

# Early-Onset, and Familial CRCs (FCC), that are not Lynch Syndrome

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# Disclosure Information

## C. Richard Boland, MD

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# Early-onset CRC without a family history suggesting Lynch Syndrome

3 recent studies

# Unexpected Lynch Syndrome among young CRC patients

- 75 CRC patients with no more than one relative with CRC, all <50 years old (mean 34.5), no FAP/UC, all from BUMC
- MSI (pentaplex PCR of 5 mononucleotide repeats)
- IHC testing of tumor tissue; MSH2, MSH6, MLH1, PMS2
- (No germline testing)
  
- 72% in the distal sigmoid colon or rectum
- MSI in 21%; abnormal IHC in 21% (n=16)
  - MSH2: 3: all MSI
  - MLH1: 3: all MSI
  - PMS2: 5: all MSI
  - MSH6: 5 (and only 2/5 had MSI)

# Does a young patient with CRC and a negative FH have LS?

- 21% of young CRC patients without an Amsterdam Family History had “cryptic” Lynch Syndrome
- 72% distal tumor location
- Over-represented by MSH6 and PMS2 abnormalities
- MSH6 cancers would be missed using usual probes for MSI (requires additional probes)
- KRAS mutations in 22% of CRCs with MMR defects
  - present in 78% of CRCs with normal MMR expression
- No BRAF mutations in any young patient

# Epicolon Study of Early-Onset CRC

- 140 CRC patients  $\leq 50$  years old, Spanish consortium (Epicolon); MSI, IHC, germline mutations in MMR genes, and MUTYH germline mutations
- Positive family histories not excluded
  - 26% had a + FH of CRC; 5.8% Amsterdam+
- 11.4% had MSI (5 MNRs), 14.3% had abnormal IHC
- 75% of the CRCs were in the distal colon
- Identified MMR germline mutations in 11 (7.8%)
- Somatic methylation of MLH1 in 1 (0.7%)
- KRAS mutations in 28%, BRAF mutations in 3.6%

