MLH1 and not even of MGMT in one case. Therefore, a tumor with MSI-H and MLH1-MMR deficiency can only be classified as “possibly sporadic” if MLH1 promotor methylation is verified^[4]. Hence, for a considerable number of patients with MMR-defects in the tumor and familial tumor clustering, the causative genetic predisposition could not be identified.

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Keywords: MLH1-MMR deficiency · MLH1 hypermethylation (CIMP) · BRAF

113 Coding microsatellite frameshift mutations in intestinal tumors of DNA mismatch repair-deficient mice

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Several DNA mismatch repair (MMR)-deficient mouse strains have been developed as models for the inherited cancer predisposing Lynch syndrome. It is completely unresolved, whether coding mononucleotide repeat (cMNR) gene mutations in these mice can contribute to intestinal tumorigenesis and whether MMR-deficient mice are a suitable molecular model of human microsatellite instability (MSI)—associated intestinal tumorigenesis. We have performed a proof-of-principle study to identify mouse cMNR-harboring genes affected by insertion/deletion mutations in MSI murine intestinal tumors. Based on bioinformatic algorithms a database of mouse cMNR-harboring genes was established. In order to determine the MSI status of intestinal matched normal/tumor tissues from MMRdeficient (Mlh1^{-/-}, Msh2^{-/-}, Msh2LoxP/LoxP) mice a panel of five mouse noncoding mononucleotide markers was used. cMNR frameshift mutations of candidate genes were determined by DNA fragment analysis. Murine MSI intestinal tumors but not normal tissues from MMR-deficient mice showed cMNR frameshift mutations in six candidate genes (Elavl3, Tmem107, Glis2, Sdccag1, Senp6, Rfc3). cMNRs of mouse Rfc3 and Elavl3 are conserved in type and length in their human orthologs that are known to be mutated in human MSI colorectal, endometrial and gastric cancer. We provide evidence for the utility of a mononucleotide marker panel for detection of MSI in murine tumors, the existence of cMNR instability in MSI murine tumors, the utility of mouse subspecies DNA for identification of polymorphic repeats, and repeat conservation among some orthologous human/mouse genes, two of them showing instability in human and mouse MSI intestinal tumors. MMR-deficient mice hence are a useful molecular model system for analyzing MSI intestinal carcinogenesis.

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Keywords: Coding microsatellite instability · MMR-DEFICIENT MICE · MSI TARGET GENES

115 BUB1 and BUB3 mutations in familial colorectal cancer and polyposis

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Purpose: Previous evidence indicates that germline mutations in the spindle assembly checkpoint genes BUB1 and BUB3 are associated with increased risk to develop colorectal cancer (CRC) at young age. BUB1/BUB3 mutated cases showed cytogenetic abnormalities in a fraction of normal cells, and some mutation carriers showed reminiscent traits of the mosaic variegated aneuploidy syndrome [1]. Here we aim to validate those findings in a cohort of familial CRC and polyposis cases without mutations in known high penetrance genes.

Methodology: Using a strategy that combines pool DNA amplification and targeted-gene massively parallel sequencing, the coding exons and exon–intron boundaries of BUB1 and BUB3 were screened for mutations in 456 Caucasian cancer patients from 441 genetically uncharacterized families with mismatch repair-proficient familial non-polyposis CRC, 60 of whom Amsterdam-positive, and in 88 unrelated adenomatous and non-adenomatous polyposes. After variant identification, cosegregation studies, in silico functional prediction of variants, and a cytogenetic analysis in lymphoblasts were performed.

Results: Four novel variants, one splice-site and three missense, were identified in four independent families. Two families met the Amsterdam criteria and the other two cases were early-onset CRC patients without family history of cancer. Cytogenetic studies are currently being performed and will be presented in the meeting.

Conclusion: BUB1/BUB3 mutations are not a major cause of familial and/or early onset CRC or polyposis, accounting for at most 0.8 % of uncharacterized cases. Further functional studies will be presented to confirm or discard the pathogenic nature of the identified variants.

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Keywords: Hereditary colorectal cancer genes · BUB1 · BUB3

116 Polymerase proofreading-associated syndrome: POLE and POLD1 mutations in hereditary colorectal cancer and polyposis

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Purpose: Germline mutations in the proofreading domains of two polymerases, POLE and POLD1, have been associated with a dominantly inherited, highly penetrant syndrome of colorectal cancer (CRC) and polyposis [1]. Here we aim to better understand the mutation spectrum and phenotypic characteristics of POLE and POLD1-associated syndrome in order to refine the recommendations for genetic testing and surveillance.

Methodology: We studied 456 Caucasian cancer patients from 441 genetically uncharacterized families with mismatch repair-proficient familial non-polyposis CRC, 60 of whom Amsterdam-positive, and in 88 unrelated adenomatous and non-adenomatous polyposes. The exonuclease domains of POLE and POLD1 were sequenced using a strategy that combines pool DNA amplification and massively parallel sequencing.

Results: The recurrent POLE L424V mutation was identified in a polyposis case [2]. Six mismatch repair proficient non-polyposis CRC families carried mutations in POLD1, all of them predicted to be functionally and/or structurally relevant. The phenotype of POLD1 mutation carriers includes CRC (8/12 carriers; 67 %), breast cancer (3/9 female carriers; 33 %, one of them diagnosed of two primary breast tumors) and endometrial cancer (2/9 female carriers; 22 %). Also, multiple metachronous primary tumors occurred in 3/12 (25 %) confirmed mutation carriers.

Conclusions: Our results identify novel potentially pathogenic variants and widen the phenotypic spectrum of the POLD1-associated syndrome, demonstrating its relevance in hereditary non-polyposis CRC cases, confirming its association with endometrial cancer predisposition and establishing a new one with breast cancer.

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Keywords: POLE/POLD1 · Polymerase proofreading-associated polyposis (PPAP) · Hereditary non-polyposis colorectal cancer

117 A whole-exome study in a family with familial predisposition to rectal and gastric cancer

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Background: We have previous published a family with rectal- and gastric cancer in a linkage study which suggested a locus on chromosome 3 [1]. The LOD score was totally dependent on one family (242), which gave a LOD for almost 3 in the region.

Aims: We wanted to find the predisposing gene, and mutation in this family.

Methods: We used whole exome sequencing, 30×, on an Illumina platform to sequence three members of the family. We also studied the whole exome for non-synonymous, variants with a MAF < 20 % and which segregated in three family member.

Results: 38 variants was found across the entire genome. After additional Sangers sequencing and segregation analysis in the whole family there was 12 variants in 12 different genes left as candidates in 4 different chromosomes, chromosome 3, 9, 12, and 22. Most suggested to be pathogenic from in-silico analysis.

Conclusions: It is possible that one of these variants alone cause the disease in this family as in the dominant disease suggested by the pedigree. However, it is also possible that more than one mutation were involved as in polygenic disease.

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Keywords: Cancer · Familial · Bioinformatics

118 Molecular-genetic analysis of the APC gene among Russian patients with classic form of FAP

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Purpose: Familial Adenomatous Polyposis (FAP)—is an important inherited colorectal cancer syndrome. The syndrome is characterised by the development of hundreds to thousands of adenomas in the colorectum. It is caused by germline mutation in the adenomatous polyposis coli (APC) gene. The aim of this study was to investigate frequency of germline mutations in the APC gene among Russian patients.

Methodology: We analyzed the APC gene of 51 patients which corresponded to 2 criteria: classic form of FAP (more than 100 colorectal polyps) and age ≤35. Germline mutations in the APC gene were analyzed by PCR, conformation-sensitive electrophoresis, Sanger sequencing and next generation sequencing.

Results: We found 33 germline mutations in the APC gene among 51 (64.7 %) patients. In most cases (32/33; 97 %) variants were frameshift and nonsense mutations. The p.Y1183X and p.1309del5 mutations were observed among two and seven non-related patients, respectively. The identified mutations were located between codons 213 and 1344 in the APC gene. Eleven out of 33 (33.3 %) hereditary mutations have not been previously described anywhere in the world: p.Q260X, p.289del4, p.445insT, p.589del11, p.785del8, p.864delC, p.903del7, p.1100del4, p.1114delC, p.Y1183X, p.Q1191X.

Conclusion: Frequency of germline mutation in the APC gene is 64.7 % (33/51) among Russian patients. Eleven mutations (33.3 %) have never been described previously anywhere in the world.

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Keywords: Familial adenomatous polyposis · APC gene · Germline mutation

121 Vaccination of MSI-H colorectal cancer patients with frameshift peptide Antigens—a phase I/IIa clinical trial

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Purpose: Microsatellite-unstable (MSI-H) colorectal cancers occurring in the context of Lynch syndrome are characterized by pronounced anti-tumoral immune responses. This immune response is likely related to the generation of frameshift peptide (FSP) antigens, which result from mismatch repair deficiency-induced insertion/deletion mutations at coding microsatellite sequences [1]. FSP antigens have been shown to be highly immunogenic antigens, which are readily recognized as foreign antigens by the immune system [2]. The generation of FSP antigens is restricted to MMR-deficient cells; therefore, we pursued the development of an FSP antigen-based vaccination approach.

Methodology: We have initiated a clinical phase I/IIa vaccination trial (Micoryx, ClinicalTrials.gov Identifier: NCT01461148) that evaluates vaccination with a combination of three FSP antigens (derived from frameshift variants of the coding microsatellite-containing genes AIM2, HT001, TAF1B) in the clinical setting. Included were patients with metastasized colorectal cancer (UICC stage III or IV) after the end of standard chemotherapy. In total, 22 patients (phase I: 6 patients, phase IIa: 16 patients) have been vaccinated.

Primary study end points were safety and toxicity (phase I) as well as the induction of cellular and humoral immune responses (phase IIa).

Results: Data from the vaccinated patients demonstrate that no FSP-associated severe adverse events have been observed after FSP vaccination. Moreover, significant FSP-specific immune responses against at least one vaccine antigen were detectable upon vaccination in all patients vaccinated per protocol. The vaccination-induced increase of humoral FSP-specific immune responses was paralleled by the induction of T cell-mediated FSP-specific immune responses in the majority of patients.

Conclusion: Our study demonstrates that vaccination with FSPs is safe and leads to the induction of pronounced FSP-specific immune responses. FSP immune therapy may represent a promising novel approach for treatment of MSI-H colorectal cancer patients. Moreover, vaccination with FSP may be used for tumor prevention in Lynch syndrome mutation carriers in the future, potentially representing the first preventive vaccine against an inherited cancer syndrome.

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Keywords: Immune therapy · Lynch syndrome · Cancer prevention

122 Clinical diagnosis of familial colorectal cancer by targeted resequencing

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Purpose: Several Novel Genes Responsible For Familial Colorectal Cancer Have Been Identified With The high speed sequencer. We, therefore, built a system to detect quickly and cheaply known germline mutations of genes responsible for hereditary gastrointestinal tract cancer syndromes using the high speed sequencer.

Methods: In order to achieve above purpose, we evaluated the built experimental system by examining the known gene adenomatous polyposis coli (APC) responsible for familial adenomatous polyposis (FAP) as the first run and then the known genes, mutL homolog 1 (MLH1) and mutS homolog 6 (MSH6) responsible for Lynch syndrome, and bone morphogenetic protein receptor, typeIA (BMPRI1A) responsible for Juvenile polyposis syndrome (JPS) as the second run. In this system, we selected the HaloPlex as the targeted capture solution and MiSeq as the high speed sequencer. The total length of the

target regions is 216,606 kb and the capture probe was designed to cover 99.07 % of them.

Results: This system exhibited 739× mean coverage and sequenced 97.35 % of the target regions with at least 30× coverage when 22 specimens were used for the first run. When confirming whether the single nucleotide variants (SNVs) obtained from data analysis are pathogenic mutations, we referred to the International Society for Gastrointestinal Hereditary Tumours Incorporated (InSiGHT) database if the obtained SNVs were included as previously reported pathogenic mutations. Using blinded specimens with defined mutations, the built experimental system correctly identified 18, 4, 2, and 1 pathogenic mutations in APC, MLH1, MSH6 and BMPRI1A, respectively, including SNVs, small insertion and deletions (Indels), and relatively large deletions.

Conclusion: These results suggested that the built system is feasible in diagnosing known genes responsible for FAP, Lynch syndrome and JPS. In future experiments, we are planning to expand the number of genes to identify pathogenic mutations responsible for other types of target diseases using this system.

Keywords: Clinical diagnosis · Familial colorectal cancer · Targeted resequencing

123 Beta2-microglobulin mutations and NK cell mediated cytotoxicity in microsatellite unstable colorectal cancer

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Purpose: Microsatellite-unstable (MSI-H) colorectal cancers (CRC) are typically characterized by signs of a pronounced anti-tumoral immune response of the host. MSI-H CRC frequently display mutations of the beta2-microglobulin (B2M) gene, which lead to a breakdown of human leukocyte antigen (HLA) class I-mediated antigen presentation. Furthermore B2 M mutations are associated with an absence of distant metastases and a prolonged relapse-free survival in MSI-H CRC patients [1–3]. The mechanism contributing to a decreased metastatic potential of B2 M-mutant, HLA class I-deficient CRC cells has been unclear. We hypothesized that NK cell-mediated tumor cell lysis may contribute to the elimination of B2 M-deficient tumor cells and thus to a decreased metastatic rate.

Methodology: We here examined the consequences of B2 M on MSI-H CRC susceptibility towards NK cell-mediated killing. Activation of NK cells upon incubation with B2 M-deficient and B2 M-proficient cancer cells was analyzed in an autologous system by CD107 degranulation assay. Moreover, cytotoxicity of NK cells in dependence of tumor cell B2 M status was measured by LDH release assay.

Results: CD107 degranulation assay revealed that tumor cells were able to activate autologous NK cells isolated from the same donor from whom the tumor cell line had been established. Activation of NK cells by B2 M-deficient autologous tumor cells was slightly, but significantly higher than activation by their B2 M-proficient counterparts (11.3 vs 8.2 %, $p = 0.007$; +interleukin 2: 2.5 vs 27.1 %, $p = 0.03$). Cytotoxicity analyses revealed that NK cells induced lysis significantly above background in B2 M-deficient ($p = 0.04$), but not B2 M-proficient autologous tumor cells.

Conclusion: Our observations are compatible with the hypothesis that the favorable prognostic effect of B2 M mutations in MSI-H CRC

may be related to a certain extent to modulation of the susceptibility of tumor cells towards NK cell-mediated cytotoxicity.

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Keywords: Beta2-microglobulin · Immune evasion · Natural killer cells

124 Molecular alterations in mismatch repair-deficient crypt foci in Lynch syndrome

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Purpose: Lynch syndrome is caused by germline mutations of DNA mismatch repair (MMR) genes, most frequently MLH1 and MSH2. Recently, a novel lesion resulting from somatic MMR gene inactivation has been described as a novel potential cancer precursor in Lynch syndrome [1]. MMR-deficient crypt foci (MMR-DCF) occur at high frequency in the intestinal mucosa from Lynch syndrome mutation carriers, but very rarely progress to cancer. In the present study, we characterized molecular alterations and clinical associations of MMR-DCF to shed light on their potential significance as cancer precursor lesions in Lynch syndrome.

Methodology: we systematically searched the intestinal mucosa from Lynch syndrome patients for MMR-DCF by immunohistochemistry. The identified lesions were characterized for alterations in microsatellite-bearing genes with proven or suspected role in malignant transformation, using multiplex PCR approaches for the amplification of six coding and three non-coding microsatellite sequences.

Results: We demonstrate that the prevalence of MMR-DCF (mean 0.84 MMR-DCF per 1 cm² mucosa in the colorectum of Lynch syndrome patients) was significantly associated with patients' age. No association with patients' gender or the MMR gene affected by germline mutation was observed. Microsatellite instability of at least one tested marker was detected in 89 % of the MMR-DCF examined, indicating an immediate onset of microsatellite instability after MMR gene inactivation. Coding microsatellite mutations were most frequent in the genes HT001 (ASTE1) with 33 %, followed by AIM2 (17 %) and BAX (10 %). Though MMR deficiency alone appears to be insufficient for malignant transformation, it leads to measurable microsatellite instability even in single MMR-deficient crypts.

Conclusion: Our data indicate that the frequency of MMR-DCF increases with patients' age. Similar patterns of coding microsatellite instability in MMR-DCF and MMR-deficient cancers suggest that certain combinations of coding microsatellite mutations, including mutations of the HT001, AIM2 and BAX gene, may contribute to the progression of MMR-deficient lesions into MMR-deficient cancers.

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Keywords: MMR-deficient crypts · Precancerous lesions · Lynch syndrome

125 Defining the inheritance pattern of MLH1 epimutations helps in the genetic counseling of families

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Constitutional epimutations in MLH1 have been identified in a subset of Lynch syndrome patients (0–2 %). Two types of constitutional MLH1 epimutations have been defined: primary epimutations, which arise de novo and are reversible between generations, and secondary epimutations, linked to in cis genetic alterations and dominantly transmitted.

Purpose: The aim of this study was the analysis of the inheritance pattern of 3 constitutional MLH1 epimutations identified in a Spanish series of Lynch syndrome patients. Two of them were previously reported[1], being one of them fully characterized.

Methodology: Mutational analysis of MLH1 coding region, promoter and intron 1 was performed by Sanger sequencing. MLH1 methylation in blood DNA was assessed by MS-MLPA. Inheritance pattern was determined by haplotype analysis in probands' first-degree relatives.

Results: The MLH1 epimutation carriers included in this study developed multiple Lynch syndrome tumors at early age. Case 1 is a 49-year-old male who was diagnosed with two colorectal cancers at ages 32 and 34. The patient has no history of cancer in his-first degree relatives. Case 2 is a 57-year-old female affected by two colorectal cancers at ages 29 and 44 and endometrial cancer at age 49. Her mother was affected by breast cancer at age of 77 years. Case 3 is a 60-years-old female diagnosed with colorectal cancer at ages 37 and 59, endometrial cancer at 43 and kidney cancer at 55. Patient's mother was diagnosed with endometrial cancer at age 50. Genetic alterations in MLH1 underlying the epimutation were not detected in probands. In the promoter region SNPs were identified: cases 1 and 3 were heterozygous for rs1800734 and case 2 was heterozygous for rs34566456. No evidence of MLH1 methylation was found in available probands' relatives: the father and two daughters of case 1, four sisters of case 2, and two sisters and two children of case 3. Haplotype analysis revealed in cases 1 and 3 that MLH1 methylation was reversed in children who inherited the proband epimutated allele. In addition, methylated allele was maternally transmitted in case 1. The lack of availability of samples precluded the parental origin analysis of the remaining two cases.

Conclusion: All three characterized MLH1 epimutations are primary epimutations. Intergenerational erasure was demonstrated in two of them. The analysis of the inheritance pattern of MLH1 epimutations is critical to assess the risk of intergenerational transmission.

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Keywords: Epimutation · MLH1 · Methylation

126 Multiplexed detection of serum antibodies against mismatch repair deficiency-induced frameshift antigens

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Purpose: Serum antibodies can be important diagnostic tools in various disease conditions by reflecting exposure to a distinct antigen [1]. In individuals with mismatch repair (MMR) deficiency-associated disease mutations in coding microsatellites are known sources for the translation of frameshift peptide (FSP) antigens. FSP antigens are considered as highly immunogenic tumor antigens due to their non-self-sequence. Immune responses against FSP antigens are potential markers of antigen exposure, which could theoretically be interesting for the identification of individuals with MMR deficiency-associated diseases such as Lynch syndrome, or for monitoring the course of disease [2]. We here describe a multiplex method using the Luminex technology allowing the high throughput detection of antibodies against multiple FSP antigens in large sets of sera.

Methodology: The approach included a set of 32 synthetic biotinylated FSPs. The FSPs represent sequences derived from mutated (−1 and −2 shift) microsatellite-containing genes with a published mutation frequency in microsatellite-unstable colorectal cancer of over 60 % (www.seltarbase.org). The antigens were fused to a FLAG epitope to ensure monitoring antigen-binding to avidin-linked microspheres in the absence of monoclonal antibodies.

Results: The FSP multiplex assay allowed detection of antibody responses against the various included FSPs. Analytical specificity of measured serum antibody reactivity was proven by the detection of immune responses in immunized rabbits and a colorectal cancer patient vaccinated with FSPs included in the assay. The measured antibody responses were comparable to peptide ELISA, and inter-assay reproducibility of the multiplex approach was excellent ($R^2 > 0.98$) for 20 sera tested against all antigens.

Conclusion: Our methodic approach represents a novel platform valuable to monitor antibody responses against FSPs. It will be used to study the diagnostic value of FSP antibody detection in MMR deficiency-associated diseases, with a particular focus on Lynch syndrome mutation carriers. Furthermore it will be a valuable tool for immune monitoring of patients in FSP-based cancer vaccine studies.

Acknowledgements: This work was funded by a grant (#109477) from the Deutsche Krebshilfe (German Cancer Aid).

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Keywords: Lynch syndrome · Immune response · Antibodies

127 Low density of FOXP3-positive cells in normal colonic mucosa is related to the presence of BETA2-microglobulin mutations in Lynch syndrome-associated colorectal cancer

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Purpose: Cancers developing in the context of Lynch syndrome are typically characterized by pronounced local immune responses of the host and dense lymphocyte infiltration. Between 30 and 40 % of Lynch syndrome-associated colorectal cancers show a breakdown of the HLA class I antigen presentation pathway as a result of Beta2-microglobulin (B2 M) mutations. This suggests that immune selection may play a role during the outgrowth of Lynch syndrome-associated cancers. In order to examine a potential relation between the host's immune surveillance and the occurrence of immune evasion phenotypes, we quantified lymphocyte infiltration in the cancer and in non-tumorous mucosa from Lynch syndrome mutation carriers and related the results to B2 M mutation status of the cancer.

Methodology: T cell infiltration was analyzed by immunohistochemistry using antibodies specific for CD3 (all T cells), CD8 (cytotoxic T cells), and FOXP3 (regulatory T cells). In total, 30 tumor samples and 76 non-tumorous mucosa samples obtained from 24 Lynch syndrome patients were included in this study. Full scans of all sections were obtained using the NDP Nanozoomer (Hamamatsu Photonics). The number of lymph follicles in the mucosa was quantified, recording primary and secondary follicles separately. Moreover, for quantification of T cell infiltration, three regions (1 mm² each) were analyzed using a quantification algorithm of VIS software suite (Visiopharm).

Results: Whereas no correlation between immune cell infiltration, Lymph follicle count and B2 M mutation status was observed in tumor tissue, we observed a significantly lower number of FOXP3-positive regulatory T cells in the tumor-adjacent normal mucosa from patients with B2 M-mutant compared to B2 M-wild type cancers. A similar trend was observed in tumor-distant mucosa from the same patients.