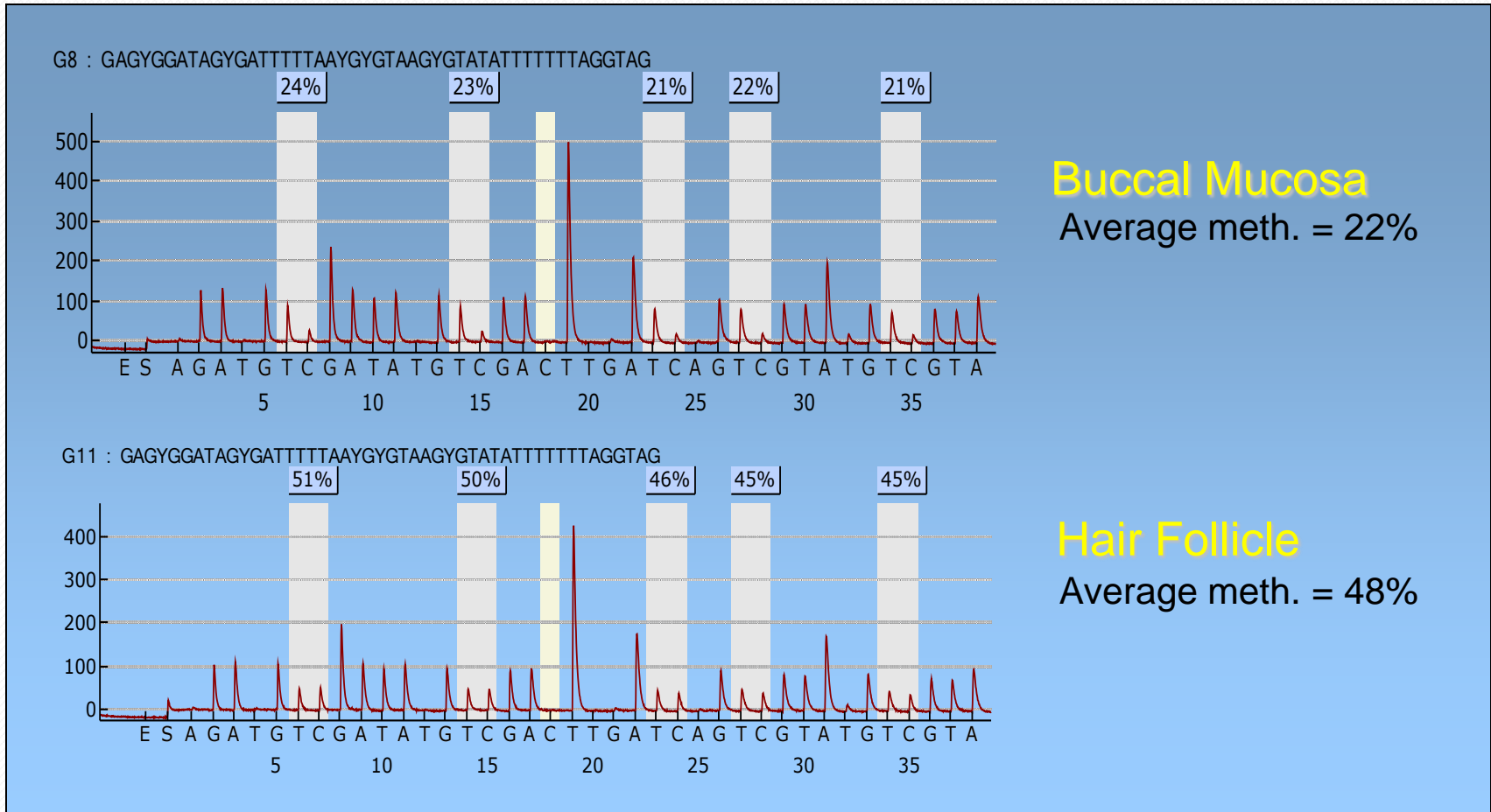
# Epicolon Study of Early-Onset CRC

- Germline mutations
  - MLH1: 4 (2.8%)
  - MSH2: 1 (0.7%)
  - MSH6: 6 (4.3%) – 2/6 were MSS
  - Biallelic MUTYH: 4 (2.8%)
- About 15% have DNA MMR defects
- Underscores the role of MSH6 and MUTYH in young CRC patients

# Soma-wide Methylation of MLH1 in a young patient with CRC

- 20 year old woman developed a 3 cm cancer in the descending colon, mucinous, Stage II (T3N0M0)
- MSI-H, loss of expression of MLH1, PMS2 and MSH6
- Negative family history
- No germline mutations in MLH1, MSH2 or MSH6 (included “rearrangement analysis”)
- Treated with adjuvant chemotherapy (12 cycles FOLFOX)
- MLH1 methylated in the tumor tissue (pyrosequencing, Deng-C)
  - Buccal mucosa DNA - 22% methylated
  - Hair follicle DNA - 48% methylated
  - PBL-LCL DNA - methylated 14% and 8%, respectively
    - PBL methylation rose to 22% after chemotherapy
    - No methylation in either parent or brother
- Somatic LOH of the unmethylated allele in the tumor
  - Methylated allele was paternal

# Buccal mucosa and hair follicle tissues also show MLH1 methylation



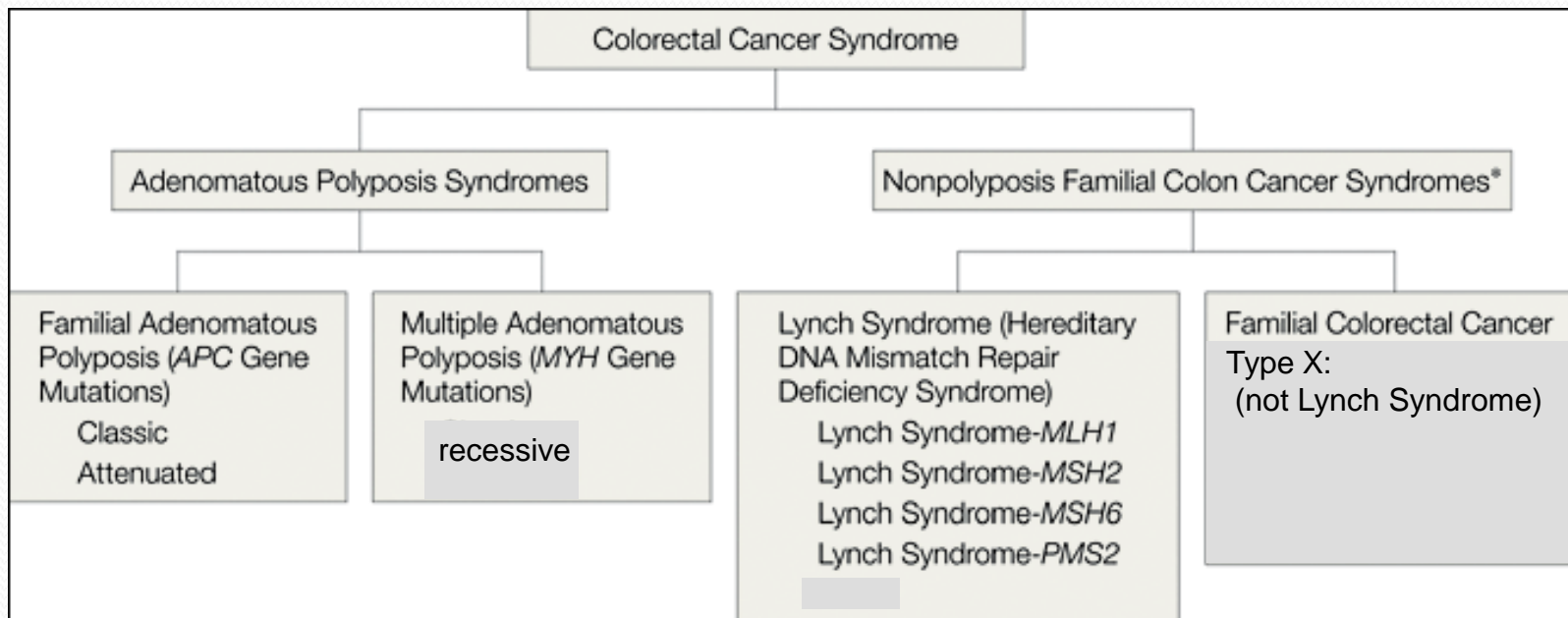
# Soma-wide Methylation of MLH1 (cont.)

- 18 year old male, cancer in ascending colon (T3N1M0), MSI-H, loss of MLH1 and PMS2 in tumor, no FH, no mutation in MLH1
- Dense hemiallelic methylation (36-50%) in tumor, PBLs, buccal DNA, saliva, hair follicles; none in parents; methyated allele was maternal, somatic loss of the non-methylated paternal allele in the tumor.
- Summary of 15 patients with constitutional MLH1 epimutations:
  - ages range from 18-67; 8/15 under age 50
  - Only 1/12 had BRAF mutations; 3/11 had KRAS mutations
  - LOH of non-methylated allele in 8/13; 2 had missense mutation 2<sup>nd</sup> hit
- Mechanism unknown

# Clinical Features of Soma-Wide Methylation of MLH1

- Uncommon (~1% of MSI tumors)
- May occur in young adults (half <50)
- Methylation of MLH1 promoter in all 3 germ tissues
- May act like Lynch Syndrome
  - produces early-onset CRC
  - full tumor spectrum unknown
- May be acquired on maternal or paternal alleles
- Tumors may occur after LOH or mutation of the unmethylated allele

# FCC Categories



Lindor, N. M. et al. JAMA 2005;293:1979-1985.

# Familial Colorectal Cancer that is not Lynch Syndrome (FCC-type X)

- Collaboration with X. Llor (Univ. Ill, Chi)
- Four groups of colorectal cancers:
  1. Amsterdam +, MSS, n=22
  2. Amsterdam +, MSI-H (Lynch Syndrome), n=21
  3. Sporadic MSS, n=92
  4. Sporadic MSI-H, n=46
- Methylation analyzed at 5 validated promoters (CIMP); LINE-1 methylation; mutations in BRAF and KRAS
- Methylation Index (MI) calculated from the 5 promoters (>5-10% meth)
  - “Low-MI” if 1-3 promoters methylated
  - “High-MI” if 4-5 promoters methylated

# Methylation Index (MI) in Syndrome X

<u>Tumor Group</u>	<u>Low MI</u>	<u>High MI</u>
MSS HNPCC (Syndrome X)	100% (22)	0
Sporadic MSS (92)	95.6% (87)	4.4% (4)
Lynch Syndrome (21)	90.5% (19)	9.5% (2)
Sporadic MSI (46)	32.6% (15)	67.4% (31)

# Line-1 Methylation in Syndrome X

<u>Tumor Group</u>	<u>% Line-1 methylation</u>	<u>Mean Rank (P =)</u>
MSS HNPCC (Syndrome X)	60.08%	56.05 (--)
Lynch Syndrome	66.29%	94.80 (p=.015)
Sporadic MSI	67.27%	105.41 (p=.001)
Sporadic MSS	65.13%	86.22 (p=.009)