Conclusion: Our study provides evidence that the occurrence of B2 M mutations is related to immune cell infiltration in normal colonic mucosa, supporting the concept that B2 M mutations in MSI-H CRC develop as a result of immunoeediting. Beyond the perspective of Lynch syndrome, our results suggest that the immune milieu may play a critical role as a host factor determining the individual risk of solid cancer development. Further studies are required to evaluate this hypothesis in a prospective setting.

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Keywords: Lymphocyte infiltration · Beta2-microglobulin · Immune selection

128 Abnormal transcripts and new fusion transcripts of MLH1 or MSH2 in Lynch-syndrome patients with chromosomal deletion, duplication or inversion

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Purpose: In seven patients suggestive of Lynch Syndrome (LS) by DNA mismatch repair (MMR)-defects in their tumors and findings of genomic rearrangements in one of the MMR genes MLH1 or MSH2, we performed cDNA-analyses to investigate their effect on the respective transcript.

Methodology: RNA was isolated from PAXgene and leucocytes from cultured blood (with and without NMD (nonsense-mediated mRNA-decay) blocked by puromycin incubation in parallel) and complementary cDNAs were generated. Primers for amplification of new fusion transcripts were specifically designed depending on genomic rearrangement findings.

Results: In one patient an MLH1-inversion previously reported by our group (Morak et al. 2011) between MLH1 breakpoint exon 15/16 and the genomically subsequent LRRFIP2 gene with antisense-orientation generated two new stable fusion transcripts in frame.

In another patient an MLH1-inversion with breakpoint between exon 1/2 generated two fusion transcripts with the genomically upstream DCLK3 gene with antisense-orientation.

In the female patient with MSH2 exon 5–16 duplicated after the complete MSH2 gene generated diverse aberrant transcripts between these two parts of the gene. Furthermore, we investigated 4 patients with different deletions in MSH2 (exon 7, exons 8–9, exons 9–16, exons 15–16) and their effect on the transcripts regarding exon skipping, usage of new polyadenylation sites and NMD. NMD was not always found in cDNA even though expected.

Conclusion: The effect of presumed pathogenic genomic rearrangements in MLH1 and MSH2 were analyzed on cDNA-level to fully understand their consequences. We detected new fusion transcripts in two different MLH1 inversion carriers and in a case with gene duplication though intact MSH2 gene. Usage of alternative polyadenylation sites was frequently found in cases with exon deletions.

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Keywords: MMR gene · Transcript analysis · New fusion transcripts

129 Common genetic variants within the TERT gene and risk of colorectal cancer for DNA mismatch repair gene mutation carriers

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Purpose: Lynch syndrome (LS) is an inherited cancer-predisposing disorder caused by germline mutations in the DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2). Carriers of a germline MMR gene mutation have a high risk of developing numerous different cancers, predominantly colorectal cancer (CRC) and endometrial cancer. However, there is a high degree of variability in

individual cancer risk observed among carriers such that large proportions of carriers have either very low or very high lifetime cancer risks suggesting the existence of modifying factors [1]. Identifying genetic modifiers of risk of CRC could help implement personalized surveillance programs based on predicted cancer risks. Previously, the rs2853668 SNP within the TERT-CLPTM1L genes has been associated with CRC risk in a GWAS meta-analysis [2] while the rs2736100 SNP has also shown an association with CRC risk [3]. The rs2075786 SNP in the promoter of hTERT has been suggested as a modifier of cancer risk in Lynch syndrome. The aim of this study was to investigate genetic variation within the hTERT gene locus on 5p15.33 as potential CRC risk modifiers in MMR gene mutation carriers.

Methodology: MMR gene mutation carriers were identified from both clinic- and population-based recruitment arms of the Australasian Colorectal Cancer Family Registry. A total of 1082 MMR gene mutation carriers (414 MLH1, 474 MSH2, 125 MSH6, 51 PMS2 and 18 EPCAM) from 330 families were genotyped for 48 SNPs within the hTERT locus using Sequenom iPLEX. We used a weighted Cox regression to estimate CRC risk per allele as well as for homozygous and heterozygous carriers of the risk allele compared with homozygous non-carriers, after correcting for ascertainment bias.

Results: Over a total of 46,757 person-years observation, 393 (36 %) carriers were diagnosed with CRC at a mean age of 43.2 (SD 12.8) years. There was no evidence of associations of risk of CRC with any of the 48 hTERT SNPs per allele as well as when homozygous and heterozygous carriers of the risk allele were compared with homozygous non-carriers.

Conclusions: Although SNPs within the hTERT gene have been associated with an increased risk of certain cancers including CRC, we found no evidence that common genetic variation in hTERT modified the risk of developing CRC in MMR gene mutation carriers.

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Keywords: Genetic modifier · Lynch syndrome · hTERT

130 Copy number variation analysis in 85 suspected Lynch syndrome families reveals novel potential causative candidate genes

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Purpose: In Up To 50 % Of Suspected Lynch Syndrome (LS) Families with Typical Signs of a MMR Defect In tumor tissue, no germline mutation in the MMR genes or EPCAM deletion can be detected. Although inversions of the MSH2 gene have been found as additional pathomechanism, a considerable amount of mutation negative patients remains. Some cases might be explained by somatic alterations of the MMR genes in tumor tissue (phenocopies), however, in a number of patients the very young age of onset or striking family history are very suggestive of an underlying hereditary cause. Loss-of-function copy number variants (CNVs) contribute significantly to the mutation spectrum of hereditary tumor syndromes and might also contain yet unidentified genes responsible for Lynch syndrome.

Methodology: Genomic DNA from 85 unrelated mutation negative patients from the German HNPCC Consortium and four patients from the University Medical Center in Leiden, Netherlands was genotyped using Illumina HumanOmniExpress Bead Array. All but two patients showed loss of MSH2 in their tumor tissue, most of them were also MSI-H. Putative CNVs were identified by QuantiSNP v.2.2 and filtered according to empirically established criteria to select rare, non-polymorphic deletions and duplications ≥ 10 kb in protein-coding genes and the regulatory regions of MSH2 which were present in not more than 0.2 % of 1320 population-based controls. CNVs that passed the filter criteria were validated by qPCR, further selected on gene level, and subsequently prioritized by gene functions and pathways.

Results: In total, 30 unique deletions (size 13–387 kb) and 18 unique duplications (size 15–788 kb) were found in 25 (21 %) and 17 (15 %) patients, respectively. Those 48 CNVs together encompass 71 protein coding genes. 33 genes were completely or partly deleted, 38 affected by duplications. None of the genes was affected in more than one patient. Five of these genes are promising candidates that are highly expressed in normal colorectal tissue. Three of these genes are involved in different cellular processes, such as cell adhesion, cell development and transformation, cell cycle checkpoint regulation, and cell volume or polarity control. One gene is known for double strand break repair and recombination and the last one possesses DNA helicase activity and is essential for the initiation of eukaryotic genome replication.

Conclusion: By applying stringent filter criteria we identified a group of rare, non-recurrent loss-of-function CNVs which might contain novel predisposing genes for LS. Our results are in accordance with

genome-wide CNV analyses in other tumor predisposition syndromes and the rarity of recently identified monogenic subtypes. The ongoing further work-up of the most promising candidates includes the detection of germline point mutations by a targeted NGS approach, a segregation analysis in families where further affected relatives are available, a screening for somatic second-hits in tumor tissue, and a pathway/network analysis.

Acknowledgements: The study was supported by the German Cancer Aid.

Keywords: Lynch syndrome · CNV analysis · Novel candidate genes

132 Deep intronic sequencing results of 71 German patients suspected of Lynch-syndrome without germline mutation detectable in the mismatch-repair genes

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Purpose: We Selected 71 Patients Suggestive of Lynch Syndrome (LS) By Dna mismatch repair (MMR)-defects in their tumors. Germline mutations/unclassified variants or deletions/duplications were not found in MSH2, EPCAM, MSH6, MLH1 and PMS2, and MLH1 promoter methylation was absent in tumors with MLH1-loss. To search for further germline pathomechanisms, we performed deep intronic sequencing of genes involved in the MMR pathway by NGS allowing for detection of variants in introns, regulatory regions and chromosomal rearrangements.

Methodology: In a custom-made gene panel, we included the complete genomic regions of MLH1, MLH3, PMS1, PMS2, MSH2, MSH3, MSH6 and EPCAM and chromosomal regions far upstream/downstream of the genes in the target region. Library preparation was performed with the SureSelectXT Reagent Kit (MSQ) and capture enrichment with a custom-made SureSelectXT Kit. We used paired-end sequencing on Illumina MiSeq and NextSeq systems. Data were analyzed using a bioinformatics pipeline consisting of BWA, Stampy, GATK, SAMtools, Pindel and snpEff.

Results: In one patient an inversion in MLH1 was detected by NGS and verified by Sanger sequencing of the new fusion points. The reads spanning the new fusion points mapped only partially to both genomic regions, the read pairs showed abnormal insert size and orientation. In the intronic regions, we selected for rare sequence variants with an allelic frequency (af) unknown (n = 1775 variants) or below 1 % (n = 1588) and 6 miRNA binding sites. 19 % of the variants were mapped into regulatory regions such as promoter/enhancer regions and might have a putative effect on transcript regulation. The effect of intronic sequence and regulatory changes have to be further investigated (e.g. on cDNA-level whether affecting transcript processing). For the detection of mosaics the coverage was not sufficient.

Conclusion: Deep intronic sequencing is a good method for the analysis of both exonic and intronic variants in the MMR genes but needs specific bioinformatical analyses. This method was used to detect further pathomechanisms such as rearrangements/inversions, regulatory defects, intronic mutations in 71 German patients with unsolved MMR-deficiency in their tumors. In many patients we found rare sequence changes of unclear significance that need further investigations by other methods, e.g. cDNA analyses. In cases with a positive family history of tumors, germline defects in one of the MMR genes are suspected, whereas in the other cases two somatic mutations in tumors might have caused the immunohistochemical loss.

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Keywords: NGS · Inversion · MMR

133 Studying cancer susceptibility genes by next-generation sequencing

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Purpose: The number of genetic causes underlying the susceptibility to gastrointestinal and other tumors has been increasing at a fast pace. The main technology currently used to evaluate these genes is Sanger sequencing, which is slow, expensive and subject to analytical error. We demonstrate here that the use of novel next-generation sequencing technology to study groups of genes is both feasible and desirable, leading to shorter times to results and lower costs per gene. Additionally, given the high degree of automation, it is very safe and reproducible.

Methodology: Forty DNA samples of patients with genetically confirmed or suspected inherited cancer syndromes were studied by next-generation sequencing. Custom amplicons were generated after sample preparation on a Tecan Evo Freedom 150 liquid handler, and digestion and adapter ligation performed by using Nextera (Illumina). The samples were barcoded and sequenced using an Illumina Miseq. Bioinformatics pipelines that come with the instrument (onboard) and developed in house were used to analyze data.

Results: Using high sequencing depth (>100×), paired-end chemistry and high quality reads, next-generation sequencing was 100 % sensitive and specific for single-base substitutions and deletions up to 19 bp (larger deletions were not tested). The analysis pipeline that comes with the instrument was not able to detect deletions larger than 5 bp, and a custom analysis pipeline had to be developed in house.

Conclusion: Next-generation sequencing of gene panels is a reliable and fast way to study cancer susceptibility syndromes. Great care and effort should be put on the bioinformatics analysis, as the solutions that come loaded in the companies' instruments are not reliable for the detection of larger deletions.

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Keywords: Next-generation sequencing · Bioinformatics · Genetics

134 Genetic analysis of the c.2059C>T mutation in the MLH1 gene

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Colorectal cancer (CRC) is the second most common cancer after breast cancer in Europe. Hereditary nonpolyposis colorectal cancer or Lynch syndrome (LS) represents approximately 2.4 % of all newly diagnosed cases of CRC and is one of the most common autosomal dominant hereditary cancer syndromes. LS is mainly caused by inherited mutations in any of the DNA mismatch repair genes MLH1, MSH2 and MSH6. Identification of a mutation in a family means that family members at risk can be offered presymptomatic carrier testing,

thus identifying individuals who are at high risk of developing the disease. These family members can then be offered various control programs to reduce LS-associated illness and death. Genetic screening of MLH1, MSH2 and MSH6 genes can detect a disease causing mutation in most families with LS. In this study, the c.2059C>T (p.Arg687Trp) mutation in MLH1 has been analyzed using a number of approaches: segregation analysis in families, association studies and haplotype analysis. Using a total of seven families carrying this mutation, segregation analysis did not show a clear segregation with disease. Association studies in a subpopulation of patients with low risk for colon cancer showed that c.2059C>T was very rare among patients, and absent in our normal controls.

Haplotype analysis will now be performed in order to elucidate if the mutation possibly represents a founder mutation in the Swedish population, or if the mutation has arisen spontaneously in different genetic backgrounds.

Keywords: Colorectal cancer · Lynch syndrome · Mutation

136 Reported MSH2 inversion and intron 1 mutation are no recurrent events in 84 mutation-negative German patients suspected of Lynch syndrome

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Purpose: A pathogenic germline mutation in one of the known causative genes can only be found in about half of the patients susceptible of Lynch syndrome. A pathogenic mutation deep in MSH2 intron 1 (c.212-478T>G) causing a splice defect by pseudo-exon inclusion was found in one family by Clendenning et al. 2011. Recently, Rhees et al. reported on a large genomic inversion including MSH2 exons 1–7 in 6 of 10 unexplained Lynch-Syndrome patients. To investigate if these two changes in MSH2 are recurrent events, we screened 84 mutation-negative German patients with MSH2-deficient tumors for these mutations. In addition, we sequenced the MSH2 promoter region and the last exon of EPCAM.

Methodology: All patients met the revised Bethesda guidelines and showed MSH2-MSH6-defects in their tumors. No pathogenic germline mutation/deletion was found in MSH2, MSH6 or EPCAM. To test for the MSH2 inversion, we performed PCR analyses for the two inversion breakpoints and a control fragment as described (Wagner et al. 2002; Rhees et al. 2014). The MSH2 intron 1 mutation locus was amplified and sequenced as described (Clendenning et al. 2011). Furthermore, we sequenced the MSH2 promoter region and EPCAM exon 9.

Results: None of our patients was tested positive for the inversion in MSH2 or harbored the intronic MSH2 mutation. Mutations in the promoter region of MSH2 or an EPCAM stop loss were also not found in our patient cohort.

Conclusion: The MSH2 inversion and MSH2 intron point mutation described by other groups could not be detected in our mutation-negative patients and are therefore no recurrent events or founder mutations in German patients with MSH2-deficient tumors. Even though somatic mutations in MSH2 have been described as a frequent

cause in germline mutation-negative patients, we still expect other pathomechanisms such as rearrangements/inversions, regulatory defects or intronic mutations in MSH2 causing Lynch syndrome in cases with positive family history. Therefore, further investigations including quantitative cDNA-analyses and deep intronic sequencing of the genes MSH2 and MSH6 by NGS are planned.

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Keywords: No founder · MSH2 inversion · MSH2 intron mutation

137 Specific bacterial sequences determination in feces identifies higher colorectal neoplasia risk subgroup among Lynch syndrome carriers

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Cancer risk in Lynch Syndrome (LS) carriers is variable and may depend upon the involved gene. Starting at early age, LS non-affected carriers follow an exhaustive surveillance through colonoscopy in order to detect pre-neoplastic lesions or colorectal cancer (CRC) in early stages. Thus, it is crucial to find a key biomarker that reflects CRC development risk. It has been shown that bacterial communities in the colonic mucosa of CRC patients differ from healthy individuals and intestinal microbiota has been proposed as a determining agent in the development and progression of CRC along its stages [1]. Recent data from our research group showed that a set of specific phylotypes determined in intestinal biopsy from CRC patients may associate with CRC risk [2].

Purpose: We aimed at defining a microbiological signature in stool sample capable of determining colorectal neoplasia risk in LS carriers.

Methodology: We designed a preliminary retrospective study to analyze intestinal microbiota in feces LS carriers (n = 30) who had a colonoscopy in the Digestive Department of Hospital Universitari Dr. Josep Trueta. Fifteen healthy controls with a normal colonoscopy were also included. Detection of specific phylotypes was performed through q-PCR of 16S rDNA bacterial sequences. The quantification of specific bacteria sequences was expressed in q-PCR cycle threshold (Ct). Ratios for the different sequences identified were calculated.

Results: Ratios were calculated for LS carriers with adenomatous polyps in their last colonoscopy (high-risk group, n = 15) and without lesions (low-risk group, n = 15). Cut-off values were defined (14.02, 24.76, 21.42 and 22 Ct values respectively) for four bacterial sequences and specific patterns for 16S rDNA were identified. Low-risk group presented elevated levels of 16S rDNA (Ct values below cut-off) in front of high risk-group that presented low levels. Levels of 16S rDNA showed a sensitivity of 80 % and an specificity of 100 % for group discrimination. No differences in 16S rDNA levels were observed between healthy controls and low-risk group.

Conclusion: Changes of specific microbiological signatures in feces may depict LS carriers harboring colorectal lesions. These

preliminary results outline a novel non-invasive approach for individualizing colonoscopy surveillance that merits validation in larger studies.

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Keywords: Lynch syndrome · Microbiome · Risk assessment

138 Exome sequencing of an Amsterdam-positive family identifies a novel causal gene for hereditary non-polyposis colorectal cancer

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Purpose: Estimates indicate that inherited factors account for over 20 % of all colorectal cancers (CRCs), but less than 6 % can be explained by rare high penetrance mutations in known genes. The identification of new genes associated with hereditary cancer will facilitate the management of patients whose predisposition is yet unexplained. Here, with the aim of identifying novel hereditary cancer genes, whole exome sequencing of CRC-affected members of a mismatch repair-proficient Amsterdam I CRC family was performed.

Methodology: The family studied had 3 members affected with CRC at ages 72, 67 and 42. Exome enrichment (Agilent SureSelect Human All Exon 50 Mb) followed by massively parallel sequencing (Illumina Hi-Seq2000) was performed on DNA extracted from peripheral-blood leukocytes of the 3 cancer-affected family members. Data analysis was performed as described [1]. Validation studies in familial cancer series and in silico and in vitro functional studies were also carried out.

Results: All cancer-affected individuals shared a novel nonsense variant in a gene involved in DNA repair that had not been previously associated with cancer predisposition. By sequencing the gene in 176 additional families, we demonstrate its implication in ~3 % (5/176) of the genetically uncharacterized Amsterdam-positive mismatch repair-proficient families. In silico prediction algorithms of function and structure, as well as in vitro DNA repair assays, support the damaging effect of the identified variants. Our findings suggest haploinsufficiency rather than a tumor suppressor-like behaviour. Moreover, whole-exome sequencing of a tumor developed by a mutation carrier showed a characteristic mutation spectrum, suggesting the accumulation of a specific type of errors due to DNA repair deficiency.

Conclusions: Our results strongly implicate a novel DNA repair gene in the inherited susceptibility to CRC, being therefore of fundamental importance for genetic counseling and genetic testing of hereditary CRC. Analysis of a larger series of cases will provide further information about the prevalence and tumor spectrum of this new syndrome.

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Keywords: Exome sequencing · New hereditary colorectal cancer gene · Hereditary non-polyposis colorectal cancer

139 Analysis of PMS2 transcripts and gene conversion in patients with suspect of Lynch syndrome

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Purpose: PMS2 transcripts from exon 1–15 were analyzed for exon skipping and sequence changes in 31 patients with isolated or combined PMS2-protein loss in their tumors but no clear pathogenic PMS2 or MLH1 germline mutation detectable. The results of the genomic PMS2 mutation/deletion screening were compared to findings on cDNA-level to investigate the PMS2 gene for exon skipping and sequence changes.

Methodology: From patient RNAs isolated from PAXgene and leucocytes from cultured blood (with and without NMD (nonsense-mediated mRNA-decay) blocked by puromycin incubation) we generated cDNAs. By LongRange (LR) PCR the complete PMS2 transcript was amplified and sequenced. Pseudogene co-amplification was excluded.

Results: One cDNA with NMD-block showed an aberrant insertion of 71 bp between exon 7 and 8, on the genomic level an insertion of a 2.2 kb SVA_F element was verified as described previously (van der Klift et al. 2012). In a female patient a splice defect with 50 % exon 4 skipping was caused by the PMS2 c.255G>A; p.Leu85Leu mutation located in the 5th nucleotide of exon 4 and noticeable in ESEfinder. 4

further patients with isolated immunohistochemical PMS2-loss had normal cDNA results. Of the 25 unsolved patients with combined MLH1-PMS2-loss the cDNA analyses of 6 patients showed diverging results, as additional and pseudogene-specific variants were found in the transcripts (in exon 13, 14 and/or 15) which were not detected on genomic level.

Conclusion: We performed PMS2 cDNA-analyses to complete mutation screening and revealed a pseudo-exon insertion in one patient, a splice defect due to a missense mutation in another patient and in six patients we found hints for PMS2 gene conversions also described previously (Auclair et al. 2007, Hayward et al. 2007) or an allelic loss in genomic mutation screening.

However, the clinical significance of PMS2 gene conversions generating the complete transcript with only few pseudogene-specific missense variants remains to be determined.

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Keywords: PMS2 · cDNA · Gene conversion

140 Novel DNA repair variants in POLH gene as possible susceptibility factors for Lynch syndrome

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Purpose: Lynch Syndrome (LS) is a cancer predisposition syndrome associated with increased risk of colorectal cancer (CRC) and other tumors at young age, representing about 5 % of all CRC diagnoses. It is caused by germline mutations in one of the mismatch repair genes (MMR) responsible for the correction errors in base pairing during DNA replication [1]. The involvement of DNA polymerase eta (POLH) in the MMR system by interacting directly with the MSH2 and MSH6 proteins was recently described [2,3]. The aim of this study is to evaluate the frequency of germline POLH variants in LS patients. **Methodology:** The coding regions of POLH, including exon–intron junctions, were Sanger sequenced in 52 unrelated patients with CRC and suspected LS. In silico analyses to predict the function of the genetic variations identified were done using SNP-info and Regulome DB databases.

Results: Overall, four different POLH DNA variants were detected in seventeen (32 %) patients, including: (1) five patients with an insertion of three nucleotides in intron 2 (rs371325034, g.43582527_43582528insGTG) that possibly changes the site of a transcription binding factor, (2) eight patients with a synonymous variation in exon 11 (c.1434G>A, rs3734690), (3) two patients with a base substitution in intron 7 (rs2307465, g.43604032A>T), which may interfere in splicing regulation, and (4) two patients with a

3'UTR substitution (rs1064260, g.43614607A>G) that influences ligation of transcription binding factors and miRNA as well. Allelic frequencies of these 4 variations were higher in LS patients (insGTG = 0.096, A = 0.038, G = 0.154 and A = 0.038) when compared to the frequencies described in the 1000 Genomes database (GTG = NA, A = 0.019, G = 0.022 and A = 0.029, respectively). **Conclusion:** In this preliminary analysis, germline POLH variants were present in 32 % of individuals with clinical criteria for LS. Further studies should be undertaken to investigate whether these variants have a phenotypic impact on tumor susceptibility in prognosis in LS patients.