# RAS/RAF Mutations in Syndrome X

<u>Tumor Group</u>	<u>BRAF</u>	<u>KRAS</u>
MSS HNPCC (22) (Syndrome X)	0	31.8% (7, all codon 12)
Lynch Syndrome (21)	0	9.5% (2, both codon 12)
Sporadic MSS (92)	2.2% (2)	39.2% (36; 25 codon 12, 11 codon 13)
Sporadic MSI (46)	28.3% (13)	4.4% (2 - codons 12 + 13)

# Genetic Alterations in Syndrome X

Familial CRC without DNA MMR inactivation:

1. Significantly lower degree of Line-1 methylation (i.e., *less global methylation*) than in all other groups
  - probably reflects global hypomethylation
2. No evidence for promoter methylation (CIMP)
  - not a cryptic form of familial CIMP
3. No BRAF mutations (consistent with absence of CIMP); KRAS mutations similar to sporadic CRC

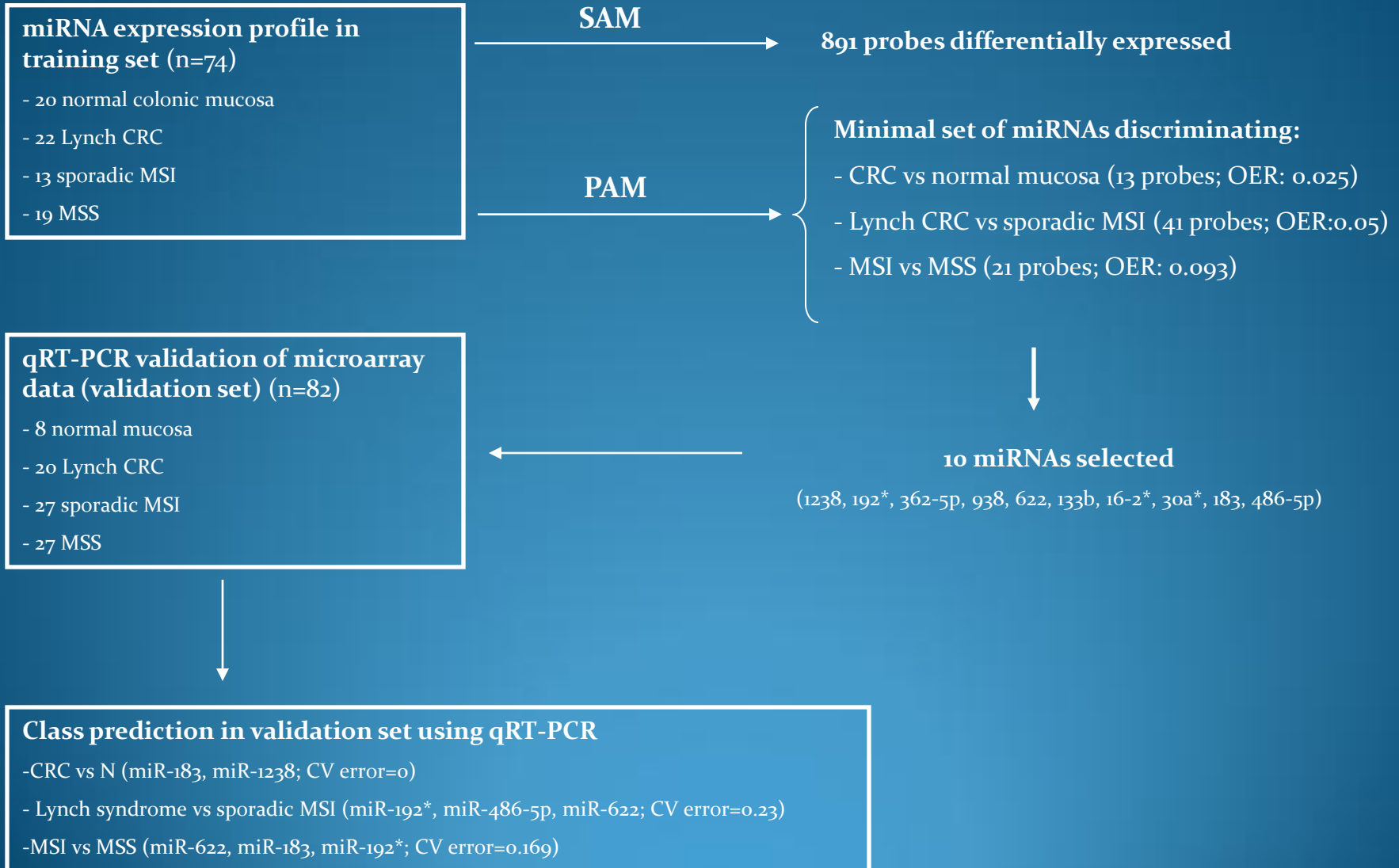
# Diagnostic Approaches to Non-Lynch Syndrome Familial CRC

- MSI tumors may be due to Lynch Syndrome (20-25% of MSI CRCs) or acquired methylation of MLH1 (75-80% of MSI CRCs); in some cases, the germline mutation is elusive
- These tumors have important phenotypic differences; the acquired form of MSI CRCs all come from a background of CIMP, with incidental methylation of MLH1
- It should be possible to find gene expression profiles that correlate with Lynch Syndrome tumors and permit distinguishing them from the acquired MSI CRCs

# miRNA Profiling in CRC

- Objectives: Using miRNAs as biomarkers for the identification of familial and non-familial colorectal cancer
  - F Balaguer, L Moreira, JJ Lozano, A Link, Y Shen, M Cuatrecasas, M Arnold, S Syngal, E Stoffel, P Bandipalliam, R Jover, X Llor, CR Boland, M Gironella, and A Goel. Unpublished.

# Experimental Approach

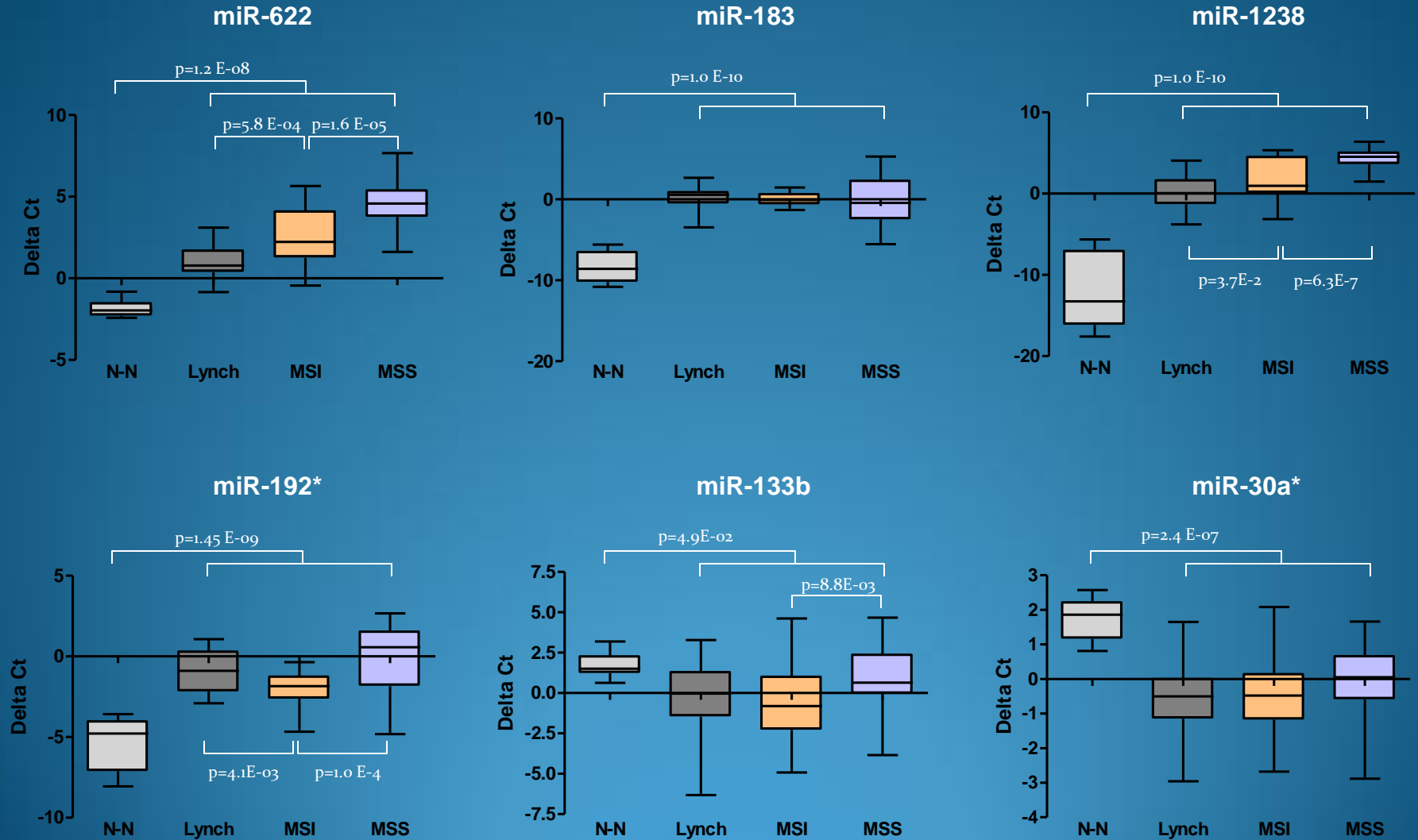


# Patient characteristics

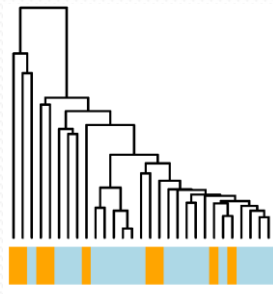
Characteristic	Non-tumor Patients (n=20)	Lynch Syndrome TS (n=22)	Lynch syndrome VS (n=13)	P value	Sporadic MSI TS (n=13)	Sporadic MSI VS (n=20)	P value	MSS TS (n=19)	MSS VS (n=19)	P value	