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Keywords: Lynch syndrome · Familial cancer · Hereditary CRC

141 A search for new colorectal cancer syndromes

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Background: We have collected 3000 consecutive colorectal cancer cases and taken a full family history of cancer for all (1,2). We aimed to verify all diagnoses, which could relate to gastrointestinal tumors, thus any kind of tumor in the abdomen. Other tumors, such as breast-, prostate- or hematological malignancies were not verified using medical records.

Aims: We wanted to use this material to search for novel syndromes involving CRC.

Methods: We studied this by comparing the number of cancer types in families with at least two close family members with CRC to the number of tumors in all the other families. The search was limited to first- and second-degree relatives and cousins. FAP and Lynch syndrome were excluded.

Results: There were significantly more other cancers in the CRC families compared to those with only a single case of CRC. In particular, gastric cancer and prostate cancer were among the most common cancers, suggesting that in some families various forms of cancer segregate as a dominant cancer predisposing trait. Figures for rare cancers were not often significant, however, breast cancer clearly do not appear to be involved in any CRC syndromes.

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Keywords: Lynch syndrome · Colorectal cancer · Immunohistochemistry

143 Simple, rapid and cost-effective methods to identify mutation in MMR genes using next-generation sequencer

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Purpose: Current approaches for molecular genetic testing of Lynch syndrome are often stepwise, time-consuming and laborious due to genetic heterogeneity. To overcome these issues, we tried to develop a comprehensive assay by RNA sequencing that detects small size mutations and splicing aberration in MMR genes (MLH1, MSH2, MSH6, PMS2) and EPCAM using RT-PCR and massively parallel next-generation sequencing on the Illumina MiSeq instrument.

Methods: Genomic DNA and total RNA was extracted with Allprep DNA/RNA kit (QIAGEN) from short-term lymphocyte cultures treated with and without puromycin prior to cell harvest. The entire coding sequences of the MMR genes were amplified by RT-PCR in one or two overlapping fragments. The RT-PCR products were tagged and fragmented (tagmented) by Nextera XT transposome. The tagmented DNA is amplified via a limited-cycle PCR program and added index and sequences required for cluster formation. Sequencing was performed with 2 × 150-bp paired-end reads on MiSeq. Sequence alignment and variant calling performed against the reference human genome (hg19) on CLC Genomics Workbench.

Results: The coding sequences of MMR genes (MLH1, MSH2, MSH6, PMS2. Total of target sequence is 13 kb) were successfully amplified by RT-PCR. Sequenced data were obtained with 2 × 150-bp paired-end reads with high-quality (>Q30) on MiSeq. More than 500-fold coverage per nucleotide across the entire targeted region was obtained. In blinded samples with defined variants by Sanger sequence, known variants were correctly identified. In addition, it need only 4 days to get the results.

Conclusion: RNA-Seq offers a rapid, powerful, cost-effective means of genetic testing for Lynch syndrome without the need for stepwise testing.

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Keywords: Next-generation sequencer · Tagmentation · RNA-seq

144 Comparative analysis of MMR deficiency screening and germline genotyping in Brazilian patients with suspected Lynch syndrome

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Pre-symptomatic diagnosis and early intervention are key factors to ensure effective cancer risk reduction in Lynch syndrome (LS) patients and ultimately decreased mortality rates from colorectal cancer (CRC) and other extracolonic tumors in families with the disease. A significant body of evidence, from studies developed in North America and Europe shows that: (a) pre-symptomatic identification of carriers has an important impact on disease management, (b) molecular diagnosis of LS is feasible; (c) cost-effectiveness studies on the inclusion of LS patients in tumor screening programs show positive results. In Brazil, available data are limited to the study of mutations and phenotypic alterations in small series of families with suspected LS. The exact frequency of MMR deficiency and prevalence of MMR gene mutations are not known in detail in families from different regions of the country. Furthermore, the performance of LS screening and diagnostic strategies proposed in other countries has not been systematically assessed in Brazilian patients considering sensitivity and specificity. Thus, the main goal of the present study was to assess, in a comparative manner, different screening and diagnostic strategies in the evaluation of Brazilian patients with suspected LS. A total of 60 unrelated probands from 4 Brazilian geographic regions were recruited for the study and provided clinical information and biologic materials after informed consent. All patients were screened for MMR deficiency by IHC (Panel of 4 antibodies: anti-mlh1, -msh2, -msh6, -pms2) and MSI was performed whenever sufficient material was available. To differentiate somatic from hereditary origin in tumors with loss of mlh1 expression in mlh1 we performed analysis of the V600E mutation in BRAF. Germline mutation analysis was done in all cases by Sanger sequencing of the entire coding region of MLH1, MSH2 and MSH6 and rearrangement testing was also performed by MLPA for MLH1, MSH2 and MSH6 in all patients. Preliminary analysis comparing screening tests of MMR deficiency with mutation testing results show that a significant proportion of cases had inconsistent findings in the comparison these two approaches, with some patients presenting germline MMR gene mutations but no evidence of loss of MMR deficiency upon screening or the reverse, evidence of MMR deficiency on screening but no evidence of germline mutations in the MMR genes. Overall, from the 60 patients analysed, we identified a pathogenic mutation in 26 (43 %) individuals. As expected, mutation prevalence was higher in probands fulfilling Amsterdam criteria (20/27, 74 %) when compared to those with the modified Bethesda criteria (6/33, 18 %). Although a significant proportion of cases were resolved with the current diagnostic approach, additional strategies should be developed to provide comprehensive genotyping in and diagnosis in probands with suspected LS.

Keywords: Hereditary cancer · Lynch syndrome · Colorectal cancer

145 Turcot syndrome: important causes of death in Lynch syndrome

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Purpose: Turcot syndrome is hereditary disorder, characterized by increased risks of colorectal and brain tumors, associated with familial adenomatous polyposis (FAP) or Lynch syndrome. FAP and Lynch syndrome are autosomal dominant disorders, and arise from mutations of APC genes and mismatch repair genes such as MLH1 and MSH2, respectively [1]. Approximately 170 cases of Turcot syndrome have been reported in literature [2]. Causative genes of Turcot syndrome, however, are still controversial. Individuals in Lynch syndrome have an estimated of 1–4 % of life time risk of brain tumor [3]. Very few studies, however, have addressed Turcot syndrome associated with Lynch syndrome.

Methodology: Affected individuals in 16 Japanese families positive for the Lynch syndrome genetic testing (12 and 4 families had MLH1 and MSH2 mutations, respectively) were evaluated for the clinical features, including brain tumor, until 2013.

Results: A total of 185 cancers from 90 patients was noted. The most frequent cancer was colorectal cancer (108 lesions), followed by stomach cancer (33 lesions), uterine cancer (19 lesions), biliary tract cancer (12 lesions), breast cancer (6 lesions), brain tumor (5 lesions) and ovarian cancer (5 lesions). The most frequent causes of cancer death was colorectal cancer (23 patients), followed by stomach cancer (12 patients), biliary tract cancer (5 patients), brain tumors (4 patients), uterine cancer (4 patients), ovarian cancer (3 patients), others (3 patients) and breast cancer (1 patient).

All of the five patients of brain tumor had MLH1 mutation, not MSH2 mutation. They consisted of 3 males, including 1 tuberous sclerosis patient, and 2 females. The mean onset age of brain tumor was 39 years old. All of the brain tumor patients, except the tuberous sclerosis one, had passed away due to the brain tumors.

Conclusion: Although the incidence of brain tumor in Lynch syndrome was not so high, the mortality was relatively high. So far, no surveillance system for brain tumor has been established in the syndrome. In Turcot syndrome associated with Lynch syndrome, we recommend brain screening using magnetic resonance imaging on an individualized basis.

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Keywords: Turcot syndrome · Lynch syndrome · Brain tumor

146 Hereditary diffuse gastric cancer: importance of molecular diagnosis in the decision making process

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Gastric cancer is the fifth most common cancer and the third leading cause of cancer deaths in both sexes worldwide according to 2012 WHO/Globocan data and genetic predisposition has been increasingly identified as an important risk factor. In this respect, as few as 1–3 % of all gastric carcinomas have an underlying highly penetrant autosomal dominant mutation as cause for the increased tumor susceptibility (1). Approximately 25–30 % of families fulfilling the criteria for hereditary diffuse gastric cancer (HDGC) have germline mutations of the CDH1 (E-cadherin) gene (2,3). We report a 78 year-old female patient, referred to genetic counseling due to her personal and familial history of diffuse gastric cancer. After the first evaluation, and since the family qualified for the diagnosis of HDGC, molecular analysis of the CDH1 gene was performed in the proband and a pathogenic germline mutation (c.1565+1 G>A) was identified. Genetic counseling was then offered to the family, with special emphasis on first-degree relatives of the proband, who were at 50 % risk for also being carriers. The proband had 10 children, two already diagnosed with gastric cancer and one of these already deceased. All 9 living children agreed to be tested for the disease causing mutation, and 4 were found to be carriers, including two cancer affected patients (with gastric and colorectal cancer). The three individuals without gastric cancer were submitted to EGD (normal) and underwent total gastrectomy. Macroscopic examination of the stomach did not show any major abnormalities, but extensive microscopic examination of different areas of the organ using specific protocols, showed multiple foci of diffuse, invasive carcinoma in all patients. We discuss the importance of genetic counselling as an essential component of the evaluation and management of HDGC. The counselling process should include not only a formal genetics evaluation but also the input from a multidisciplinary team comprising those with relevant expertise in gastric surgery, gastroenterology, pathology, nutrition and psychology. Ideally, the full team should be engaged in both the pre- and post-testing phases, but MDT involvement is mandatory in the post-test setting (5).

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Keywords: Hereditary diffuse gastric cancer · CDH1 · HDGC

147 Familial adenomatous polyposis registry: a private center' report

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Background: Familial adenomatous polyposis is best model of colorectal cancer prevention. This disease has hundreds to thousands of preneoplastic lesions (adenomas) and the surgical treatment avoid colorectal cancer (CRC). The screening in Colorectal Cancer Registry context has shown a decrease in mortality and incidence.

Objective: Presented the Registry. Show the results of its performance (early detection and colorectal cancer prevention).

Materials and methods: Inclusion in a database of all individuals diagnosed with PAF (index case), and their first-degree relatives (population at risk). We performed: anamnesis, physical examination, familial pedigree, colonoscopies, detection of extracolonic manifestations, genetic analysis (sequencing massively parallel sequencing (NGS), the panel for PAF (APC-MUTYH).), Family and genetic counseling.

Results: Individuals registered: 217 (47 families), 96 diagnosed with FAP and 2 MAP (MUTYH associated polyposis); 55 of them followed in our institution. Of the 20 families studied genetically, the mutation was found in the APC gene in 15 (75 %), 2 in the MUTYH gene and two no mutation was found. Four first-degree relatives studied did not inherit the mutation. 1/31 (3.22) of call up patients presented CRC at diagnosis time, and 13/25 who consulted symptomatic (52 %), of which seven late stages. Three patients developed cancer in call up patients during the control: two rectal and one in sigma; two of them unbanded the control. Surgeries: 28 total colectomy with ileorectal- anastomosis (IRA), 12 coloproctectomies with J pouch reservoir and IRA was converted to J pouch.

Conclusions: Work in hereditary Colorectal Cancer Registry has proven that is possible to reduce the incidence of CRC and detect colorectal cancer early staged. The genetic study allows to define the diagnosis and detect carriers to take early measures and adequate and timely decisions. For individuals who have not inherited the familial mutation gives personal and family relief.

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Keywords: Registry · Prevention · CRC

148 MMR-deficient crypt foci as cancer precursors in Lynch syndrome—evidence from tumor histology

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Purpose: Colorectal adenoma formation with subsequent inactivation of the DNA mismatch repair (MMR) system has commonly been proposed as a progression model of colorectal tumor formation in Lynch syndrome. However, recently a novel lesion with pre-cancerous potential, the MMR-deficient crypt focus, has been described in Lynch syndrome mutation carriers [1]. MMR-deficient crypt foci lack a polypous appearance and are characterized by lack of functional MMR in crypts with normal appearance or only slight structural alterations. MMR-deficient crypt foci might give rise to an alternative pathway of Lynch syndrome cancer formation, which is initiated by MMR inactivation. Due to their non-polypous appearance, MMR-deficient crypt foci may potentially be responsible for the formation of cancers that escape colonoscopy detection. In the present study we aimed to analyze manifest Lynch syndrome-associated cancers for potential signs of adenoma- or MMR-deficient crypt focus-initiated tumor development and to narrow down the potential frequency of both pathways.

Methodology: MSI-H colorectal cancers (Lynch syndrome, n = 40; sporadic MSI-H, n = 34) were histologically examined. Tumor sections were HE-stained and evaluated for the presence of polypous formations (adenomas, serrated polyps) adjacent to the invasive cancers. Comparative analyses were performed for Lynch syndrome-associated and sporadic cancers, and potential associations with histological growth pattern and stage were examined.

Results: We were able to identify polypous regions adjacent to the invasive cancer in 15 (37.5 %) out of 40 Lynch syndrome-associated cancers and in 17 (50.0 %) out of 34 sporadic MSI-H colorectal cancers. Evidence of large pedunculated adenomas was lacking in all Lynch syndrome-associated cancers analyzed. No significant differences of growth patterns or tumor stage were observed between cancers showing polypous regions compared to their polyp-free counterparts. A growth pattern suggestive of immediate invasive growth initiated in non-polypous mucosa were observed in 7 (17.5 %) out of 40 Lynch syndrome-associated cancers, but only in 2/34 (5.9 %) of sporadic MSI-H colorectal cancers.

Conclusion: Our data demonstrate that histology examination of full-blown invasive cancers can provide information about the history of pre-cancer evolution. Our results suggest that at least more than one-third of cancers in Lynch syndrome follow the previously described adenoma-carcinoma pathway of tumor development. The results further suggest that MMR-deficient crypts may be relevant pre-cancerous lesions in a subset of Lynch syndrome-associated cancers. Molecular analyses are warranted to differentiate between adenoma- and MMR-deficient crypt foci-driven cancers in Lynch syndrome, particularly with regard to identify potential markers occurring in interval cancers.

Acknowledgements: The study has been funded in part by grants of the Deutsche Krebshilfe (DKH) and the Deutsche Forschungsgemeinschaft (DFG).

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Keywords: Lynch syndrome · Adenoma · MMR-deficient crypts

149 Characteristics of the patients with suspected Lynch syndrome interviewed in the clinic of gastrooncologia of the UNIFESP

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Introduction: Lynch syndrome (LS) corresponds to 5 % of colorectal cancer (CRC). Mutations in repair genes MLH1 and MSH2, and less frequently on MSH6 and PMS2 cause this syndrome. The SL is also characterized by the presence of extracolonic tumors such as gastric, bile ducts, endometrial, renal pelvis cancer and glioblastoma multiforme. The clinical diagnosis is based on the criteria of Amsterdam II and Bethesda reviewed.

Purpose: To select patients with CRC or gastric cancer with suspected of LS by the criterious of Bethesda using the electronic medical record for subsequent construction of heredogram.

Methodology: Among patients with CRC and gastric cancer on treatment by the Oncology Group of the Gastroenterology of UNIFESP, from April 2012 to October 2014, we selected 102 patients who met at least one of the Bethesda criteria reviewed. The study was approved by the Ethics Committee and all patients were informed and signed the consent form. The information was initially collected by the electronic medical records and the patients were interviewed by the same investigator in his periodic return to the clinic. In the medical consultation, the pedigree had been done based on information provided by the patients. Sex, age, tumor location, histological type and degree of differentiation were also collected. In a second phase the genetic tests will be done to confirm the clinical diagnosis.

Results: Among the 102 patients interviewed, 54 were men, 43.3 % had <50 years and 55.6 % had a family history suggestive of LS by the criteria of Amsterdam II. Regarding the location, 37 % were in the left colon, 23 % in the right colon, 22 % at rectum and 18 % in the stomach. 37 % of tumors were well differentiated, 46 % moderately differentiated and 17 % poorly differentiated. Between the patients, 49 (48 %) obtained an evaluation of a specialized genetic service, and 21 (48 %) were considered suspect of LS by the pedigree. The mean age of these patients was 59.1 years (SD = 12.79), 18 were localized in the colon, 7 (33 %) in the sigmoid, 6 (29 %) in right colon, 5 (24 %) in the rectum and 3 (14 %) in the stomach. The majority of the tumors (n = 13, 62 %) was moderately differentiated, 5 (24 %) well differentiated and 3 (14 %) poorly differentiated. Five of them (21 %) had mucinous component. It was also observed that besides CRC, other cancers associated with LS had been diagnosed in first or second degree relatives independent of the age.

Conclusion: Almost half of the patients with at least one positive Bethesda criteria were diagnosed with Lynch syndrome by the clinical history and the heredogram. A previous interview with the preparation of the pedigree, facilitates the identification of carriers of Lynch syndrome.

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Keywords: Colorectal cancer · Lynch syndrome · Mismatch repair

150 Exome sequencing identified potential causative candidate genes for hyperplastic polyposis syndrome

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Purpose: Hyperplastic polyposis syndrome (HPS), also known as serrated polyposis syndrome (SPS), is a yet poorly defined colorectal cancer (CRC) predisposition characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. A serrated polyp-CRC sequence (serrated pathway) has been postulated, however, to date, only few molecular signatures of serrated neoplasia (BRAF, KRAS mutations, CpG Island Methylation, microsatellite instability) were described in a subset of HPS patients and neither the etiology of the syndrome nor the distinct genetic alterations during tumorigenesis have been identified.

Methodology: To uncover predisposing causative genes, the exomes of 11 unrelated and clinically well characterized HPS patients with sporadic appearance were sequenced (Illumina HiSeq platform) using leukocyte DNA. The variants were filtered for rare truncating germline mutations (nonsense, frameshift, highly conserved splice sites) assuming a monogenic disease model. For data analysis and variant filtering the GATK software and in-house tools (VARBANK pipeline) were applied.

Results: Altogether, 260 rare truncating germline variants were identified. After stringent filtering steps including quality scores, the comparison with large datasets from population-based controls, detailed manual investigations of the variants and data mining according to functions and pathways, 135 unique variants in 132 genes remained. Each patient harboured several variants (range 9–16). Six genes were affected by biallelic variants (recessive model) in at least one patient and 19 genes by heterozygous variants (dominant model) in at least two patients. The majority of these genes is supposed to be associated with cancer or is involved in molecular and cellular functions related to tumorigenesis such as DNA repair or apoptosis. Another 53 genes, which are affected by heterozygous variants in only one of the patients, are regarded as interesting candidates according to functional scores and known somatic mutations in colorectal tumours. In a validation cohort of 20 unrelated HPS patients, three of the candidate genes were affected by additional truncating point mutations.

Conclusions: Using exome sequencing we identified new potentially causative genes for HPS, some of them are recurrently mutated. However, the number of variants per patient is also in line with a more oligogenic etiology of polyp predisposition. The current work-up includes the validation of all variants by Sanger sequencing, testing of relatives to determine the phase of assumed biallelic variants and segregation with the phenotype where applicable. All validated variants are included in a pathway and network analysis.

Acknowledgement: The study was supported by the German Cancer Aid

Keywords: Hyperplastic polyposis syndrome · next generation sequencing · Novel candidate genes

151 Randomized comparison of surveillance intervals in familial colorectal cancer

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Purpose: Colonoscopic surveillance is recommended for individuals with familial colorectal cancer (CRC). However, the appropriate screening interval has not yet been determined. The aim of this randomized controlled trial was to compare a 3-year with a 6-year screening interval.

Methodology: Individuals aged between 45 and 65 years with one first-degree relative with CRC < 50 years or two first-degree relatives with CRC were selected. Subjects were excluded if they had 3 or more adenomas at baseline colonoscopy, while those with 0–2 adenomas were randomized into two groups: A) colonoscopy at 6 years and B) colonoscopy at 3 and 6 years. The primary outcome measure was advanced adenomatous polyps (AAP). Risk factors studied included gender, age, type of family history and baseline endoscopic findings.

Results: 528 patients with 0–2 adenomas at baseline colonoscopy, were randomized into two groups (A = 262, B = 266). The proportion of subjects with AAP at the first follow-up examination at 6 years in A was higher than the proportion of subjects with AAP at 3 years in B, however the difference was not statistically significant. There was also no statistically significant difference in the proportion of participants with AAP at the final follow-up examination between both groups. Male gender, age and (proximal) adenoma at baseline were significant predicting factors for adenoma. No significant predictors were found for AAP.

Conclusion: Our findings demonstrate that a 6-year surveillance interval in familial CRC is safe.

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Keywords: Familial colorectal cancer · Surveillance · Interval

152 Genomic alterations in hereditary colorectal cancer in negative cases for mismatch repair genes mutations

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Colorectal cancer (CRC) is one of the most common neoplasms in worldwide. Lynch Syndrome (LS) represents the major hereditary disease associated with CRC, mostly caused by germline mutations in mismatch repair (MMR) genes. However, approximately 50 % of LS cases have no mutation in MMR genes, suggesting the involvement of new genes associated with CRC predisposition. In this study, we used two different microarray platforms: Agilent 4 × 180 K and Affymetrix CytoScan HD (1.9 million copy number probes and 750 thousand SNP probes), to interrogate the germline alterations in 11 patients with LS and without pathogenic mutations in the MMR genes. Beyond CNVs, Affymetrix platform also allows to detect copy-neutral loss of heterozygosity (cnLOH). The data were analyzed using Genomic Workbench v6.5 (Agilent Technologies) and Chromosome Analysis Suite v2.1 (Affymetrix) softwares. The results were compared with the Database of Genomic Variants (DGV), 100 healthy Brazilian individuals (evaluated with 180 K Agilent platform) and 1038 phenotypically healthy individuals (Affymetrix database). As expected, the higher resolution platform identified almost three times more CNVs than the 180 K Agilent platform (179–68) and almost the double of rare CNVs (≤ 1 % of reference databases) (45–28). It was not detected cnLOH. The differences observed could be explained by technical procedures, as well as the coverage and the number of probes and the type of analysis for the detection of CNVs (software and algorithm). Five cases presented in common six rare CNVs confirmed by both array platforms, including four regions encompassing genes associated with cancer: gain of 9p24.3 (DOCK8 and KANK1), loss of 2p23.3 (DNMT3A), gain in 9p21.2 (TEK) and loss of 3p12.3 (ROBO1). Interestingly, by using the Affymetrix platform, two unrelated cases presented loss of 2p22.3 covering the last four exons of BIRC6 gene, recently identified as potential therapeutic target in CRC. In overall, we identified rare genomic alterations with potential to be used as predisposition genes associated with Lynch Syndrome

Acknowledgements: Financial Support: FAPESP, CNPq.