<b>Age (<math>\pm</math>standard deviation)</b>	64.45 (15.81)	46.32 (12.54)	49.54 (10.14)	0.438	67.38 (10.25)	63.95 (13.94)	0.451	67.11 (12.04)	64.89 (13.86)	0.603	
<b>Sex, n (%)</b>											
- Males	10 (50)	9 (40.9)	10 (76.9)	0.08	4 (30.8)	12 (60)	1	8 (42.1)	7 (36.8)	1	
- Females	10 (50)	13 (59.1)	3 (23.1)		9 (69.2)	8 (40)		11 (57.9)	12 (63.2)		
<b>Race, n (%)</b>											
- Caucasians	21 (100)	21 (95.4)	13 (100)	1	13 (100)	20 (100)	1	14 (73.7)	12 (63.2)	0.630	
- African-americans	-	-	-		-	-		-	1 (5.3)		2 (10.5)
- Hispanics	-	-	-		-	-		-	3 (15.8)		4 (21)
- Others	-	1 (4.6)	-		-	-		-	1 (5.2)		1 (5.3)
<b>Tumor location, n (%)</b>											
- Proximal		16 (72.3)	4 (30.8)	0.217	8 (61.5)	18 (90)	0.008	5 (26.3)	5 (26.3)	1	
- Distal		6 (27.7)	5 (38.5)		5 (38.5)	-		14 (73.7)	12 (63.2)		
- Unknown		-	4 (30.8)		-	2 (10)		-	2 (10.5)		
<b>Tumor stage, n (%)</b>											
- I		4 (18.2)	4 (30.8)	0.324	1 (7.7)	1 (5)	0.189	3 (15.8)	1 (5.3)	0.261	
- II		8 (36.4)	3 (23)		9 (69.2)	6 (30)		9 (47.4)	11 (57.9)		
- III		8 (36.4)	1 (0.8)		2 (15.4)	5 (25)		6 (31.6)	3 (15.8)		
- IV		1 (4.5)	1 (0.8)		1 (7.7)	6 (30)		1 (5.2)	4 (21)		
- Unknown		1 (4.5)	4 (30.8)		-	2 (10)		-	-		



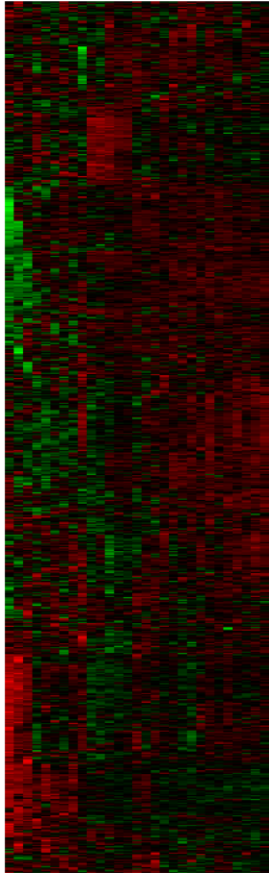
# qRT-PCR validation of differentially expressed miRNAs



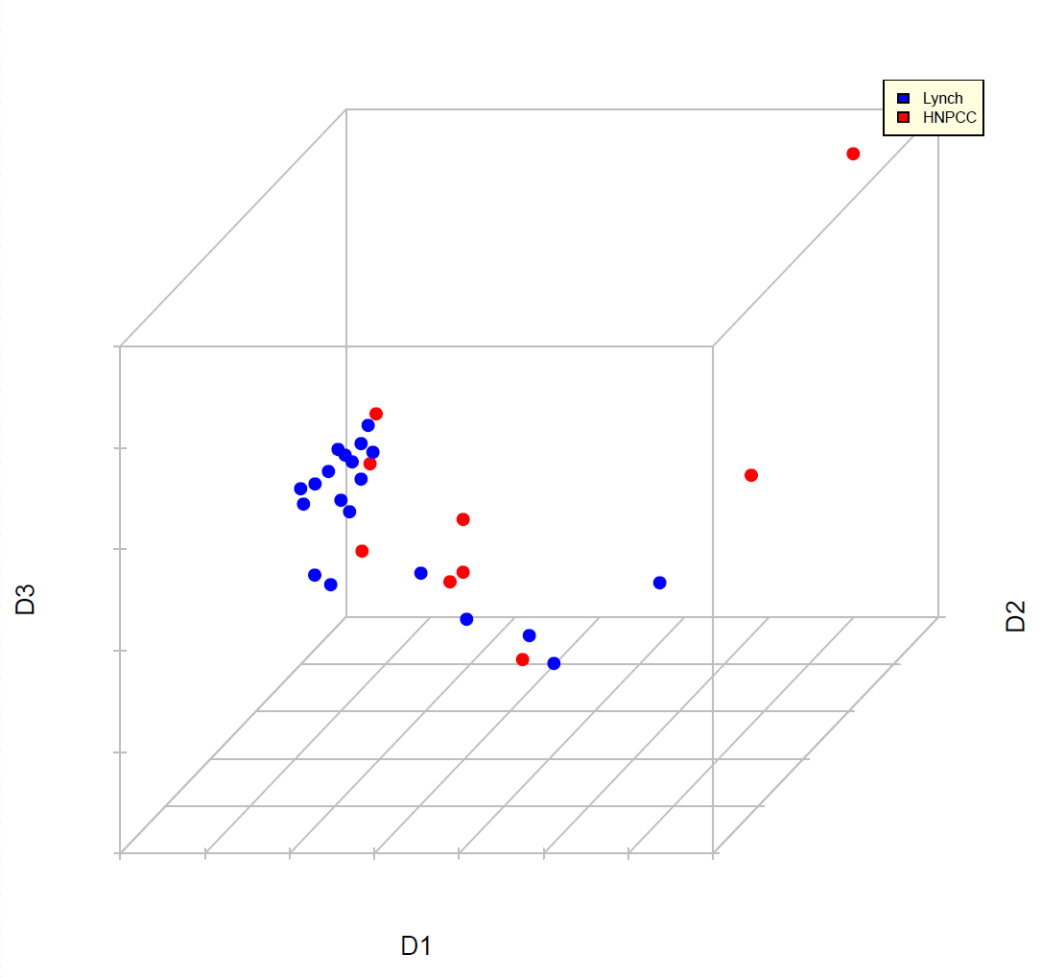
# Lynch syndrome with/without identifiable germline mutations show similar miRNA expression profiles