Keywords: Hereditary colorectal cancer · Lynch syndrome · Copy number variations

153 Identification of familial colorectal cancer through the Dutch population screening program: the results of a pilot study

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Purpose: A population screening program for colorectal cancer (CRC) has been started in The Netherlands since January 2014 for individuals aged from 55 to 75 years. The aim of this study was to evaluate the proportion of individuals in the Dutch screening program with a positive immunochemical fecal occult blood test (iFOBT) that fulfill the criteria for familial colorectal cancer (FCC) and to evaluate

the proportion of participants that needs genetic counseling based on their family history and/or endoscopic findings.

Methodology: In this cross-sectional study, 387 participants aged from 55 to 75 years with a positive iFOBT were included. The participants were invited for colonoscopy. Subsequently, detailed family history was obtained at the intake at the outpatient clinic, by means of a questionnaire about their family history for CRC to assess the familial risk.

Results: Of the 387 participants, 325 participants (84.0 %) completed colonoscopy and familial risk assessment. In the present study, 51 (15.7 %) participants were found to have a positive family history for CRC and 20.3 % had a positive family history for a Lynch syndrome associated tumor. It was found that 3 % of the participants fulfilled the FCC Criteria and 0.6 % the Bethesda Criteria. None of the participants fulfilled the Amsterdam Criteria. Multiple adenomas (>10) were found in 15 participants (4.6 %). No cases of serrated polyposis were detected. Based on endoscopic findings and family history, 21 participants (6.4 %) should be referred to the clinical geneticist. Based on family history, 10 participants (3.1 %) need referral for surveillance colonoscopy.

Conclusion: The importance of a carefully taken family history is emphasized by the identification of a substantial proportion of patients that needs genetic counseling and/or colonoscopic surveillance in this study.

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Keywords: Familial colorectal cancer · Population screening program · Family history

154 Chemoprevention—a cautionary tale

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Purpose: We describe a patient in whom the use of indomethacin suppositories successfully reduced and controlled rectal adenoma growth. Within 18 months the patient was virtually polyp free but then presented with a Dukes' B rectal cancer. There have been previous reports of similar scenarios.

Methodology: We describe the case from the medical records. Mrs A is a 67 year old patient with familial adenomatous polyposis (FAP). She underwent a total colectomy with ileo-rectal anastomosis in 1981 at the age of 34. She had her surgery and all her follow-up to date at our institution. A surveillance flexible sigmoidoscopy in October 2012 revealed that significant polyp progression had occurred over the previous 6 month period. In March 2012 the endoscopist estimated there to be 30–40 polyps <4 mm in the rectum, plus one 15 mm polyp which was removed and proved to be a tubulovillous adenoma with low grade dysplasia. The same endoscopist in October 2012 estimated the rectal polyp count to be >100; between 60 and 70 polyps were removed including the largest at 8 mm which was found to be a tubular adenoma showing low grade dysplasia and a focus of high grade dysplasia. At Mrs A's clinic appointment in November 2012, surgical intervention in the form of completion proctectomy was discussed but the patient favoured close surveillance and medical

therapy. Indomethacin suppositories, 100 mg nocte, were therefore prescribed and endoscopic surveillance was increased to 3 monthly. February 2013: chromo-endoscopy revealed a significant reduction in polyp burden (4 polyps \leq 2 mm). Histology revealed tubular adenomas with low grade dysplasia. May 2013: No polyps identified within the rectum. One aphthous ulcer was seen at the ileo rectal anastomosis. Histology revealed mild chronic inflammation possibly related to previous polypectomy sites. September 2013: Using chromo-endoscopy no polyps were identified within the rectum. At this point surveillance reverted back to 6 monthly due to the low polyp count. March 2014: Mrs A postponed her endoscopy appointment. June 2014: A large ulcerated lesion was noted in the upper rectum, distal to the anastomosis. Histology confirmed an ulcerated moderately differentiated invasive adenocarcinoma with an adjacent tubular adenoma. July 2014: Following discussion with Mrs A, she underwent excision of rectum and formation of an end ileostomy. Final staging was pT3N0(0/17)V0ROM0; Dukes' B.

Results: A dramatic reduction in polyp burden with chemoprevention agents can lead to a false sense of security.

Conclusions: We question the validity of a reduction in cancer risk when polyp burden has been reduced by chemoprevention. A formal review of outcomes of chemoprevention agents is required (presented separately).

Keywords: Polyposis · Chromo-endoscopy · Chemoprevention

157 Routine MSI screening of Brazilian colorectal carcinoma patients: the experience of 1013 cases from Hospital de Cancer de Barretos

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Background: Colorectal cancer (CRC) is the second in incidence and the fourth in mortality worldwide. There are three pathways in the pathogenesis of CRC: chromosome instability, microsatellite instability (MSI) and CpG island methylator phenotype. These pathways are closely related and some tumors may harbor alterations in multiple pathways. The MSI is the less common and is more frequently related with hereditary non-polyposis colorectal cancer (HNPCC) Syndrome. In Brazil, CRC incidence is increasing and the frequency of MSI and its biological and clinical impact in CRC tumors are largely unexplored. The goals of this study are: (1) to compare two methodologies of MSI determination, namely molecular and immunohistochemistry; (2) to correlate MSI phenotype with patients' clinic-pathological features; and (3) to determine the patients ancestry by molecular markers and correlate with the MSI phenotype.

Materials and Methods: One thousand thirteen CRC patients were enrolled in the study. The MSI evaluation was performed using a multiplex PCR comprising 5 markers (NR27, NR21, NR24, BAT25, and BAT26), and by immunohistochemistry analysis of MMR enzymes (MLH1, MSH2, MSH6 and PMS2). Patients' genetic ancestry was evaluated using a panel of 46 AIMs. **RESULTS:** MSS status was observed in 86.0 % (871/1013), MSI-L in 4.0 % (41/1013) and MSI-H in 10.0 % (101/1013) of cases. Loss of at least one MMR was observed in 10.3 % of cases, been MLH1/PMS2 responsible for 53.3 % of the cases. We observed a concordance of 95.5 % between both methodologies. Genetic ancestry showed that the average ancestry proportion was of 73.2 % of European background, followed

by 12.9 % of African, 7.1 % of Asian and 6.9 % of Amerindian. No statistical difference was observed between distinct MSI status patients and their genetic ancestry.

Discussion: We showed that both MSI and MMR immunohistochemistry and suitable methodologies for routine assessment of MSI in Brazilian population. The MSI frequencies identified in Brazilian CRC patients are in agreement with the international literature, and do meet seem to be related their genetic ancestry.

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Keywords: MSI · Ancestry · Biomarker

159 Proteins status in the pouch mucosa after proctocolectomy in FAP patients

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Background: Familial adenomatous polyposis (FAP) is an inherited disease characterized by the development of hundreds to thousands colorectal adenomas leading to 100 % lifetime risk of colorectal cancer. A prophylactic colectomy or a restorative proctocolectomy using an ileal J-pouch is recommended for patients with FAP to prevent colorectal cancer.

Methods: The aim of this study was to compare the status of 16 proteins (MUC 1, 2, 5AC, 6, CTNNB1, Ki67, CDX2, p53, APC, BCL2, COX2, and PKC α , λ , δ , ϵ , ι) related to homeostasis and carcinogenesis of gastrointestinal mucosa in the ileal J-pouch mucosa with matched ileum and colon mucosa prior the surgery (proctocolectomy), having at least 8 years of follow-up, in patients with

FAP. Changes of protein expression are tissue-dependent and may reflect the ileal pouch mucosa in a new function as a reservoir.

Results: The samples consisted of 7 patients, 3 (42.9 %) males and 4 (57.1 %) females, with a mean age of 39.3 years (range 24–55 years). Ileal J-pouch biopsy microscopic examination revealed 2 cases (28.6 %) with microadenomas. Expression of MUC2 and CDX2 was strongly positive in ileal J-pouch mucosa when compared with ileum and colon mucosa prior the surgery. In contrast, other proteins showed similar expression in all groups.

Conclusion: The changes of tissue-dependent protein expression may just reflect the adaptation of the ileal J-pouch mucosa according to a new physiological status and not yet the carcinogenesis in this tissue. Further studies are necessary to better understand the whole process of tumor formation in ileal pouches.

MUC1,2,3,5AC,6 (mucin 1, 2, 3, 5AC, 6 cell surface associated); CTNNB1 (catenin beta 1); CDX2 (caudal type homeobox 2); p53 (tumor protein 53); APC (adenomatous polyposis coli); BCL2 (B-cell CLL/lymphoma 2); COX2 (mitochondrially encoded cytochrome oxidaseII); PKC (protein kinase C).

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Keywords: Familial adenomatous polyposis · Ileal J-pouch mucosa · Carcinogenesis

160 Uptake of genetic testing among relatives of Lynch syndrome carriers in a United States cancer genetics registry

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Purpose: Cost-effectiveness analyses estimate that for population-based screening for Lynch Syndrome (LS) to be economically beneficial at least three additional relatives of each DNA mismatch repair (MMR) gene mutation carrier should undergo genetic testing. Prior studies examining uptake of genetic testing in families with LS have been limited by small sample sizes and failure to delineate family members beyond first-degree relatives in their analysis. We sought to estimate the uptake of genetic testing among at-risk family members by (1) comparing numbers of individuals tested for LS using full sequencing of MMR genes and site-specific testing and (2) surveying individuals from families with confirmed MMR mutations to determine how often genetic test results are communicated to at-risk family members.

Methodology: We reviewed results of genetic tests performed for individuals evaluated for LS at the University of Michigan from 2004 to 2014 and compared the number of full gene sequencing versus site-specific tests over time. Subjects who tested positive for MMR gene mutations or who had a known LS mutation in their family were mailed a survey >1 year after disclosure of their genetic test result asking if any additional relatives had undergone genetic testing.

Results: Records of genetic tests for MMR gene mutations were available for 556 individuals, 453 (81.5 %) were tested using full gene sequencing and 103 (18.5 %) with site-specific tests. The number of individuals evaluated for LS increased exponentially during this 10 year period, with uptake of full gene sequencing and site-specific tests increasing 14-fold and fivefold, respectively. The largest increases in site-specific tests were observed after 2010, with an average of 2.3 site-specific tests performed for every 1 MMR mutation carrier identified by full sequencing. Overall, pathogenic mutations were identified in 68/453 (15 %) of individuals evaluated with full gene sequencing, compared with 48/103 (47 %) of those evaluated with site-specific tests. Subjects from 21 LS families reported that a total of 74 additional family members underwent genetic testing. The average number of first-degree and second-degree relatives tested per proband was 2.7 (range 0–7) and 0.7 (range 0–4), respectively.

Conclusion: For each MMR mutation carrier identified, on average between 2 and 3 at-risk family members undergo predictive genetic testing. Variability in uptake of genetic testing among first and second degree relatives suggests a need for interventions to facilitate communication about genetic information in LS families.

161 The largest public Lynch syndrome registry in Argentina: description of our 10 year-experience

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Purpose: although only a minority of the total cases, Lynch syndrome (LS) represents around 150 cases of colorectal cancer (CRC) per year in Argentina, which through timely investigation and intervention could potentially be prevented. The optimal management of this syndrome involves specialized familial cancer units and registries that facilitate a multidisciplinary approach, with a demonstrated improvement in both the incidence of CRC and the overall survival in affected families. Here we describe the structure, the management and the patients characteristics of the largest public Lynch Syndrome Registry in Argentina, located in a public metropolitan hospital in Buenos Aires, along with our improvements during the last 10 years.

Methodology: a review of all the registered families since 1999 was undertaken. We evaluated families characteristics, molecular tests and surveillance colonoscopies results, and we analyzed the increase over time of the number of families recruited and of the molecular tests done.

Results: we recruited 648 families with Amsterdam (92 = 14 %), Bethesda (501 = 77 %) or CRC < 70 (55 = 8.5 %) criteria for LS attended at the Oncology Section of our hospital. Interestingly, 193 (30 %) reached only the Bethesda 1 criteria. During the period 2009–2014, in which two gastroenterologists full-time were incorporated to the registry, we recruited 490 families, 3 times more than during the 1999–2008 period. 279 (43 %) of our index patients had no social insurance. Immunohistochemistry (IHC) for mismatch-repair (MMR) proteins was started at our hospital in 2010, and since then 428 (66 %) patients were analyzed. 266 (41 %) cases did microsatellite instability (MSI) analysis in the private context, since no one does it freely in Argentina. During 2014 our Hospital has become the first and only public center that does MSI, and we have evaluated already 36 cases. We actually have 21 (3.2 %) confirmed Lynch syndrome families, 14 (2 %) Lynch-like syndrome patients, 8 (5 %)

Familial CRC syndrome X, 230 (35 %) cases with an intact MMR, 260 (40 %) patients that still need IHC and/or MSI, and 115 (18 %) cases with MMR deficiency that still need to be genetically studied but have no possibility because these tests are not done freely in Argentina. Lastly, we have analyzed so far 919 colonoscopies in the context of CRC surveillance, 519 from our index patients and 327 from their first-degree relatives. We identified 37 (5.7 %) high risk adenomas and 22 (3.4 %) CRCs; of these 18 (82 %) were early tumors (Stage I-II) and only 3 (13.5 %) were stage III and 1 (4.5 %) stage IV.

Conclusion: we have achieved with great effort and dedication of our human resource, along with funding and managerial support from the CONICET and the Argentine National Cancer Institute, one of the largest LS Registries in South America. Our registry also represents the first public hospital in our country to do IHC and MSI for LS freely. Nevertheless, we still must keep on working to achieve more permanent human resource and the realization of more genetic tests, so we can finally democratize sequencing and reach the whole community, without distinction of socio-economic status.

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Keywords: Lynch syndrome · Registry · Experience

162 Detection of suspicious families of Lynch syndrome within a colorectal cancer screening program

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Introduction: There is no literature reporting the identification of cases suspicious of Lynch Syndrome (LS) within a screening program for colorectal cancer. This is relevant specially in asymptomatic patients with family history of colorectal cancer related to LS. Patients with family history of cancer and those diagnosed with colorectal cancer during screening, should be assessed by a health survey considering Amsterdam and Bethesda's criteria.

Aim: To identify families suspicious of LS using Amsterdam and Bethesda's criteria within a colorectal cancer screening program in Chile.

Methods: Personal and familial information was obtained from the 5300 patients' program data base. Two groups were identified: (1) patients with the diagnoses of colorectal cancer (CRC) detected and treated according to the program and (2) asymptomatic individuals with a cancer history in first degree relatives. Both groups were contacted to collect information about family history and to build their genealogy.

Results: A total of 241 individual were identified: Group 1 (CRC) 69 patients and Group 2 (Family history) 172 individuals. In Group 1, 51 patients (74 %) and in Group 2, 115 (67 %) agreed to participate. In Group 1, three Bethesda patients were identified with CRC, diagnosed at 62, 65 and 74 years old, all of them with family history of cancer related with LS, and one Amsterdam II family, with mother and son with CRC diagnosed at 49 and 58 years old respectively, and a sister with uterine cancer at the age of 30. In Group 2, three patients (2.6 %) had a first degree relative (mother, daughter, sister) with CRC diagnosed before 50 years old, fulfilling the Bethesda criteria. In summary, in these 7 families, 54 individuals with higher risk of cancer in comparison with the general population, were detected. They were advised to have a clinical surveillance and molecular tests according with the actual guidelines.

Conclusion: This study demonstrates that the use of a health survey as part of a colorectal cancer screening program allowed to detect 2.6 % LS suspicious cases in asymptomatic individuals with a family history of cancer.

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Keywords: Lynch syndrome · Colorectal cancer screening · High risk

164 Causative novel POLE mutations in hereditary colorectal cancer syndromes

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Background In families with Familial Adenomatous Polyposis (FAP) it is today possible to find almost all of the disease-causing mutations responsible for the classical polyposis syndrome. However, in patients with less than 100 polyps only a fraction of the disease-causing mutations can be identified. The low detection rate implicates the probable presence of additional disease-causing genes still to be identified. FAP is caused by autosomal dominantly inherited mutations in the APC (Adenomatous polyposis coli) gene. In families with

less than 100 polyps 20–30 % of the cases will exhibit a germline APC mutation. MUTYH is responsible for the recessive polyposis syndrome MUTYH Associated Polyposis (MAP). Recently a new CRC syndrome, polymerase proof reading associated polyposis (PPAP) was described. This syndrome is characterized by a dominantly inherited predisposition to the development of a variable number of colorectal adenomas and carcinomas. The aim of this study was to sequence the exonuclease domain of POLE in 88 index patients with a familial history of polyposis or non-polyposis and/or early onset CRC that had previously tested negative for mutations in APC, MUTYH and/or mismatch-repair genes MSH2, MLH1, MSH6 and PMS2.

Method: In one large family exome sequencing was performed in four family members and for the remaining 87, index patients mutation screening of the exonuclease domain (ex3–14) was conducted.

Results and Conclusion: We have identified two novel mutations in the exonuclease domain. The first mutation was identified from the exome sequencing in a large Swedish family with CRC. The POLE: c.1089C>A, p.Asn363Lys mutation is directly involved in DNA binding. Theoretical prediction of the amino acid substitution suggests a profound effect of the substrate binding capability and a severe impairment of the catalytic activity. Family members carrying this mutation demonstrate a high penetrant predisposition not only to CRC but also to extra-intestinal tumours such as ovarian, endometrial and brain. The second mutation located in POLE: c.1274A>G, p.Lys425Arg was also found to be directly involved in DNA binding, according to theoretical predictions. It was found in a patient with early onset CRC. In summary, theoretical predictions of the variant's functionality and segregation analysis in the families strongly suggest a pathogenic nature of these mutations. Screening the proofreading domains of POLE should be considered in routine genetic diagnostics

Keywords: POLE · Mutation · Exome sequencing

165 Gains in genes encoding zinc-finger proteins are candidates to be involved as predisposition factor risks in patients with multiple primary tumors and from families with history of gastrointestinal tumors

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Background: Multiple primary tumors (MPT) are the major cause of mortality and morbidity in patients that survived after the treatment of a first malignant neoplasm. High risk of a second primary tumor development has been suggested as associated with radiotherapy or chemotherapy used as treatments for the first cancer. Potential risk factors also included unhealthy lifestyle, genetic predisposition, aging, environmental exposition or an interaction of these factors. We described genomic alterations in seven patients with MPT and personal or familial history of gastrointestinal cancer.

Patients and Methods: Seven patients with multiple primary tumors and with family history of cancer, including gastrointestinal tumors were evaluated by genomic alterations (CytoScan HD Array Platform

(Affymetrix). Chromosome Analysis Suite (ChAS) software (v.2.0.1) was used for analysis considering at least 50 markers for gains; 25 for losses and a minimum of 5 Mb for cn-LOHs. Data from 1038 phenotypically healthy individuals (Affymetrix) and from Database of Genomic Variants were used as reference. Only alterations found in <1 % (rare) or never described (new rare) of the reference population were considered.

Results: The patients with MPT presented 3–9 rare and or new genomic alterations. Three patients presented gains involving genes that encode proteins containing zinc fingers that may act as transcriptional regulators (ZNF107, ZNF138, ZNF273, ZNF407, ZNF516). In addition, two other cases presented gains of ERBB4 gene. This gene is a member of the epidermal growth factor receptor subfamily. The protein encoded by ERBB4 binds to and is activated by neuregulins and other factors and induces a variety of cellular responses including cellular proliferation and differentiation. Mutations in this gene have been associated with cancer.

Conclusion: The genomic alterations herein reported pointed out genes with potential to be associated with high risk of cancer predisposition in families with history of gastrointestinal cancer.

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Keywords: Multiple tumors · Familial cancer · Copy number variations

166 Familial adenomatous polyposis presenting in a child as multiple primary hepatic tumors

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This is a case report of a female child referred to us at the age of 11 years with a diagnosis of biliary cirrhosis, hepatic insufficiency, status-post liver transplantation and with identification of six hepatic tumors upon pathology examination of the liver. The tumors included three hepatoblastomas of the pure epithelial fetal type, two well differentiated trabecular hepatocarcinomas and one biliary duct adenoma. Physical examination did not show minor or major malformations but there was evidence of neurodevelopmental delay in infancy and childhood. Parents were apparently healthy and unrelated and there was no significant family history of cancer. Differential diagnosis included Wilson's disease, viral infectious diseases of the liver, Progressive familial intrahepatic cholestasis (PFIC), and familial adenomatous polyposis. Sequencing of the APC gene revealed a novel germline mutation, p.Arg2415fX10 in heterozygosity which creates a premature stop codon and is thus predicted to be pathogenic. We describe the phenotypic characteristics of the disease in this FAP case with unusual clinical presentation.

Keywords: Familial adenomatous polyposis · APC gene · Liver cancer

167 Grupo Colaborativo Uruguayo (GCU): first outputs in molecular characterization of colorectal hereditary cancers

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Purpose: To describe the first catalog of mutations found in Uruguayan families with colon cancer susceptibility.

Methodology: Based on the GCU database, 298 out of 710 family records were reviewed to meet the criteria for clinical classification into CRC risk groups according to international guidelines. A genetic query, including the collecting of a family and personal history and all clinical records were performed. The patients selection was made following the respective international clinical criteria described in NCCN guides 2014: Amsterdam (I and II) and Bethesda for Lynch Syndrome; CHROMPET for Li-Fraumeni syndrome; polyp count and histologic type in case of MYH-associated polyposis or Familial adenomatous polyposis (FAP). For those patients who met Amsterdam or Bethesda criteria, the determination of microsatellite instability was added to complete the assessment. The DNA samples were submitted to one or more of the following techniques: SSCP, DGGE, Sanger and NGS techniques. The analyzed genes were: MLH1, MSH2, MSH6, MYT, APC, PMS1, PMS2, p53 and APC. Clinical significance validation of the detected genetic variants was checked on international databases. DNA samples were obtained from peripheral blood. In all cases a written informed consent specific for the present analysis was obtained.

Results: A total of 66 samples were sequenced. 20 deleterious mutations were detected: 14 for Lynch Sd (72 % microsatellite instability positive), 3 not previously described; 5 in the polyposis, 1 of them new; 1 in Li-Fraumeni.

Conclusion: The GCU is a nonprofit multidisciplinary group created in 1996. Its main task consists in working in registry, diagnosis, surveillance and monitoring of patients with colorectal hereditary cancer and their families. Based on the sequence results catalog of gene variants was created. This will help us to a better comprehension of the pool of genetic variants in our population and to identify our own risk gene variants. Further analysis of variants of unknown clinical significance are programmed to be held in an international collaboration

Acknowledgments: Fundación Génesis Uruguay

Keywords: Gene variant · Sequencing · Gene data analysis

168 Universal screening method with microsatellite instability and immunohistochemistry for detection of Lynch syndrome: preliminary experience at Clínica Las Condes-Chile

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Introduction: Traditionally, individuals suspicious of Lynch Syndrome have been selected according to the Amsterdam and Bethesda clinical criteria, which sensibility for mutation detection is 50 and 72 % respectively. Nowadays, the implementation of microsatellite instability (MSI) and/or immunohistochemistry (IHC) for MLH1, MSH2, MSH6 and PMS2, is being promoted to assess the MMR system function for all colorectal cancer. Universal screening would increase the sensibility for mutation detection and enlarge the group

of high risk families, taking into account those who were discarded for not meet traditional criteria.

Aim: To increase detection of cases suspicious of Lynch Syndrome through the implementation of MSI and IHC studies in patients who do not fulfill Amsterdam criteria.

Methods: We included colorectal cancer patients treated by surgery without neoadjuvant treatment with chemo/radiotherapy, who do not fulfill Amsterdam criteria. Cancer specimens were studied for MSI with 7 microsatellite markers and immunohistochemistry for 4 MMR proteins. In those tumors with MLH1 expression loss, MLH1 promoter methylation and BRAF V600E mutation were analyzed to discard sporadic cases.

Results: From the 112 enrolled patients, 83 have MSI and IHC information. High-MSI was identified in 11 tumors (13 %), all of them with expression loss of at least one of the 4 MMR proteins. 9/11 showed MLH1 loss and, the majority of them, PMS2 loss as well. For the purpose of discard sporadic cases, MLH1 methylation and BRAF mutation studies was performed in 8/9 tumors. In this group, one case suspicious of LS was identified, corresponding to a 54 years old right colon cancer patient without family history. Besides, two more cases were identified; one with MSH2/MSH6 heterodimer expression loss and other one with PMS2 loss, both patients diagnosed with right colon cancer at 62 and 61 years old without family history, respectively. On the other hand, in the group with MSS tumors, a 49 year old left colon cancer patient with a MSH6 protein expression loss was identified. MSH6 expression loss with no affection to the MMR function, does not discard a mutation, given to the redundant function between MSH6 and MSH3.

Conclusion: In summary, with the routine implementation of MSI and IHC, we identified 4 cases (4.8 %) that do not fulfill the traditional clinical criteria (Amsterdam and Bethesda) and, according to its tumoral characteristics, are candidates for genetic studies. These patients and their first degree relatives must receive clinical recommendations depending on the results of the genetic studies.

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Keywords: Universal screening · Immunohistochemistry · Suspicious Lynch syndrome

169 Novel juvenile polyposis syndrome mutations

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Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder characterized by multiple juvenile polyps (JP) mainly in the colon with increased risk of colorectal cancer [1]. Subsets of patients develop severe gastric polyposis with increased risk of gastric cancer [2]. Hereditary Hemorrhagic Telangiectasia (HHT) has been reported in association with a subset of individuals with JP and SMAD4 mutations [3]. Of fifty patients evaluated for polyposis in 2013 two JPS patients with novel mutations were diagnosed.

1st: 27yo, Druz descent male. 7 years post rt hemi-colectomy for multiple hyperplastic, and JPs resulting in recurrent rectal bleeding and anemia. Sequencing and MLPA for SMAD4 were negative. BMPR1A sequencing yielded c.367G>T (p.123E>*(STOP), a novel, de novo, nonsense mutation. His gastroscopy is normal. On follow up 8 years post colectomy multiple small mixed hamartomas and hyperplastic polyps 2–10 mm in size are present throughout his remaining colon and treated endoscopically so far.

2nd: A 39 yo, Jewish North African descent. At 32 years total colectomy for multiple adenomatous polyps suspicious of Familial Adenomatous Polyposis. Gastroscopy detected multiple hyperplastic polyps. APC sequencing and two common MUTYH mutations were normal. Due to severe bulky gastric polyposis a revision of the pathology was performed and a clinical diagnosis of JPS was made. A total gastrectomy was performed for persistent anemia and cancer risk. Epistaxis and telangiectases on his back and chest raised the possibility of HHT and the diagnosis was established based on the Curacao criteria. Subsequently, a small pulmonary AVM was suspected based on a bubble echo and Chest CT. Sequencing of SMAD4 found c.406_407delGT (p.V136CfsX6), a de novo, novel frameshift mutation.

JPS composes 10 % of the polyposis syndromes. About 20 % of SMAD4 patients have JPS-HHT combined syndrome. Phenotypically, patients with SMAD4 mutations may have gastric polyposis with a significant risk of gastric cancer. These JPS patients, illustrate the need for careful history collection and thorough pathological analysis, that should guide the genetic evaluation. Genetic workup of JPS patients is important for family counseling and to establish the need for HHT evaluation in patients with SMAD4 mutations preferably prior to surgical procedures.

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Keywords: Juvenile polyposis · Hereditary hemorrhagic telangiectasia · Management

170 Nursing consultation—a feasible strategy for hereditary cancer center in a Public University Hospital

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Purpose: Hereditary cancer syndromes (HCS) are genetic diseases in which cancer become more prevalent in individuals of the same family. The discovery of a germline mutation in a healthy carrier can trigger measures to prevent and reduce cancer risk. In patients with cancer the presence of a mutation can set choices in relation to specific therapeutic approach and define future preventive measures. We created a specialized multidisciplinary group for identification and management hereditary cancer patients and their families. In this

context was proposed an initial nursing consultation in order to identify suspected cases with hereditary component, aiming to optimize the medical consultation.

Methodology: The nurse team was trained by medical staff in an attempt to promote the ability to extract a genetic history, build a proper pedigree and infer the pattern of genetic inheritance. Aiming to strategically intervene positively in the care of patients with suspected familial cancer we established a multidisciplinary care flowchart, consisting of a pre nursing consultation, and subsequent referral to medical consultation of all cases with possible HCS. The multidisciplinary team was composed by a medical geneticist, medical oncologists, nurses and a psychologist. We analyzed all consecutive patients attended from Oct/2013 to Oct/2014.

Results: On these 13 months, 274 initial consultations were scheduled with the nursing team and 179 patients attended to the consult. Of these, 43 % were from patients with gastrointestinal tumors (n = 78); after multi disciplinary team discussion, 41 were referred for medical consultation, 20 were discharged and 17 are awaiting pathology review and/or evidence of tumors in the family. The possible diagnoses evidenced after medical consultation were: Lynch Syndrome (n = 8), AFAP or MUTHY (n = 2), NF1 (n = 1) and HBOC (n = 1). At the time of evaluation 6 patients were awaiting consultation, 4 were without a definition of diagnosis, 4 missed. There 15 patients discharged after medical evaluation, in the absence of a hereditary syndrome diagnosis.

Conclusion: The multidisciplinary care for patients with suspected HCS is performed in several centers of excellence, optimizes resources and facilitates medical care. There is a shortage of these services in the Brazilian public health system and the implementation of hereditary cancer clinics should be encouraged. Within our team, the role of nursing proved to be critical because not only is the main key for the screening of patients referred, but is also the first specialized professional to communicate information to the patient on the role of heredity in cancer. The development of programs with a nursing consultation within this area may optimize resources and contribute for the planning of actions related to prevention and early detection of cancer in the this context.

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Keywords: Nursing consultation · Hereditary syndrome · Public service

171 Screening strategies in multiple endocrine neoplasia type 1 (MEN-1): improved diagnoses to gastroenteropancreatic endocrine tumor

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Introduction: Multiple endocrine neoplasia (MEN-1) is a rare, autosomal dominant inherited disorder. The presence of MEN1 is defined as in an individual there are two of the three main MEN1-related endocrine tumors (hyperparathyroidism, enteropancreatic endocrine tumor and pituitary tumor). MEN1 is considered family when a person has MEN1 and at least one first-degree relative presents the

minimum of one characteristic of MEN1, that is, the presence of tumor in at least one of the three most frequently affected tissues[1–3]. MEN1 is associated with high morbidity mainly due to the presence of gastroenteropancreatic tumors, in which gastrinoma is the most often one leading to gastric hypersecretion, some times fatal stomach bleeding [4].

Objective: The present study aimed to characterizing the epidemiological profile (geographical, genealogical and clinical) of patients with MEN1, through the characterization of their biochemical and clinical data.

Materials and methods: The project was submitted to the Ethics Committee in Research of the Federal University of Ceara for validation. Both geographical and genealogical investigation of affected patients as well as their families at risk was conducted by means of a questionnaire prepared for the study and was applied in period to 2010–2014. Then the relatives at risk was invited (all first-degree relatives of patients diagnosed MEN1) and the same questionnaire was applied. Therefore, “snowball technique” was used, in which each case found will look for other possible cases of the same event[5]. Individuals with high clinical suspicion detected after application of the questionnaire was subject to screening laboratory tests that consist of calcium, phosphorus, PTH, prolactin and gastrin. They were followed according to Guideline for following patients with MEN-1.

Results: Analyzing our series of patients with MEN-1 in regular follow-up until 2014, we found that around 50 % of cases are from a well-defined geographical region of our state, and this data needs more care investigation. In 2010 our series was composed to 08 families and a total for 36 patients, 22 % (08/36) gastrinomas, 11 % (04/36) no functional pancreatic lesions and 2.7 % (01/36) insulinomas. After 4 years used “snowball technique” our series have 10 families with a total of 41 patients, 26 % (11/41) gastrinomas, 9.7 % (04/41) no functional pancreatic lesions and 2.4 % (01/41) insulinomas. In the total of our series we have 24 % (10/41) of patients that was submitted for surgery approach.

Conclusions: The “snowball technique” was economic and important strategy for diagnosis new cases. This syndrome is associated to aggressive gastrinomas and no functional pancreatic tumors that have impact in morbimortality[4]. Gastrinomas associated with MEN-1 often present aggressive behavior inducing locoregional metastases and early diagnosis of the syndrome becomes necessary[2–4]. Additionally, until this moment, not all patients underwent assessment of conventional radiology exams and new diagnosis may increase during the follow up

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Keywords: MEN-1 · Familial gastrinoma · Gastroenteropancreatic tumors

172 Germline MLH1 mutations in individuals with PMS2 deficient tumours

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Purpose: Lynch syndrome is caused by mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 as well as EPCAM. Immunohistochemistry (IHC) is used to identify MMR protein expression in tumours and guide germline testing. Tumours solely PMS2 IHC-deficient prompt germline testing of the PMS2 gene; however, in rare circumstances, MLH1 germline mutations have also been linked to this tumour phenotype [1]. This study investigates the underlying germline cause in individuals whose tumours are solely PMS2 IHC-deficient.

Methodology: Individuals with PMS2 deficient tumours were identified through the REB-approved Familial Gastrointestinal Cancer Registry. IHC, germline analysis, family history and cancer histology records were reviewed. Individuals with biallelic MMR mutations were excluded.

Results: Thirty individuals from 29 families had PMS2 IHC-deficient tumours where MLH1 was IHC-proficient. Twenty-nine tumours were colorectal cancers (CRC) and one was an endometrial cancer. Pathogenic or predicted pathogenic PMS2 mutations were identified in 53 % (16/30) of individuals. Six (20 %) individuals from five families had MLH1 mutations. Eight (27 %) individuals had no germline mutation in PMS2; of those, five also had no identifiable mutation in MLH1. Three of the six (50 %) individuals with MLH1 mutations had strong nuclear MLH1 staining and the other three had weak nuclear MLH1 staining. Family history review showed that 83 % of the MLH1 carriers (n = 6) met Amsterdam criteria compared with 13 % of the PMS2 carriers (n = 16). Mean age of CRC diagnosis for MLH1 mutation carriers was 50 years (SD = 19.8), and for PMS2 mutation carriers was 45 (SD = 10.7).

Conclusion: MLH1 mutations were found in 20 % of individuals whose CRC or endometrial cancers were PMS2 deficient with strong or weak MLH1 nuclear staining. With PMS2 deficiency, weak MLH1 staining may prompt MLH1 germline testing. However, MLH1 germline testing should also be offered to individuals with strong MLH1 staining, when PMS2 germline analysis is uninformative. Discrepancies in IHC were observed between tumors in the same individual as well as between individuals in the same family. Sensitivity and specificity of IHC testing is pathologist and centre-dependent. It is important to consider IHC on multiple tumours and/or relatives in families suggestive of Lynch Syndrome when germline mutations are not identified.

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Keywords: MLH1 · PMS2 · Lynch

173 Prevalence of gastrointestinal tumors in Li-Fraumeni syndrome

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Li-Fraumeni syndrome (LFS; OMIM # 151623) is an autosomal dominant disorder associated with multiple early-onset childhood and adult cancers. The molecular basis of LFS is the presence of pathogenic germline mutations in TP53 tumor suppressor. Carriers have a 50 % risk to develop cancer before the age of 40 years and the lifetime risk of cancer in germline TP53 mutation carriers is 90 % by age 60 years. Mutation carriers are at increased risk for multiple tumors of the full LFS spectrum, in particular ACC (adrenocortical carcinoma), choroid plexus carcinoma, and premenopausal breast cancer. Gastrointestinal tumors have been reported in LFS families but are not considered as core tumors of the syndrome. A cohort of 210 Brazilian LFS TP53 mutation carriers from 73 families from the Oncogenetics Department of AC Camargo Cancer Center was analysed. The tumor profile in this cohort revealed some gastrointestinal tumors not typically associated with LFS: gastric cancer (4 patients; 1.9 %; mean age at diagnosis of 53.2 years old), colorectal cancer (4 patients; 1.9 %; mean age at diagnosis of 40 years old) and 2 patients with cancer of ampulla de Vater at the age of 41 and 60. Gastrointestinal tumors are a part of LFS spectrum in Brazilian LFS population, supporting the need for endoscopy and colonoscopy screening in TP53 mutation carriers.

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Keywords: Li Fraumeni · Gastrointestinal tumors · TP53

174 Searching for novel polyposis associated genes through whole exome sequencing of APC/MUTYH mutation-negative patients

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Purpose: Germline mutations in APC and MUTYH genes accounts for 85–90 % of the genetic cause of adenomatous polyposis. The remaining 10–15 % of patients with multiple colorectal adenomas does not harbor mutations in these genes, suggesting that other yet unknown polyposis-predisposing genes could exist.

Methodology: Thus, the aim of this study was to investigate novel susceptibility genes by whole exome sequencing of negative polyposis patients screened for APC and MUTYH mutations. In a previous study [1], 23 unrelated polyposis patients were screened for APC/MUTYH point mutations and genomic rearrangements, with 21 patients being identified as mutated in this cohort (91 %). Two patients were negative for mutations in the evaluated genes and were screened for mutations in other genes through exome sequencing at SOLiD 5500xl platform.

Results: The percentage of bases covered at least 20× was 68 and 74 % for the two patients. We identified a total of 10 novel loss-of-function variants (stop codon, frameshift or splice site mutations) and 158 novel missense variants. Of these, 6 missense variants occurred in tumor suppressor genes and oncogenes frequently mutated or altered in cancer (TET1, NCOR1, RAF1, MCC, MTOR and MARK4). One gene (KIF14) was mutated in both patients (two different novel missense variants) and other two genes, SAMD9 and SRRM2, presented two distinct missense variants in each patient (possibly biallelic mutations). Moreover, the discovery of the involvement of DNA polymerase genes, including POLD1 and POLE, as novel polyposis susceptibility genes [2], prompted us to investigate for possibly damaging mutation in genes of DNA polymerase families. One patient was found to harbor a novel missense variant classified in silico as probably damaging in the POLQ gene, a polymerase involved in DNA double-strand breaks repair. The identified p.Lys2155Asn variant occurs in POLQ polymerase domain, in a region associated with the enzyme processing efficiency. The association of this and other selected candidates with the polyposis phenotype will be further investigated.

Conclusion: Our results show that whole exome sequencing efforts of APC/MUTYH negative patients, associated with stringent criteria of candidate selection based on gene/variant function, may aid to the recognition of novel putative polyposis genes.

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Keywords: Polyposis · Exome · Susceptibility genes

175 Back to the future—limitations of next generation screening strategies for Lynch syndrome

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Background: Lynch Syndrome is a highly penetrant, autosomal dominant, multi-system cancer disorder caused mainly by heritable defects in the highly conserved DNA mismatch-repair (MMR) genes hMSH2, hMLH1, hMSH6 & hPMS2 [1]. Most tumours from patients with Lynch Syndrome have a characteristic molecular signature resulting from the involvement of defective MMR, i.e., the presence of microsatellite instability (MSI) and/or the absence of MMR protein expression by immunohistochemistry (IHC) [2]. Identification of a

pathogenic germline mutation is extremely important because it enables pre-symptomatic testing of family members and structured surveillance of mutation carriers. Due to the heterogeneity of the mutation spectrum of the MMR genes, mutation analysis is time-consuming and expensive, therefore, screening strategies are required to pre-select those families that are likely to harbour a deleterious mutation. Various criteria (Amsterdam & Bethesda) have not proved definitive for identifying patients who may harbour a mutation. Pathogenic mutations are identified in approximately 60 % of microsatellite instability-high (MSI-H) cancer patients fulfilling clinical criteria for Lynch syndrome [3]. Next Generation Sequencing (NGS) technologies offer significant advantages from the traditional Sanger method of genetic testing with regard to massively parallel analysis, high throughput, and reduced cost. However these newer platforms are not without limitations in relation to sensitivity.

Aims and Hypothesis: Our examination of a large Irish kindred satisfying the stringent Amsterdam criteria for Lynch syndrome aims to highlight limitations of diagnosis based solely on NGS.

Methods: A comprehensive family history was taken from the proband. Molecular genetic studies are described in which NGS testing was then compared to a more traditional protocol comprising IHC, MSI, Southern blotting and Sanger sequencing.

Results: NGS testing did not identify any variants in the MMR genes tested (MSH2, MLH1 and MSH6). IHC and MSI testing provided very strong evidence of Lynch syndrome. Investigation by Southern blotting and Sanger sequencing detected a MSH2 intronic rearrangement in an affected 1st cousin with endometrial cancer displaying MSI and loss of expression of MSH2 & MSH6 proteins on IHC.

Conclusion: In this kindred, NGS as a primary molecular diagnostic modality would fail to identify an intronic MSH2 rearrangement potentially leading to defective DNA MMR. In conjunction with a comprehensively developed family history, tumour IHC analysis of all four MMR proteins (hMSH2, hMLH1, hMSH6 & hPMS2) and MSI testing remains the optimum screening strategy for Lynch syndrome. Consequently, consideration of employing traditional genetic techniques such as Southern blotting and Sanger sequencing still has relevance in classic Lynch syndrome kindreds with no mutation identified by NGS.

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Keywords: Lynch syndrome · Microsatellite instability · Mismatch repair (MMR)

176 Survival rate of patients who develop cancer in rectal stump after colectomy and IRA in FAP patients

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Purpose: Patients with classic familial adenomatous polyposis (FAP) undergoing Total Colectomy with ileum-rectum anastomosis (IRA) could develop cancer in the rectal stump [1–2]. The purpose of this study was to evaluate clinical features and survival rate after developing cancer in rectal stump in patients with FAP.

Methodology: The database of Hereditary Digestive Tumor Registry at Fondazione IRCCS Istituto Tumori of Milan was reviewed. Patients diagnosed with classic FAP underwent Total Colectomy/IRA between 1935 and 2014 were included in the study, and patients who developed cancer in rectal stump were identified. The survival rate of the patients who developed a cancer in rectal stump was assessed using Kaplan–Meier method.

Results: From a total of 697 patients undergone total colectomy with IRA, 49 patients (7 %) developed a cancer in the rectal stump. The median (range) age at diagnosis of cancer in the rectal stump, for the 49 patients, was 42 years (21–67), the APC mutation was pathogenetic in 43 (88 %) patients and in 12 patients (24 %) the mutation location was identified between codon 1061 and 1309. Median (range) interval from Total Colectomy/IRA and developing cancer in rectal stump was 157 months (12–486). The stage of cancer in rectal stump was A/B in 38 pts (77.5 %) while stage C/D in 11 pts (22.5 %). With a median (range) follow-up of 88.3 months (12–368) after developing cancer in rectal stump the survival rate at 10 years was 72 %.

Conclusion: Within the present series the cancer in rectal stump is a quite long term risk, with a prognosis that may support the conservative approach at first surgery in FAP patients.

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Keywords: FAP · Surgery · Rectal cancer

177 Value of mismatch repair deficiency in predicting response after neoadjuvant chemoradiation for rectal carcinoma

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Background: Neoadjuvant chemoradiation followed by radical surgery is the treatment of choice for patients with locally advanced rectal cancer. Several molecular markers have been tested as possible predictors of response. In our study we evaluated the role of mismatch repair deficiency (dMMR) as predictor of complete response to neoadjuvant chemoradiation in rectal adenocarcinomas.

Methods: This was retrospective analysis, from a prospective database. We evaluated 109 patients with adenocarcinomas of the rectum, located up to 8 cm from dentate line, clinically staged as cT3/T4 or cN+. Immunohistochemistry for mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) was carried out in pre-treatment biopsy specimens. The primary endpoint was pathologic complete response (cPR). Tumor regression was a secondary endpoint.

Results: we observed clinical complete response in 17.2 % of patients. 18.3 % had pathologic complete response after neoadjuvant therapy. 67 % of patients had good pathologic tumor regression (less than 25 % of tumor viable cells). dMMR was found in only 1.8 % of pre-treatment tumor samples. Clinical complete response was significantly associated with pathologic complete response, but the positive predictive value was only 43.8 %. The two patients with dMMR in tumor samples had clinical and pathologic complete response, which was a significant association ($p = 0.032$).

Conclusions: dMMR was found in only 1.83 % (02/109) and had a significant association with pathologic complete response. However, the very low frequency of dMMR limits the use of this tool in predicting response to preoperative chemoradiation in rectal cancer.

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Keywords: Rectal cancer · Mismatch repair · Chemoradiation

178 Variants of unknown significance in DNA mismatch repair genes: results from a hospital based hereditary cancer registry

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Purpose: Patients that present with features suggestive of a Lynch syndrome predisposition are evaluated at the high-risk clinic at MD Anderson cancer center. These patients are often diagnosed with a variant of unknown significance (VUS) in the DNA mismatch repair (MMR) genes. The purpose of this study was to characterize VUS detected in the high-risk clinic using tumor pathology, reported data and in silico tools at RNA and protein level.

Methodology: The MD Anderson Cancer Center cancer genetics database was queried for all VUS in MMR genetic test results. In addition to collecting germline mutation information, we also collected results of the microsatellite instability (MSI) and immunohistochemistry (IHC) analyses from tumors analyzed. For the characterization of the VUS, we used different in silico tools: LOVD for reported mutations, Spliceport, NNSplice and Softberry for RNA, and Sift, Polyphen2, Mutation Assessor, FATHAMM, and Mutation Taster for protein classification. Finally, we classified the variants in four categories: Damaging (predicted as damaging by protein in silico tools and LOVD or aberrant splicing at RNA level), Probably Damaging (predicted or reported in LOVD as probably damaging with No effect at RNA level), Inconclusive (Non correlation between in silico predictions and LOVD) and Neutral (predicted as Neutral by LOVD and RNA and protein in silico tools).