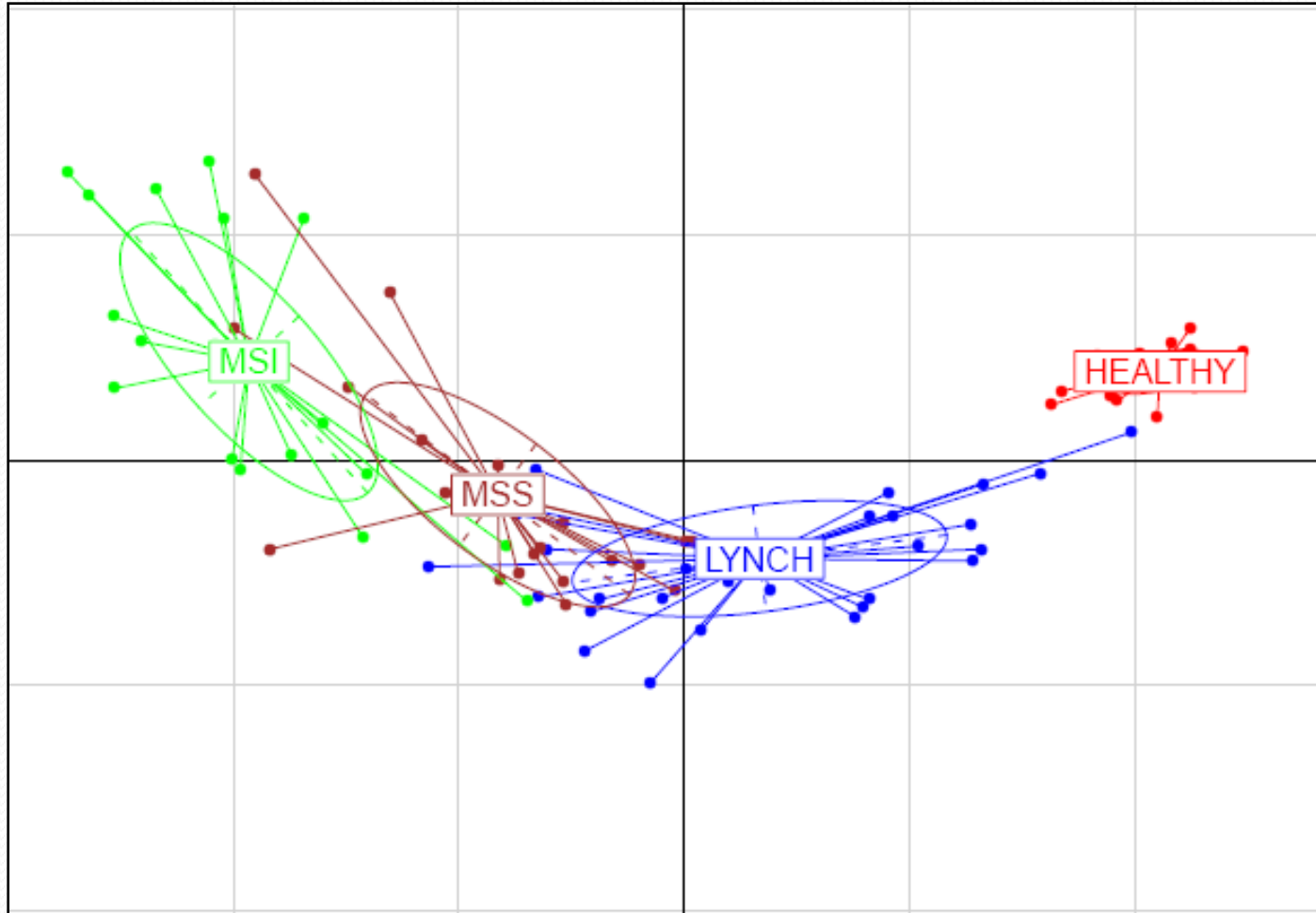
■ Lynch without mutation  
■ Lynch with mutation



HNPCC1  
Lynch10  
HNPCC2  
Lynch11  
Lynch20  
Lynch21  
Lynch22  
Lynch3  
Lynch4  
Lynch5  
Lynch6  
Lynch7  
Lynch8  
Lynch9  
HNPCC3  
Lynch12  
Lynch13  
Lynch14  
Lynch15  
Lynch16  
Lynch17  
Lynch18  
Lynch19  
HNPCC4  
Lynch2  
Lynch1  
Lynch12



# Discrimination of samples using between group analysis (BGA)



# Summary: miRNA profiling

- Our data with miRNA suggest that it is feasible to:
  - separate hereditary (LS) from acquired defects (CIMP) in DNA MMR genes
  - identify Lynch syndrome tumors, *even when we cannot find the germline defect*

# Conclusions:

## Early Onset and Familial CRC

- Among young CRC patients without an obvious family history to suggest Lynch Syndrome, ~20% have cryptic LS, frequently with mutations in MSH6 and PMS2
  - also consider biallelic MUTYH
- Soma-wide methylation of MLH1 (“constitutional epimutation”) is not common, but occurs in young people and may mimic Lynch Syndrome
- Familial CRC-type X is associated with Line-1 hypomethylation, and is not a form of familial CIMP
- It may be possible to characterize LS tumors in which the germline mutation is not yet found using miRNA profiling