Results: A total of 53 VUS in MMR genes were identified of which 44 were unique. The VUS distribution was MLH1 42 %, MSH2 29 %, MSH6 13 %, and PMS2 16 %. Five patients had more than one VUS. MSI status was analyzed in 75 % of the tumors, the MSI results were high (80 %), low (3 %) and stable (15 %). IHC was positive for MLH1/PMS2 (38 %), MSH2/MSH6 (27 %), PMS2 (15 %), MSH6 (8 %), MLH1/PMS2/MSH6 (8 %), MSH2/MSH6/PMS2 (4 %) and isolated loss of MLH1 or MSH2 staining (0 %). Based on LOVD results, VUS were: 5 pathogenic, 12 likely pathogenic, 2 likely not pathogenic, 7 unclassified and 18 not available (NA). In silico RNA results were: 2 aberrant splice site, 4 inconclusive, 32 no effect, and 6 NA. In silico protein analysis: 24 damaging, 7 probably damaging, 8 neutral and 5 NA. Summary results combining in silico and reported data: 21 pathogenic, 7 probably pathogenic, 8 inconclusive and 8 neutral.

Conclusion: Our data did not show isolated loss of MLH1 or MSH2 staining although isolated MSH6 and PMS2 loss were observed. The majority of VUS analyzed did not show a predictive effect at the RNA level. For that reason, in silico protein analysis are more informative than RNA analysis. Incorporating in silico data and reported data may assist in reclassifying VUS in high-risk clinics.

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Keywords: Variant of unknown significance · DNA mismatch repair · In silico

179 Homozygous PMS2 c.137G>T (p.Ser46Ile) mutation causing constitutional mismatch repair deficiency (CMMR-D): extending the CMMR-D phenotype

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Background: Heterozygous germline mutations in mismatch repair (MMR) genes MSH2, MLH1, MSH6 and PMS2 cause Lynch syndrome (LS). Biallelic mismatch gene mutations cause a syndrome referred to as 'constitutional mismatch repair-deficiency' (CMMR-D) [1]. There is a markedly different tumour spectrum and onset times observed in these familial cancer syndromes. Approximately one half of CMMR-D patients develop brain tumours, and/or digestive tract cancers while a third develop haematological malignancies. Brain tumours and haematological malignancies are mainly diagnosed in the first decade of life, and colorectal cancer (CRC) and small bowel cancer in the second and third decades of life. However, there appears to be a different phenotypic expression within CMMR-D depending on the mutated MMR genes, we report on a case with CMMR-D caused by a biallelic PMS2 missense mutation and review the literature for evidence of 'extended' CMMR-D phenotype.

Materials and Methods: In addition to this case presentation we will investigate the literature for evidence of a genotype/phenotype correlation in CMMR-D based on MMR mutation type and age of diagnosis. Pooled analysis of published CMMR-D cases will be evaluated for age of diagnosis and mutation type using Fisher's exact test.

Results: We describe an 26 year old woman of Irish ancestry who presented with a rectal carcinoma and commenced neo-adjuvant chemotherapy. Following 2 cycles of FOLFOX, ovarian biopsy identified a poorly differentiated ovarian carcinoma. Immunohistochemistry (IHC) for mismatch repair protein expression completed on biopsy material demonstrated complete loss of staining of the MMR protein PMS2 and normal staining for MSH2, MLH1 and MSH6. MSI studies identified instability in three of the five mononucleotide repeats (BAT25, NR-21 and NR-27) assayed. Sequence analysis of exon 2 of the PMS2 gene identified a homozygous G to T base substitution at nucleotide position 137 (c.137G>T) resulting in the substitution of the amino acid serine for isoleucine at codon 46 p.(Ser46Ile). This missense change has been reported as pathogenic in the literature when seen heterozygously in Lynch syndrome patients. The proband's paternal uncle was diagnosed with CRC at age 36 and her paternal grandfather died from rectal cancer at age 60 and there were no cases of cancer reported on the maternal lineage. Co-segregation testing has confirmed that the proband's parents are unaffected carriers and that there is consanguinity in the family. The proband has one café-au-lait (CAL) spot.

Conclusion: Constitutional mismatch repair deficiency (CMMRD) is a distinct childhood cancer predisposition to mainly haematological, brain and intestinal tumours. Confirmation of a homozygous PMS2 mutation carrier with albeit synchronous Lynch syndrome tumours in a young adult suggests a genotype and phenotype that represents an intermediate between LS and CMMRD.

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Keywords: Constitutional mismatch repair-deficiency (CMMR-D) · Homozygous · Postmeiotic segregation increased 2 (PMS2)

180 Tumor development after colonic surgery in familial adenomatous polyposis

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Introduction: In familial adenomatous polyposis (FAP), prophylactic colonic surgery is considered the treatment of choice to avoid the development of colorectal cancer (CRC). An hereditary CRC registry allows to identify FAP associated tumors, which become the main cause of morbidity and mortality and impact the life expectancy of these patients.

Aim: To identify the FAP associated tumors in a cohort of patients with FAP being followed-up after colonic surgery.

Methods: We included all patients who underwent colectomy from June 1999 to June 2014 registered in the FAP data base. Demographic and clinical data was collected at the moment of surgery, and, from the following-up information, the mortality and the associated tumor diagnosis rate were analyzed.

Results: 27 patients from 23 families were identified. Mean age of surgery was 30.1 yo (i:8–68). During a mean period of 49.4 months, 17 gastrointestinal tract adenomas (63 %) were diagnosed, with a median appearance of 29 months (i:5–131). 11 patients had duodenal adenomas, 3 of them with ampullomas who required endoscopic ampullectomy and 2 patients with profused adenomas in the remnant rectum, who underwent proctectomy and ileal-pouch. The second most frequent diagnosis in this series was desmoid tumors, present in 8 patients (30 %), with a median appearance of 28 months (i:12–97). In 4 cases, the tumors were mesenteric, in 1 patient it was localized in the abdominal wall and 3 patients had tumors in both sites. Surgical resection was the treatment for 4 cases and 5 patients received chemotherapy with tamoxifen or vinorelbine or doxorubicin. One patient (4 %) also had a thyroid papilar tumor. Only 8/27 patients do not develop other tumors during the follow-up period. Only one patient died due to metastatic CRC.

Conclusion: Hereditary CRC registry and the follow-up of FAP allow an early diagnosis and treatment of associated tumors with an adequate long term survival.

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Keywords: Familal adenomatous polyposis · Hereditary colorectal cancer · Associated tumors

181 Study group on hereditary tumors—GETH—a South American initiative

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The Hereditary Colorectal Tumor Registry was organized in 1992 in Sao Paulo. After 11 years, in February 2003, the Brazilian Study Group on Hereditary Tumors—GBETH was founded. In 2005 and 2007 the group published two updated books on hereditary cancer. In 2007, with the participation of professionals from Argentina, Chile and Uruguay, the GBETH became the Study Group on Hereditary Tumors—GETH (www.geth.org.br). In 2006, the GETH organized the First International Symposium with the presence of several

international guests and more than 170 registered participants. Currently the GETH website has been one of the major tools for the integration of group members. The website has a restricted area for members, which allows entering the forum with thematic panels on hereditary cancer, as well as giving access to all meetings held from 2014, which have been recorded in video, as well as the newsletters written during the last years (+200). The GETH performs periodic clinical meetings with live broadcast on web streaming and simultaneous recording in high definition at Sirio-Libanes Hospital, in Sao Paulo. Live access can be made by streaming, via the WEB, through any computer or even mobile phone, in real time. The possibility of distant participation is fundamental in Brazil and South America.

The project in which the GETH is currently working on is to establish the South American Collaboration of Registries on Hereditary Cancer on a WEB platform. The idea is to use non-proprietary software to register the data of families with suspected hereditary predisposition to cancer. This tool is being built by the Engineering/Computer Science Department from the University of São Paulo (already in functional testing and security) with the goal of each project participant having individualized access to their own data, using login and password. Thus, the information from each institution is safeguarded against access by other participants in the collaboration. Even hierarchical access within the same institution can be achieved according to pre-determined decisions by the participants of the project itself. However, it is essential that all collaborators of the Registry have the same clinical and molecular data for possible future research or collaborations. Other benefits that the system offers are distant access via Internet from any computer or mobile phone, using a standardized data storage system to participants. On September 21, 2014, the South American Workshop of Hereditary Cancer—WSACH 2014 was held in Sao Paulo. The main objective of this meeting was to bring together the leaders from Brazil and South America in the area of Hereditary Cancer to a large panel in order to start the South American Collaboration of Registries on Hereditary Cancer. It was an invitation-only event, with 70 participants representing 35 different institutions/universities from all South America. We are at this moment beginning a forum on the WEB to establish the basis of this collaboration.

Keywords: Collaboration · Web based software · Database

182 Gastrointestinal tumors in families of pediatric patients with cancer

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Purpose: Identify the frequency of gastrointestinal tumors in families of children with cancer, relating them to the child's diagnosis or with other hereditary cancer predisposition syndromes and the need of diagnostic or preventive actions.

Methodology: We reviewed the charts of all patients seen from January 2012 to August 2014, as well as the patients database of the Oncogenetics Clinic at the Instituto de Oncologia Pediátrica-Grupo de Apoio ao Adolescente e à Criança com Câncer, São Paulo, Brazil.

Results: The charts of 180 families (201 patients) were reviewed. A conclusive diagnosis was not obtained in 53 families (26.8 %), and these were classified either as suspected malformation syndromes associated with cancer (26/13.1 %) or suspected familial/hereditary cancer (27/13.6 %). More than half of the families with a conclusive diagnosis had either neurofibromatosis (53/26.8 %) or retinoblastoma

(49/24.7 %). Only 24.5 % of the families with neurofibromatosis had history of gastrointestinal tumors, and just 01 family raised the suspicion of a concurrent cancer predisposition syndrome, the others seemed to have sporadic colorectal cancer not related to a predisposition syndrome or to neurofibromatosis. Among the families with retinoblastoma, 36.7 % had a positive history of gastrointestinal cancer, but, when selecting just families with confirmed hereditary retinoblastoma (33), 39.4 % had history of gastrointestinal tumors. Since an excess risk of non-ocular cancers were seen in these families, a proportion of the affected relatives could be in fact carriers of the mutated retinoblastoma gene.

Among the 180 families, gastrointestinal tumors were found in 57 (31.7 %). In 19 of these 57 families (33 %), the presence of gastrointestinal tumors among relatives prompts an investigation of a specific hereditary predisposition syndrome or adoption of cancer preventive measurements. Ten of the 19 families had a suspicion of colorectal predisposition syndrome, 7 of Li-Fraumeni syndrome, 01 of Multiple Endocrine Neoplasia and 01 of BRCA-related cancer predisposition syndrome (these last two in families with pancreatic cancer). In 13 families, gastrointestinal tumors were associated with the child's syndromic diagnosis.

Conclusions: The presence of gastrointestinal tumors among relatives of children with cancer may help the identification of a specific tumor predisposition syndrome. It also allow the recognition of concurrent hereditary cancer risks, not related to the child's diagnosis, but meaningful to family members, resulting in actionable knowledge that can prompt preventive care measures.

Keywords: Hereditary cancer predisposition · Pediatric cancer · Gastrointestinal tumors

183 Serrated polyposis. Diagnosis and management

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Purpose: Hyperplastic or serrated polyposis is a rare syndrome of colorectal cancer predisposition, three distinct subtypes of serrated lesions are include like hyperplastic (70 % of all serrated polyps), sessile serrated adenoma/polyp (SSA/P) (25 %) and traditional serrated adenoma (<2 %). Patterns of inheritance of are not obvious and the clinical definition is relatively arbitrary. Colorectal cancer prevalence is around 0 a 50 % (1–5). The aim of our study is to analyze prevalence of colorectal cancer, clinical characteristics, motive of diagnosis and management of these patients.

Methodology: Between January 2009 and November 2014 the Polyposis Registry incorporated 17 affected patients with Hyperplastic/Serrated polyposis, all of them index cases, they belong to 17 families. We analyzed retrospectively their demographic characteristics, age at cancer diagnosis, prospective preoperative or retrospective postoperative diagnosis, upper gastrointestinal polyposis associated as extra-intestinal manifestations, endoscopic and surgery treatment, pathological Stage (S), and prevalence of high risk adenomas associated and colorectal adenocarcinomas. Data were obtained from the Registry data base. Descriptive retrospective observational study.

Results: We evaluated 17 serrated polyposis patients, all of them index cases, nine were male (64.2 %), mean age was 48.9 years ranging from 28 to 71; one patient (5.88 %) had duodenal polyps in assessment by the multidisciplinary team. The diagnosis was done in prospective form before surgery in 11 patients (64.7 %) through the

colonoscopy assessment and in retrospective form after surgery in six (35.2 %) by the histopathologic study of the surgical specimen. The mean age at cancer diagnosis was 60.5 years ranging from 54 to 71. The surgical procedures were four total colectomy and ileorectal anastomosis and two proctocolectomy with ileal pouch and ileoanal anastomosis. We found four patient with adenocarcinoma (23.52 %) two localized in colon and two in rectum. There were two cases Stage I (T1 N0 M0), one Stage II (T3 N0 M0), one Stage III (ypT2 ypN1 ypM0). High risk adenomas were found in two of the adenocarcinoma cases (50 %). The management of the non operated 11 patients was done through endoscopic polyp resection and they will stay in a surveillance endoscopic protocol.

Conclusion: The patients at polyps or colorectals cancer diagnosis were older than in classical adenomatous polyposis. In our Registry the first four patients in this serie were diagnosed in retrospective postoperative form because they were initially attended with a colorectal cancer developed. Only one patient had an extra-intestinal manifestation associated. The serrated polyposis may be accuracy managed by endoscopic therapy in patients without colorectal cancer.

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Keywords: Serrated polyposis · Colorectal cancer · Diagnosis management

184 Implications of genetic testing for adenomatous polyposis syndrome in Japan

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Purpose: We have carried out genetic testing of the APC gene for 132 families with familial adenomatous polyposis (FAP) in Japan. The rate of detection of APC germline mutation using PCR-DNA sequencing method was approximately 70 percent. Moreover, some FAP-families have a recessive inheritance manner. Alterations of the MUTYH gene, whose product is a group of base excision repair (BER) enzymes, caused some FAP-patients, especially cases with attenuated form. We studied the relationship between alterations of the APC gene or the MUTYH gene and adenomatous polyposis.

Methodology: We analyzed the total coding region of the APC gene. In next, we analyzed the total coding region of the MUTYH gene. Furthermore, we adopted Multiplex Ligation-dependent Probe

Amplification (MLPA) method to detect the wide-range genomic abnormalities.

Results: In families with adenomatous polyposis syndrome, 95 families (72.0 %) were detected deleterious mutations of the APC gene. 5 families (3.8 %) carried biallelic mutations of the MUTYH gene. Then, MLPA method clarified the alterations of the six families, 5 in APC gene and one in MUTYH gene respectively. The detection rate of alterations improved to 106 of 132 families (80.3 %) in our laboratory.

Conclusion: We were able to improve the precision of the genetic testing. It was clarified that alterations of the MUTYH gene are related to a cause of tumorigenesis of multiple colorectal tumors as well as those of the APC gene. Each family with adenomatous polyposis syndrome has an autosomal inheritance manner or an autosomal recessive inheritance manner. We think that improvement of the genetic testing for adenomatous polyposis syndrome is necessary for the reliable genetic counseling service and the appropriate management.

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Keywords: Familial adenomatous polyposis · APC · MUTYH

185 Frequency and management of duodenal adenomas in patients with familial adenomatous polyposis

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Purpose: Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited disorder, which results from a germ line mutation in the adenomatous polyposis coli (APC) gene. Many studies have shown that adenomas in the duodenum can be found in 50–90 % of FAP cases. (1) The risk of developing duodenal cancer is relatively low (5 %) and appears to be related to the Spigelman stage, but it is considerably high in relation to general population. (2) The options of

treatment are pharmacological, endoscopic and surgical therapies. The surgical treatments include local surgical procedures (duodenotomy with polypectomy and/or ampullectomy), pancreas-sparing duodenectomy and (pylorus-sparing) pancreaticoduodenectomy (Whipple's procedure). (3) The objective of our paper is to estimate the prevalence, the results of endoscopic surveillance and to show the different treatment modalities of duodenal adenomatosis in patients with FAP.

Methodology: Between January 1975 and November 2014 the Polyposis Registry has 2094 individuals including affected patients and their relatives, 715 FAP cases, 650 classical FAP (90 %) and 45 attenuated form (10 %); they belong to 337 families. We analyzed retrospectively the number of gastroduodenoscopy, duodenal affected patients, their demographic characteristics, Spigelman stage (S) distribution, medical and surgery treatment in the different stages and specific choice of procedure, prevalence of adenocarcinoma. Data were obtained from patients who had undergone a gastroduodenoscopy consulting the Registry data base. The endoscopic and histological findings were used to classify the duodenal adenomas according to the Spigelman classification.

Results: We evaluated 286 patients with gastroduodenoscopy, 136 (47.55 %) were done in index case patients and 131 (45.8 %) in relatives called patients, 20 cases (0.7 %) were controlled in other centers. Duodenal polyps were found in 99 cases (34.6 %), 42 male patients (14.7 %), mean age was 39.7 years ranging from 16 to 70. We found 31 cases (10.8 %) in Spigelman stage 0, 33 Stage I (11.5 %), 15 Stage II (5.3 %), two stage III (0.7 %) and nine (3.1 %) stage IV. There were nine cases without initial classification. The management of duodenal polyposis in Stage 0-I-II was clinical control and the interval for upper gastrointestinal endoscopy was in relation to Spigelman classification. Celecoxib was used in six cases (two in S I, one in S II, three in S IV) 800 mg. per day during 1 year, without changes in S after cancelling the drug. A patient with duodenal disease Spigelman III is actually programing an elective surgery. Thirteen patients were operated, nine S IV and four with no registered initial S (they were derivatived from another institution). In S IV cases were done: one segmental duodenal resection, eight pancreaticoduodenectomy, two of them had an ampullectomy and resection of duodenal polyp previously. Five cases of prophylactic pancreaticoduodenectomy were done in our Institution and there was only one case of duodenal adenocarcinoma and it was Stage I (T1 N0 M0). In the nine initial S no registered patients were done four surgical procedures: two segmental duodenal resection, an ampullectomy and one pancreaticoduodenectomy.

Conclusion: Current screening protocols of the upper gastrointestinal tract usually detect duodenal disease at a premalignant stage. Surgical procedures are the main treatment options for patients with Spigelman IV stage. In our patients there was only one case of adenocarcinoma which was found after a prophylactic surgery in the surgical specimens study.

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Keywords: Duodenal polyps · Familial adenomatous polyposis

186 Characterization of a population with suspected Lynch syndrome in a university cancer center in Sao Paulo

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Purpose: Lynch Syndrome (LS) is due to germline mutations in the DNA mismatch repair (MMR) genes, most commonly MLH1 and MSH2, but also MSH6 and PMS2, and more recently in the EpCAM gene. In addition to a high lifetime risk of colon cancer, LS is characterized by an early age at diagnosis of colon cancer, a preponderance of right-sided tumors, susceptibility to multiple primary cancers (mainly endometrial), and by peculiar phenotypic and genotypic features, such as microsatellite instability and hypermethylation of the MLH1 gene promoter. The best cost-effective strategies to screen LS families have been advocated to test all colorectal tumors with immunohistochemistry of four MMR proteins plus BRAF V600E mutation testing and/or MLH1 hypermethylation testing. The identification of patients with Lynch syndrome is important to plan the follow up of cancer patients and parents and relatives at cancer risk. We evaluated the patients attended at the hereditary tumors clinic (HTC) to better understand the patients profile in a university cancer hospital.

Methodology: We conducted a retrospective analysis of all medical records from January/2010 to October/2014. The following variables were evaluated: age, sex, primary site, treatment performed, initial stage, secondary neoplasia, immunohistochemistry, follow-up examinations, Bethesda and Amsterdam scores, time from 1st consultation in ICESP up consultation in HTC. We considered immunohistochemistry suggestive of LS: absence MHL1 of expression without BRAF mutation and absence of MSH2 expression and/or MSH6 and PMS2. There were also included all first-degree relatives of index cases that are observed in our service.

Results: 106 patients suspected of LS were treated at our HTC since 2010, 53 (50.5 %) male. 66 of them were patients in cancer treatment or follow-up, while 39 were relatives of patients with Lynch syndrome. Right colon was the most common primary site (46 %), followed by left colon (23 %), rectum (14 %), endometrial (8.6 %), transverse colon (5.8 %) and urotelial cancer (1.44 %). Among patients with colorectal cancer, 52.5 % were Stage II, 35.6 % Stage III, 8.4 % stage IV. 62.7 % of them were treated with adjuvant chemotherapy. MSH6 protein was absent in 37 patients, MSH2 protein in 30, MLH1 protein in 20 patients (everyone wild-type BRAF) and PMS2 protein was absent in 13 patients. During follow-up, 15 patients were diagnosed with second cancer. The right colon was the most common site (33.3 %), followed by urotelial cancer (26.6 %). The youngest patient with cancer was 23 years old, and the median age was 32. 16 patients were diagnosed with adenomatous polyps during follow-up, 2 with in situ adenocarcinoma and 5 with invasive cancer. No relatives underwent prophylactic surgery, while four patients that had primary colorectal tumor did prophylactic hysterectomy. The mean time between the first appointment to cancer treatment and the first appointment in HTC was 439 days.

Conclusion: These data demonstrate the considerable number of patients possibly affected by Lynch syndrome, which if properly found may lead to identification of the mutation carriers in need of proper monitoring aiming to prevent cancer in these families. Further,

demonstrate the importance of molecular tests for proof of correct diagnosis and identification of family carriers. Important to note that despite being within a service specialized in oncology the average time to be referred to our HTC was over 1 year, demonstrating the lack of attention given yet to genetic counseling by medical oncologists and surgeons.

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Keywords: Lynch syndrome · screening · Brazilian

187 Mutation spectrum in South American Lynch syndrome families

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Background: Genetic counselling and testing for Lynch syndrome have recently been introduced in several South American countries, though yet not available in the public health care system. Methods: We compiled data from publications and hereditary cancer registries to characterize the Lynch syndrome mutation spectrum in South America. In total, data from 267 families that fulfilled the Amsterdam criteria and/or the Bethesda guidelines from Argentina, Brazil, Chile, Colombia and Uruguay were included.

Results: Disease-predisposing mutations were identified in 37 % of the families and affected MLH1 in 60 % and MSH2 in 40 %. Half of the mutations have not previously been reported and potential founder effects were identified in Brazil and in Colombia.

Conclusion: The South American Lynch syndrome mutation spectrum includes multiple new mutations, identifies potential founder effects and is useful for future development of genetic testing in this continent.

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Keywords: Lynch syndrome · South America · Mutation

188 High prevalence of Li-Fraumeni syndrome in South and Southeastern Brazil due to a founder mutation

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Li-Fraumeni Syndrome (LFS; OMIM # 151623) is an autosomal dominant syndrome that predisposes to a larger spectrum of tumors in pediatric and adult population most notably breast cancer, sarcoma, brain tumors, adrenocortical carcinoma, and colorectal cancer. The molecular basis of LFS is the presence of pathogenic germline mutations in TP53 tumor suppressor. Carriers have a 50 % risk to develop cancer before the age of 40 and the lifetime risk of cancer in germline TP53 mutation carriers is 90 % by the age of 60. Recent studies in cancer-prone families of South and Southeastern Brazil have identified a founder germline TP53 mutation (c.1010G>A, p.R337H) at an unusually high prevalence of about 1–300 subjects (0.3 %). This mutation occurs in the oligomerization domain and has lower penetrance than the majority of TP53 germline mutation presented in DNA binding domain of TP53 gene. Moreover, due to genetic modifiers in p.R337H carriers, tumors tend to occur at a later age compared to other TP53 mutation carriers and a variety of neoplasias not included in LFS core tumors are also observed in this population. A high prevalence of TP53 mutation carriers related to the founder mutation in South and Southeastern Brazil become a public health issue. Efforts have been implemented by the Cancer National Network in order to prepare the health care professionals for the identification of carriers, referral patients to genetic counseling, molecular tests and propose strategies of screening for Brazilian TP53 carriers in order to make early diagnosis and reduce the need of long therapies and mortality related to cancer.

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Keywords: Li Fraumeni · TP53 · R337H

189 Profile of gastrointestinal tumors in a large cancer genetics clinic in Brazil

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Profile of gastrointestinal tumors in a large Cancer Genetics Clinic in Brazil

A total of more than 2000 patients are seen each year at the Oncogenetic Department of AC Camargo Cancer Center, Sao Paulo, Brazil. The Oncogenetic clinic, which covers the full range of hereditary cancer syndromes, has grown from serving about 350 patients in 2000 to more than 2000 patients annually in 2013. The Oncogenetic clinic offers a comprehensive genetic counseling with a multidisciplinary team (physicians, clinical geneticist, nurse and psychologist) for many hereditary cancer syndromes. Considering the high volume of patients seen, a wide variety of gastrointestinal tumors is observed. More than 50 % of the patients have Hereditary breast-ovarian cancer syndrome (HBOC) or Li-Fraumeni syndrome (LFS), both syndromes with predisposition to gastrointestinal tumors: pancreatic and gastric cancer related to BRCA2 germline mutation in HBOC and a large profile of gastrointestinal tumors in LFS. Lynch syndrome is observed in 15 % of the patients and Familial adenomatous polyposis (FAP) in 7 %. Hereditary diffuse gastric syndrome is present in 1 % of the patients; less frequent syndromes as Hereditary paraganglioma-pheochromocytoma syndrome (PGL/PCC), in which the carriers are at elevated risk of development of GIST, Familial melanoma/pancreas cancer linked to germline CDKN2A mutations and other hereditary polyposis are also observed.

The Oncogenetic Department offers an interdisciplinary approach to the diagnosis, treatment and management of patients and their families with hereditary cancer syndromes. For asymptomatic carriers, follow up is planned according to international guidelines for specific hereditary cancer syndrome.

190 Yearly gastroscopy in MLH1 and MSH2 mutation carriers—an endoscopy too far?

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Purpose: The cumulative risk of gastric cancer in Lynch Syndrome is reported to be 0.2–13 % by the age 70 years (yrs). Family history of gastric cancer is also a poor predictor of risk². Consideration of surveillance gastroscopy in Lynch Syndrome, particularly in MLH1 and MSH2 carriers¹, has been proposed². In May 2011 the New Zealand Familial Gastrointestinal Cancer Service (NZFGICS) made the recommendation that yearly gastroscopy be considered in individuals with an MLH1 or MSH2 mismatch repair gene mutation. To determine the appropriateness of this recommendation in New Zealand, where the endoscopy resource is constrained, an audit of the outcome of surveillance gastroscopy was proposed. A secondary aim, to contextualize the audit, was to identify any confirmed upper gastrointestinal (GI) or small bowel malignancy in these mutation carriers.

Methodology: The NZFGICS progeny database was searched to identify MLH1 and MSH2 mutation carriers (excluding EPCAM) who had been referred for a surveillance gastroscopy after 01/5/2011

and were identified to have (1) malignant or significant upper GI pathology at a surveillance gastroscopy (2) any confirmed malignant upper GI or small bowel pathology. The search included procedures and pathology reported to 01/11/2014.

Results: The NZFGICS database identified 126 MLH1 (66 female) and 152 MSH2 (85 female) mutation carriers. Since 1/5/2011 475 referrals for consideration of gastroscopy were made in 225 living carriers who had consented to be part of the service surveillance programme. Three hundred and twenty gastroscopies were performed in 177 individuals. A first surveillance gastroscopy identified short segment Barrett's oesophagus in a 70 year old male MSH2 mutation carrier and at a further surveillance gastroscopy performed 15 months later, in association with colonoscopy, a 1.5 cm nodular gastro-oesophageal junction adenocarcinoma was identified. A 60 year old female MSH2 mutation carrier was identified at the first surveillance procedure in November 2011 to have a 3 cm duodenal cap cancer. Both underwent curative surgical resection. In addition to these surveillance identified upper GI cancers, symptomatic assessment (before surveillance gastroscopy was introduced) identified two MLH1 mutation carriers with gastric adenocarcinoma at ages 34 and 45 years, four small bowel cancers (mean age 51 years), a duodenal cancer age 60 years and an oesophageal squamous cell carcinoma age 83 years. Two jejunal cancers (mean age 59 years) presented symptomatically after the introduction of surveillance gastroscopy.

Conclusion: In our service 160 gastroscopies in MLH1 and MSH2 mutation carriers were needed to identify one resectable upper GI cancer. Yearly surveillance gastroscopy in NZ may be too frequent and the more recent recommendation¹ of surveillance gastroscopy every 2–3 years, based on patient risk factors, from the age of 30–35 years, may be more appropriate.

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Keywords: Gastroscopy · Surveillance · Lynch

192 Frequency of CDH1 germline mutations in early-onset gastric cancer in Brazil

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Purpose: To examine the frequency of CDH1 germline mutations in a population-based series of early-onset gastric cancer (EOGC, <50 years old) in Brazil, which is considered a high-incidence country for gastric cancer.

Methodology: From October 2013 to October 2014 a total of 51 unrelated and consecutive patients attending a Brazilian public hospital with EOGC were enrolled and all CDH1 exons and intronic boundaries were sequenced. Clinico-pathological features were

extracted from electronic medical records. All patients signed informed consent before study registration to have blood specimens drawn and analyzed.

Results: Of 51 patients, 59 % were female and the mean age at gastric cancer diagnosis was 38 years (range 21–50); 22 % reported family history of gastric cancer in first- or second-degree relatives. The majority of the tumors were diffuse (82 %), poorly differentiated (76 %), and located in the middle and distal-third of the stomach (59 %). One germline deleterious mutation (c.1849G>A, p.A617T) was identified in 2 unrelated female patients with diffuse EOGC (42 and 47 years) and without family history of gastric cancer. The overall frequency of germline CDH1 mutations was 3.9 % (2/51) for EOGC.

Conclusion: To our knowledge, this is the largest population-based study investigating the contribution of CDH1 germline mutations in early onset gastric cancer in Brazil. For a high-incidence country for gastric cancer, mutation frequency was higher than expected [1,2]. This finding warrants further validation in larger cohort studies.

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Keywords: e-Cadherin · Hereditary diffuse gastric cancer · CDH1

193 Controlling polyposis with colonoscopy: an update

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Introduction: Patients with oligopolyposis (<100 polyps) are often recommended to have a prophylactic colectomy. There is an alternative: colonoscopic control of the polyps. This can be difficult as there are sometimes many polyps and sometimes large flat lesions. We have been controlling polyposis with colonoscopy in many patients for several years and here we report our latest data.

Methods: Any patient with a hereditary colorectal cancer syndrome, or with more than 10 and less than 100 synchronous adenomas, and who had at least three colonoscopies, was eligible for the study. A single endoscopist colonoscopy database was searched and suitable patients added to the study. Their entire colonoscopy experience was summarized by abstracting the number of polyps on their first examination, the number on their last examination, the size of the largest polyp on the first and last examination, and the total number of polyps removed. Results were stratified according to syndrome.

Results: There were 42 patients, mean age 64, including 11 with MYH associated polyposis (MAP)(age 58), 14 with serrated polyposis (age 65), 7 with non specific oligopolyposis (age 67), 5 with PTEN mutation (age 41), 2 with attenuated familial adenomatous polyposis (age 65), 1 with Lynch syndrome, and 2 with hereditary mixed polyposis (age 73). Patients had a mean of 5.2 colonoscopies each over a mean of 6.6 years follow-up. The average number of polyps on first colonoscopy was 27 compared to 8 at the most recent. The average overall size of the largest polyp at the first examination was 24 mm compared to 11 mm for the largest polyp at the most recent examination. Overall average total number of polyps removed was

47. There were no cancers. MAP patients had a mean of 5.8 colonoscopies each over a mean of 4.9 years follow-up. The average number of polyps on first colonoscopy was 53 compared to 14 at the most recent. The average overall size of the largest polyp at the first examination was 22 mm compared to 10 mm for the largest polyp at the most recent examination. Overall average total number of polyps removed was 62. Serrated polyposis patients had a mean of 4.5 colonoscopies each over a mean of 6.7 years follow-up. The average number of polyps on first colonoscopy was 19 compared to 6 at the most recent. The average overall size of the largest polyp at the first examination was 29 mm compared to 12 mm for the largest polyp at the most recent examination. Overall average total number of polyps removed was 37.

Conclusion: Patients with oligopolyposis can be managed by yearly colonoscopy, at least over the short term. This saves patients the hazards and sequelae of surgery.

Keywords: Oligopolyposis · Colonoscopy · Polypectomy

194 Analysis of the susceptibility to colorectal cancer in Brazilian individuals through genotyping known single nucleotide polymorphisms: a replication study

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Background: Colorectal cancer (CRC) is one of the most prevalent cancers worldwide, the third most common cancer in men and the second one in women, and the fourth leading cause of all cancer deaths. CRC incidence rates have been increasing specially in developing countries, such as Brazil, where is the third most frequent cancer in both genders. Etiology of CRC is multifactorial, both environmental and genetic risk factors interacting with each other, of whom family history plays a special role. CRC heritability is about 35 % and Mendelian syndromes respond for 6 % of the cases. Recently, genome-wide association studies (GWAS) have showed that part of the risk is due to common low penetrant variants, such as single nucleotide polymorphisms (SNPs). Approximately 20 SNPs have been discovered through GWAS from European-descent populations, each one with modest size effects on the CRC risk, but collectively, make great part of populational risk. Non-European replication studies have not achieved enough power to detect significant association.

Aims: to identify ten SNPs previously detected in European populations in the Brazilian population; to calculate allelic and genotypic frequencies of the ten SNPs in cases and controls; to detect effect sizes of the risk alleles and correlate risk with clinical pathological characteristics and family history.

Methods: 1467 individuals (727 cases and 740 controls) were included. Ten SNPs were genotyped (rs6983267, rs4939827, rs4779584, rs16892766, rs10795668, rs4444235, rs9929218, rs10411210, rs961253, rs3802842).

Results: 51 % of cases were male, with mean age at diagnosis of 57 years-old; 30 % fulfilled Bethesda criteria; 3 % were advanced adenomas; rectal and stage III tumors were most frequent at diagnosis, with pericolic/perirectal invasion and without distant metastasis; grade 2 differentiated tubular tumors predominated. The majority of patients were alive and healthy and about one third had no CRC family history; 52 % of controls were female with mean age of 52 years-old. Half of the ten SNPs (rs6983267, rs4939827, rs4779584, rs961253, rs3802842) significantly associated with CRC risk after correction for multiple tests in most genetic models, whereas two tended to be associated (rs10795668 and rs10411210). rs6983267 had the most significant association, strongest statistical

power and greater effect size on CRC risk. rs4939827 was the only one to be protective. Lack of association among rs16892766, rs4444235 and rs9929218 was most likely due to insufficient power by the small sample size. Correction for eventual false positives through ancestry stratification was not performed, although it is necessary for admixed populations. However, risk allele frequencies did not significantly differ from European as their effects were similarly small. Genotype-phenotype correlations showed rs6983267 as a good prognostic factor and rs961253 as associated with revised Bethesda criteria. Nevertheless, clinical application of these factors is limited by the lack of prospective studies.

Conclusion: This study partially replicated European GWAS and reinforced the need to stratify the Brazilian population.

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Keywords: Susceptibility · SNP · Colorectal cancer

197 Mutation spectrum and risk of cancer in African American families with Lynch syndrome

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Purpose: African Americans (AA) have the highest colorectal cancer (CRC) incidence and mortality of all US populations [1]. There is paucity of data on CRC genetic risk factors among AA. Specifically, no studies have determined cancer risks and mismatch repair (MMR) gene mutation spectrum in AA with the most common inherited CRC syndrome, Lynch syndrome (LS), and the contribution of this

syndrome to cancer disparities [2]. The aim of this study is to characterize phenotype, mutation spectrum, and risks of cancers in AA with LS.

Methodology: AA with a deleterious mutations or variants of unknown significance (VUS) in MMR genes from 13 US referral centers were analyzed for personal and familial cancer histories. Modified segregation analysis was used to calculate age- and sex-specific cancer incidence for AA with deleterious mismatch repair (MMR) gene mutations.

Results: Fifty-seven unrelated AA families were identified of which 50 had deleterious mutations [30 MLH1 (60 %), 11 MSH2 (22 %), 3 MSH6 (6 %), and 6 PMS2 (12 %)] and 7 had a VUS. Eight recurrent mutations accounted for 38 % of all deleterious mutations and 12 novel mutations were identified in MMR genes. Of 911 relatives (462 males and 449 females) in 50 AA families with deleterious mutations, the cumulative risk of CRC at the age of 80 years was estimated to be 36.2 % (95 % CI, 10.6–83.7 %) and 29.7 % (95 % CI, 8.39–75.9 %) for male and female carriers, respectively. CRC risk was significantly elevated for individuals with mutations in MLH1 or MSH2 [HR 13.9 (95 % CI, 3.44–56.6)] and those less than age 50 [HR 25.1 (95 % CI, 1.76–358)].

Conclusion: This is the largest series of AA families with LS reported to date. Our estimates of CRC cumulative risk for AA mutation carriers overlap with those in mutation carriers of European descent [3]. Almost two-thirds of mutations were found in MLH1 including recurrent and novel mutations. Differences observed in the mutation spectrum likely reflect genetic diversity in this population. Efforts to increase identification of LS in AA are needed.

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Keywords: African American ancestry · Colorectal cancer · Lynch syndrome

198 Better education is needed for both HNPCC family members and their providers

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Background: Lynch Syndrome accounts for 2–4 % of all colorectal cancers (CRC). Colonoscopic screening is recommended for known Lynch mutation carriers as well as in patients who meet the Amsterdam II criteria for Hereditary Non-Polyposis Colorectal Cancer (HNPCC). Although aggressive colonoscopic screening can

reduce CRC incidence and overall mortality, most patients with Lynch syndrome are not adequately screened.

Goal: To compare the knowledge of CRC screening guidelines between members of families that meet clinical criteria for HNPCC and their treating endoscopists and to assess the rates of genetic counseling and testing.

Methods: The Family Health Promotion Project (FHPP) was a randomized controlled trial of a telephone-based educational and barriers counseling intervention to promote colonoscopy screening in members of high-risk families [1]. Of the 632 FHPP participants, 165 were from families who met Amsterdam II criteria for HNPCC; for this group the telephone intervention included specific recommendations for colonoscopic screening every 1–2 years. The HNPCC participants were surveyed regarding their knowledge of recommended screening guidelines and their attitudes/beliefs towards genetic testing at baseline, 6, 12 and 24 months after the intervention. Colonoscopy and pathology reports as well as the endoscopist's follow-up recommendations were obtained for those participants who underwent colonoscopy during the study period. Participants were sent a supplemental questionnaire after completion of the 24 month study period querying details of whether genetic testing was performed and how it was handled by providers.

Results: The FHPP intervention increased colonoscopy screening by 10 % in the 165 HNPCC participants [2], 95 of whom underwent colonoscopy during the 2 year study period. Only 26 % of participants reported that they thought they should have colonoscopy every 1–2 years at the end of the study (24 m) and only 30 % of their endoscopists recommended a 1–2 year follow up colonoscopy. Based on the colonoscopy reports, only 20 % (n = 17) listed Lynch Syndrome or HNPCC as the primary indication for the procedure but 15 (88 %) of these recommended a 1–2 year surveillance interval. There was a 65 % concordance between endoscopist recommendations and participant reports regarding screening intervals and this was not substantially impacted by the telephone-based intervention. Of the 165 HNPCC participants, 91 (55 %) completed the supplemental questionnaire; only 33 % (30) of the respondents reported having ever been advised to undergo genetic testing, only 24 % (n = 22) had discussed genetic testing with their physicians, and only 21 % (n = 19) reported having undergone genetic testing, eight reported testing positive for a genetic mutation and six reported that they did not know the results of their genetic testing.

Conclusions: Unaffected members of families that meet Amsterdam II criteria for HNPCC have a suboptimal knowledge of colonoscopy screening guidelines and only a minority (26 %) of these participants reported having undergone a formal genetic evaluation. Only a small minority of endoscopists recognized that their patients had HNPCC or gave them appropriate screening recommendations. The high concordance between endoscopist recommendations and participant's knowledge of screening intervals despite an intervention promoting colonoscopic screening, suggests that educational interventions for healthcare providers as well as patients are critically important to improve identification of and proper screening for members of HNPCC families.

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Keywords: Hereditary non-polyposis colorectal cancer · Genetic testing · Screening

199 Experience with pancreas-sparing duodenectomy for familial adenomatous polyposis

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Purpose: Duodenal adenomas are a common finding in patients with familial adenomatous polyposis (FAP), being seen in up to 90 % of adults with the condition. Individuals with Spiegelman stage IV adenomas [1] are at high risk of developing duodenal carcinoma [2] that is the leading cause of death in FAP that have undergone colectomy [3]. These patients are traditionally treated by pancreatoduodenectomy (PD) though an alternate approach is pancreas-sparing duodenectomy (PSD) [4]. We report present a 22-year experience with PSD for the treatment of duodenal polyps in FAP.

Methodology: The departmental prospectively maintained database containing all patients undergoing PSD from 1992 to 2013 was interrogated. Data analyzed included demographic features, perioperative management, histopathological findings, and outcome. Phone interviews were conducted to confirm current status of patient at follow-up.

Results: Fifty-four patients underwent PSD during the study period, all for Spiegelman stage IV polyps. An unsuspected invasive cancer was found in one patient on final pathology. The mean operative time was 305 ± 70 min with a mean blood loss of 300 ± 170 mL. There was one peri-operative mortality, unrelated to the operative procedure. Thirteen patients (24 %) had an immediate post-operative complication including eight (15 %) biliary/pancreatic leaks, and 1 (2 %) enteric anastomotic leak. Pancreatitis was observed in 4 (10 %). 42 (78 %) of patients were available for follow-up. Recurrent polyps were found in 16 (34 %). Of these, only 3 (19 %) patients required operative intervention, two proximal jejunal resections and one PD for development of a polyp at the ampullary anastomosis.

Conclusion: Our experience with PSD reinforces its value as a definitive prophylactic procedure for duodenal polyposis in FAP and allows for full preservation of pancreatic function.

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Keywords: Familial adenomatous polyposis · Duodenal adenoma · Pancreas sparing duodenectomy

200 Increasing incidence of colorectal cancer (CRC) among young adults in the U.S. challenges insight and current epidemiologic tools to explain and reverse the trend

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Purpose: The Incidence of colorectal cancer (CRC) in the United States in individuals aged 50 and older is rapidly decreasing, dropping 3.9 % per year for the decade 2001–2010 for total drop of 30 % (RS). In marked contrast, recent literature details significant increases in the incidence of CRC among young adults (YA) aged 50 and younger for the same decade. (1&2) Effective strategies to reverse this trend require an improved understanding of its causes. To pursue this understanding we: 1 Reviewed current literature to define the characteristics of this increasing incidence including the contribution of known CRC predisposition alleles to the trend. 2. Analyzed current ascertainment practices and data fields of US population based cancer registries.

Methodology: Under expert guidance we searched PUBMED for current literature pertaining to YA CRC. Multiple search terms were modeled and an optimal strategy defined. Separately we conducted a detailed analysis of official descriptors of the U.S. Centers for Disease Control National Program of Cancer Registries (NPCR) (<http://www.cdc.gov/cancer/npcr>) and the NCI Surveillance and End Result Program (<http://seer.cancer.gov>).

Results: Multiple permutations of the search terms “Population Based”, “Young Adult” and “Early Age Onset” Colorectal Cancer yielded 1309 results for detailed review. Eight of 1309 articles (0.06 %) provided population based analysis of YA CRC incidence trends in the US documenting a 20 % increase for colon cancer (1998–2007) and a staggering 75 % increase for rectal cancer (1973–2007). Importantly, one study reported 86 % of YA CRC patients were symptomatic at diagnosis. Three independent papers estimated the contribution of familial/hereditary CRC to overall YA CRC incidence in US to be 20–22 %. Review of the CDC SEER and NCI National Cancer Registries documented population based incidence trends consistent with those reported above. However, neither NPCR or the SEER registries “collect information about risk factors”. No information is available from current US population based cancer registries regarding family history, obesity, activity, diabetes, tobacco or alcohol consumption.

Conclusions: These results confirm well referenced population based evidence of a significant increase in colorectal cancer in the US among young adults. Current US population based cancer registries are neither designed or resourced to accrue basic information on CRC risk factors, including family history, obesity, diet, tobacco and alcohol use. Over 99 % of the 1309 papers reviewed do not have a population based approach and virtually all reported etiologic laboratory efforts focused exclusively on defining the percentage of Lynch syndrome cases; the minority of cases by far. Progress in reversing YA CRC incidence and mortality will require significant redesign of and investment in epidemiologic tools as well as the deployment of considerable intellectual resources. Therefore we believe our results challenge the InSiGHT community to expand their scientific focus to include the vast majority of YA CRC cases which do NOT present with family history or other evidence of known hereditary CRC syndromes.

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Keywords: Young adult · Colorectal cancer · Epidemiology

201 Results of high/moderate cancer gene panel tests in an ethnically diverse patient population

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Purpose and Background: Kaiser Permanente Southern California (KPSC) provides health care services in an integrated health care system serving 3.8 million health plan members at 14 medical centers. Since the summer of 2014 our licensed genetic counselors and clinical geneticists have been offering testing for inherited cancer susceptibility via a High/Moderate Risk cancer gene panel when the patient’s clinical presentation suggests the possibility of more than one cancer syndrome. The panel includes 20 cancer susceptibility genes: APC, ATM, BMPR1, BRCA1, BRCA2, CDH1, CDKN2, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, PTEN, SMAD4, STK11, TP53, and VHL. All tests were performed by the same laboratory using next generation sequencing (NGS) for sequencing and exon-level array CGH or MLPA for deletion/duplication testing. Results are recorded in our department’s genetic testing database and in the patient’s electronic medical record.

Methods: We report our results for the first 314 patients who were tested via the cancer gene panel described above. Patient demographics: The patients tested ranged from 22 to 81 years of age; there were 294 females and 20 males. Race/Ethnicity: Latino/Hispanic = 90 (28.7 %), Western/Northern European = 85 (27 %), Asian = 37 (11.8 %), African American = 20 (6.4 %), Ashkenazi Jewish = 16 (5 %), Native American = 14 (4.5 %), and Other/Unknown = 53 (16.6 %). 213/314 patients tested had both a personal and family history of cancer, 77 only had a family history of cancer, 20 had only a personal history of cancer.

Results: Results of our first 314 high/moderate risk cancer gene panel are as follows: No mutation was detected in 167/314 patients (53 %), at least one variant of unknown clinical significance (VUS) was detected in 103 patients (32 %) and at least one pathogenic mutation was detected in 45 patients (14.3 %). Variants in ATM (21), APC (16) and CHEK2 (9) made up 45 % of all variants detected. We detected a total of 47 pathogenic mutations in the following genes: BRCA2 = 12, BRCA1 = 8, MUTYH = 8 (all heterozygous), CHEK2 = 5, ATM = 4, MLH1 = 2, PALB2, and one mutation in each of the following genes: APC, BMPR1A, CDH1, PMS2, PTEN, and TP53. Among the 45 patients with pathogenic mutations some patients were found to carry more than one pathogenic mutation, for example: (a) a Hispanic woman diagnosed with diffuse gastric cancer at age 44 years and a family history of breast cancer had two pathogenic mutations: one in BRCA2 and one in STK11, and (b) a Western European man with a personal history of juvenile and adenomatous polyps and a close relative with very early (less than 15 years of age at diagnosis) colorectal cancer had pathogenic mutations in both BMPRI and TP53. Of those with a pathogenic mutation 32 patients had both a personal and family history of cancer/

polyps. 18/45 (40 %) of those with a pathogenic mutation were of Latino/Hispanic background.

Conclusions: The significant proportion of VUS results is surprising considering that our panel includes only high/moderate risk cancer genes and we purposely omitted poorly characterized genes. The high proportion of Latino/Hispanic patients who were offered a cancer gene panel and were positive for a pathogenic mutation suggests that cancer gene panels may be a more appropriate testing strategy for this population.

Keywords: Cancer gene panels · Ethnic diversity · VUS

202 Genotype-phenotype correlation in Brazilian patients with familial adenomatous polyposis (FAP)

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Background: The incidence of Colorectal Cancer (CRC) is variable around the world and it is attributed to different aspects like diet habits and inheritance conditions presented in different populations. FAP is an autosomal dominant disorder caused by a mutation in the tumor suppressor gene adenomatous polyposis coli (APC) located on chromosome 5(5q21-22). The colonic phenotype manifestation follows a classical complete penetrance pattern, while extra-colonic clinical presentation is highly variable. The identification of mutations is useful in defining subgroups of patients at high risk for extra-colonics lesions and guide therapeutic decisions. Objective: To establish correlation between genotype and phenotype in patients diagnosed with FAP in a Brazilian cohort.

Methods: this is a prospective observational study carried out in the Department of Cancer Genetics of the Barretos Cancer Hospital, from January 2010 to December 2014. The study enrolled only patients with FAP with detected mutation in the APC gene.

Results: Thirty-five different families were evaluated, being ninety-nine patients. Classical colonic adenomatous polyposis phenotype was detected in 94 cases (94.9 %) and profuse in 5 cases (5.1 %); 50.5 % were female and 49.5 % were male. The age ranged from 12 to 67 years (mean of 30.7 years; median of 29 years). Fifty five (55.6 %) cases had stop codon mutations detected, 39 (39.4 %) cases presented with frameshift mutations, rearrangement in 3 cases (3 %), aberrant splicing in 1 case (1 %) and association between nonsense

and rearrangement in 1 case (1 %). The most part of mutations detected between the exons 9 and 16. A total of 34 cases of cancer were diagnosed, including CRC (85.3 %), thyroid cancer (5.9 %), stomach cancer (5.9 %) and central nervous system (CNS) (2.9 %). Seven cases (7.1 %) were diagnosed with desmoids and nine cases (9 %) of congenital hypertrophy of retinal pigment epithelium (CHRPE). Eight four (84.8 %) patients are alive and free of cancer and 3 (3 %) cancer related deaths were recorded. Conclusions: This report showed a strong genotype-phenotype correlation among patients harboring FAP diagnosis. It may be an important tool for risk assessment for colonic and extra-colonic manifestations, helping the clinical management of these patients.

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Keywords: Familial adenomatous polyposis · Genotype · Phenotype

204 Desmoids in familial adenomatous polyposis (FAP) characterization of patients at Barretos Cancer Hospital

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Background: Desmoids are characterized by fibroblastic proliferation usually located in deep soft tissue. It represents less than 3 % of soft tissue tumors. The incidence is 3–4 cases/million, with expected 900 newly cases per year in the United States. Most cases are diagnosed

APC Mutation	Adenoma Stomach/ Duodenum	Adenoma Papilla Duodenal	Thyroid Nodules And Biopsy	Desmoid	CNS Tumor	Osteoma And Jaw	Cranial, Face	CHRPE
Exon 1 To 16	1	0	0	0	0	1		0
Exon 6	11	4	2	1	0	2		0
Exon 8	0	0	0	1	0	1		1
Exon 9	4	1	1	1	0	1		0
Exon 10	1	0	0	0	0	1		0
Exon 13	4	1	1	0	0	4		0
Exon 14	1	0	0	0	0	1		0
Exon 16	45	15	6	6	1	2		8
Intronic	0	0	0	0	0	0		0
Exon 15	2	2	0	0	0	2		0
Total	78	23	6	9	1	15		9

on ages ranging from 15 to 60 years, with a peak around 30 years, and with higher incidence in women. Desmoids may be associated to FAP, as an extra-colonic manifestation. The incidence of desmoids in FAP ranges from 3 to 32 %, according to the genotype-phenotype correlation with the site of the APC mutation, mainly downstream of 1309 codon, with bigger risk (6×). Mutations detected after codon 1464 are associated with a higher risk (10–20×) for extra or intra abdominal desmoids.

Objective: To establish correlation among specific APC mutations and characteristics of patients harboring FAP and desmoids.

Methods: APC mutations were classified in low and high aggressive profile and were correlated to the clinical FAP presentation.

Results: Between January of 2010 and December 2014, nine cases (9 %) among ninety-nine patients harboring FAP and APC mutations were diagnosed with desmoids. The mutations were described in exon 6 (1 case), exon 8 (1 case), exon 9 (1 case) and exon 16 (6 cases), according the table below. Tumor location at diagnosis occurred in rectus abdominis in 2 cases, mesentery in 4 cases and pelvis in 3 cases. One patient died due to desmoids mesentery complications.

Conclusions: Due to the small number of cases we didn't find a strong genotype-phenotype correlation between the site of the APC mutation, location of the desmoids, aggressiveness of the colonic polyposis or other extra-colonic manifestation.

APC Mutation	Patients With FAP	CRC	CNS Tumor	Thyroid Cancer	Gastric Cancer	Desmoids	Osteoma	CHRPE
p.Arg213Ter(c.637C>T)	11	2	0	2	0	1	2	0
	Classic					mesentery		
p.Arg302Ter(c.904C>T)	4	3	0	0	0	1	0	0
	Classic					pelvis		
p.Gln1041Ter(c.3121C>T)	2	1	0	0	0	1	0	0
	Classic					rectus abdominis		
p.Asp849Glufs*11 (c.2547_2550delTAGA)	1	1	0	0	0	1	0	0
	Classic					rectus abdominis		
p.Tyr986Ter(c.2958T>G)	13	2	1	0	1	3	0	0
	Classic					1 mesentery 2 pelvis		
p.Asn1017Metfs*4 (c.3050_3053delATGA)	1	0	0	0	0	1	0	0
	Classic					mesentery		
p.Arg232Ter(c.794C>T)	1	1	0	0	0	1	0	1
	Profuse					mesentery		

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Keywords: Desmoid tumor · Familial adenomatous polyposis · APC

208 Macrolide induced read-through of APC nonsense mutations in familial adenomatous polyposis

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide.

Approximately 85 % of colorectal adenomas or carcinomas show Adenomatous Polyposis Coli (APC) gene, a classical tumor suppressor, loss of function. Familial adenomatous Polyposis (FAP) is caused by dominant germline APC gene mutation. In a subset of FAP patients, APC loss occurs due to nonsense stop codon mutation that leads to expression of a truncated, non-functional protein. It has been shown that various nonsense mutations can be ameliorated by treatment with aminoglycosides antibiotics or other compounds, which lead to mutation read-through and expression of full length, functional protein (1). Interestingly, we have reported that members of the macrolide antibiotic family could induce read-through of APC nonsense stop mutations in tissue culture and in mice models (2).

Aim: Determine proof of concept for the ability of macrolides to induce APC mutation-read-through in patients suffering from FAP caused by APC nonsense mutations.

Methods and results: 1. We have recently constructed a novel reporter plasmid where the expression level of the blue fluorescent protein (BFP) is determined by read-through levels of stop codon sequences. Using this method we can demonstrate that different types of macrolide antibiotics lead to read-through of various human APC nonsense stop mutations. We have tested 4 specific APC mutations from FAP patients and found various levels of read-through induction. 2. Our preliminary in vivo experiments in Min mice show that

macrolide treatment of polyps caused by APC nonsense mutations lead to a reduction in both the number and the size of the polyps. Gene arrays show that the treatment also leads to differential gene expression. 3. We are currently conducting a clinical trial in APC-nonsense-mutation-induced FAP and expect to have soon initial results with our recruited patients.

Conclusions: We have constructed tools for testing APC FAP mutations read-through effect by different compounds. Initial results demonstrate that some APC nonsense mutations, more than others, are sensitive to read-through macrolide treatment. Macrolide antibiotics can cause read-through of APC nonsense mutations in mice. Based on our preliminary results and on published data, we are moving into clinical trial, to offer additional treatment for FAP patients and to widen our perspective for sporadic neoplastic processes.

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Keywords: APC gene · Nonsense mutation · Readthrough · Familial adenomatous polyposis (FAP)

209 Colonoscopy for FAP under 12 years of age: Why is it done?

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Purpose: The first Paediatric Nurse Practitioner in polyposis at our institution noted that some children were undergoing colonoscopy under the age of 12 years despite the fact that our guidelines recommend screening from 12 years of age for familial adenomatous polyposis (FAP). The cases were reviewed to document the reason for early colonoscopy.

Methodology: All endoscopic procedures are recorded on the departmental database. A search to identify children from FAP families who had a colonoscopy under the age of 12 was carried out. Relevant data were exported to an Excel file and analysed.

Results: 30 children had a colonoscopy under 12 years of age. 28 were from families in which the APC mutation had been identified and 2 were from families with clinically diagnosed FAP. Of these 2, 1 had a colonoscopy aged 11 because his mother had died from colon cancer in polyposis and the father and child were extremely anxious. No polyps were found and he remains polyp free at age 19. The other had colonoscopy at age 11 years due to bleeding per rectum, increased frequency of defaecation and a family history of clinical FAP. No polyps were found and he remains in a screening programme. Of the 28 children with an APC mutation, 15 had been screened <12 years old to assess suitability for recruitment to a clinical trial. 10/15 were recruited to the study, the remaining 5/15 children had too many polyps for inclusion. Of the remaining 13 children who had colonoscopy <12 years, 6 presented with symptoms. 5 children had bleeding per rectum and 1 had faecal incontinence and abdominal pain. Of the 5 children with bleeding, 4 went on to have a restorative proctocolectomy (RPC) between the ages of 4 and 8 years. These

children all had a mutation in exon 15 between codons 1309 and 1491. They had between several hundred and a1000 polyps throughout the large bowel. The other 2 children with an APC mutation and symptoms had scanty polyps and remain in a surveillance programme. One child had a total proctocolectomy (TPC) for FAP and Hirshsprungs at age 5 years at another institution, no other history is available. The remaining 6/30 children who underwent colonoscopy <12 years of age were between 9 and 11 years old and had from as few as 11 polyps to dense polyposis. 4/6 children went on to have colectomy with ileo rectal anastomosis (IRA) between 11 years and 16 years with 300–3000 polyps counted on histology. There was no documentation of why the children in this group had genetic testing and colonoscopy before the recommended age of 12 years. 1 Child did however have the testing performed at a different institution and 1 child was referred by an optician following diagnosis of congenital hypertrophy of the retinal pigment epithelium (CHRPE) and underwent genetic testing at age 9 years.

Conclusion: In this study group of children with FAP, 50 % had early colonoscopy to assess suitability for enrolment in a clinical trial and 30 % because of signs or symptoms. The study highlights the importance to document reasons for deviation from guidelines. In addition, the high polyp burden requiring surgery at a young age in some confirms that there should be no hesitation in performing a colonoscopy early if a child is symptomatic.

Symptomatic = 6 + 1Hirs + 1no mutation + 1CHRPE = 9 = 30 %
Keywords: Polyposis · FAP · Proctocolectomy

210 Preliminary results of the E-learning course in genetic counseling for hereditary cancer

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Introduction: In developed countries there are specialized professionals in genetic counseling of hereditary cancer, who are responsible for identifying individuals at high risk of developing cancer and genetic studies guide. In Latin America there are very few genetic counselors due to the lack of educational program. Most of this work is performed by physicians and researchers in cancer genetics. In 2013, we conducted for seven months an e-learning course in genetic counseling for Latin American health professionals, thereby facilitating the training of more professionals. One year after the graduation of the first students (3 physicians, 2 nurses, 1 biochemist and 1 medical technician), we evaluated the usefulness of this course in the clinical practice.

Aim: To evaluate the usefulness in the clinical practice of an e-learning course in genetic counseling on hereditary cancer.

Methods: After graduation, were contacted via email to answer a survey. This instrument considered 7 items: to belong to a high risk oncologic group, to implement hereditary cancer registries, to form interdisciplinary team, to increase the derivation to genetic studies, to participate in national and international congresses for the diffusion of experiences, and to participate in the design of clinic or scientific projects.

Results: 5/7 (71.4 %) students answered the survey. 100 % of the students are part of a high risk oncologic group where they are working in a hereditary cancer registries, and they are supported by an interdisciplinary team. 80 % of the students are referring patients to a genetic studies. In terms of knowledge exchange, 40 % of them have

attended to a high risk congress this year and 80 % of them are planning to participate at Insight 2015.

Conclusion: The e-learning course in genetic counseling on hereditary might benefits high risk patients and families due to it favors the formation of high risk tumors work-up groups and motivates the creation of projects and continuous medical education.

211 Microsatellite instability status and pathological features of gastric cancer in Lynch syndrome

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Objectives: Lynch syndrome is an inherited disease caused by a mutation in one of the mismatch repair genes and is characterized by elevated risk of a variety of cancers. The aims of this study were to elucidate pathological features of gastric cancer (GC) in Lynch syndrome and to evaluate the benefits of microsatellite instability (MSI) testing for GC as a screening test for Lynch syndrome.

Methods: Microsatellite instability (MSI) status and pathological features were evaluated in 50 sporadic GCs and 7 Lynch syndrome associated GCs. To assess MSI status, we used the NCI panel, which includes 5 markers (D2S123, D5S346, D17S250, BAT25 and BAT26). The pathological features include signet/mucinous histology, medullary-type pattern, intra-tumoral lymphocytic infiltration and Crohn's-like lymphoid reaction were assessed on sections stained with Haematoxylin and Eosin.

Results: Of the 57 GCs, 9 exhibited MSI-high (MSI-H), 6 exhibited MSI-low (MSI-L), and 42 exhibited MS-stable (MSS). Seven MSI-H GCs were associated with Lynch syndrome (MLH1:5, MSH2: 2). Then, pathological features of the MSI-H GCs were compared with those of the MSI-L/MSS GCs. The MSI-H GCs were more likely to show medullary carcinoma (MSI-H vs MSI-L/MSS: 22.2 vs 4.6 %, $p = 0.052$), intraepithelial lymphocytosis (MSI-H vs MSI-L/MSS: 22.2 vs 4.6 %, $p = 0.052$). On the other hand, no difference was shown in signet/mucinous histology (MSI-H vs MSI-L/MSS: 20.8 vs 11.1 %, $p = 0.498$), and Crohn's-like lymphoid reaction (MSI-H vs MSI-L/MSS: 25 vs 33.3 %, $p = 0.602$).

Conclusion: One hundred percent of Lynch syndrome associated gastric cancers ($n = 7$) showed MSI-H, whereas only 4 % of sporadic gastric cancers ($n = 50$) showed MSI-H. These results suggested that MSI testing in gastric cancer is very useful screening tests for Lynch syndrome in Japan.

Keywords: Microsatellite instability · Gastric cancer · Lynch syndrome

212 Hereditary cancer predisposition: clinical profile of patients in a private center in Brazil

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Purpose: Patients who fill criteria for investigation of familial cancer susceptibility syndromes are often identified in oncology practice. The aim of this study was to evaluate demographic characteristics of patients at a gastrointestinal department in a private center, focusing on features that suggest the presence of a hereditary cancer predisposition.

Methodology: We reviewed electronic medical files of 178 consecutive patients registered ingastrointestinal tumors clinic at our center between January 2013 and June 2014. Collected data included gender, age at diagnosis, primary site of cancer, presence of multiple tumors, family history and data of geneticist evaluation (hereditary syndrome hypothesis and diagnosis). A descriptive analysis was performed using software Microsoft Excel© 2010.

Results: Among all 178 patients, 145 had a positive family (any degree) history of cancer: 30 (20.7 %) had at least one relative (any degree) with cancer diagnosed at age younger than 50 years old (yo); 72 (49.6 %) had at least 2 cases of cancer in the family; 69 (47.6 %) had more than 2 cases of cancer in the family; 110 (75.86 %) had a first degree relative with cancer. Cancer diagnosis was confirmed in 137 out of 178 patients. Most of them were woman (58.4 %) and 30 patients (21.9 %) were younger than 50 yo, with a median age of 45 yo (20–50). Most primary sites were: colon (35.8 %), gastric (14.6 %), rectal (11 %) and pancreatic (10.2 %). Multiple tumors were present in 3 patients (2.19 %). Overall, 39 patients were referred for genetic evaluation and counseling: 14 (35.9 %) of them were younger than 50 yo, 9 (23 %) had one relative with cancer diagnosed at age younger than 50 yo and more than 2 cases of cancer in the family, 34 (87.2 %) had a first degree relative with cancer. Only 2 patients, in fact attended oncogenetic evaluation. Hereditary syndrome hypothesis in these two patients were Lynch syndrome and Hereditary Breast and Ovarian Cancer/Hereditary Breast and Colon Cancer. The genetic investigation in these patients is not yet concluded.

Conclusion: In our study population, there is a high incidence of patients with criteria for investigation of hereditary familial cancers. These patients should be recognized by their medical oncologists and referred to genetic evaluation. Nevertheless, the progress of the investigation still come up against difficulties of access to genetic evaluation covered by health insurance, high cost exams and lack of specialized laboratories. Education of patients and health staff about importance of genetic testing, reminders in electronic medical files (directed to medical staff) including questions about family history, as well as the incorporation of a geneticist at medical staff, assuring to patients easy access to this specialty, are some tools which have been chosen in our center to minimize these difficulties.

Keywords: Gastrointestinal · Hereditary · Colon cancer

213 Sedation colonoscopy for children—Is this better? Audit

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Purpose: Previously children aged 17 and under at our institution underwent colonoscopic surveillance under general anaesthetic (GA). As part of service development of a transition service for young adults moving from children to adult care, it was agreed that those aged 14–17, with a polyposis syndrome or inflammatory bowel disease, should be given a choice between GA and sedation for colonoscopy. A Standard Operational Procedure (SOP) was written to safely implement this service. The purpose of the audit, which is ongoing, is to

monitor the effectiveness of the new SOP and identify improvements that are required.

Methodology: Two forms were designed: (1) A questionnaire, based on recognised satisfaction tools to be completed by the child, not the parent, following the procedure, prior to discharge but only after they have fully recovered. (2) A form to be completed by the paediatric nurse responsible for the sedation list during the endoscopy and the recovery period. These two forms were reviewed and approved at our Institution by the audit team prior to use. All children aged 14–17 years inclusive who agree to undergo the procedure under sedation rather than GA are included. 7 children have been offered sedation but opted for GA for various reasons and 1 child age 12 wanted sedation. The paediatric nurse responsible for the sedation list explains the rationale for this review to the parent and child prior to asking for verbal consent. It is made clear that the child's care will not be compromised if they do not wish to take part. This is done before any section of the audit is completed.

Results: Between June 3rd and September 10th 2014 six paediatric sedation lists took place. 15 children, 10 of whom had a polyposis syndrome, underwent a colonoscopy under sedation. Full results will be presented on the poster. The following were of particular interest:

- 14 out of 15 questionnaires were completed by patients
- Only 3 of 15 were given an endoscopy patient information leaflet prior to the procedure, however 100 % of patients said that they were given enough information about the test
- None of the children were given an opportunity to see the endoscopy unit prior to their procedure
- Complete colonoscopy was achieved in all cases
- There were no adverse events related to the procedure or sedation
- Maximum time to recovery was 120 min but some children were not discharged until much later; the reason for this was not identified.
- 64 % of patients experienced mild discomfort during the procedure and 78 % said that they did not experience any discomfort after completion
- 50 % were worried about procedure but 100 % said they would have it done this way again in the future

Conclusion

Despite the fact that all patients said they had sufficient information prior to the procedure, only 3 of the 15 had been given an information sheet. This indicates that the verbal information given to the children was effective but further investigation is required. A nurse led discharge policy with clarity of the discharge criteria needs to be developed and implemented in order to speed up discharge. Colonoscopy can be done under sedation in 14–17 years age group with good outcomes.

Keywords: Colonoscopy · Children · Sedation

6th Biennial InSiGHT Meeting 2015—Participants List

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