

**ACTIVATING  $\beta$ -CATENIN MUTATIONS IN LYNCH  
SYNDROME TUMOURS ARE INDICATIVE OF AN  
*MLH1* GERMLINE DEFECT AND MAY BE  
SELECTED BASED ON APOPTOSIS RESISTANCE**

**<sup>1</sup>Inês Francisco, <sup>1</sup>C Albuquerque, <sup>1</sup>B Filipe, <sup>2</sup>P Lage, <sup>2</sup>R Sousa, <sup>2</sup>I Claro, <sup>2</sup>P Rodrigues,  
<sup>2</sup>M Cravo, <sup>2</sup>P Fidalgo, <sup>2</sup>C Nobre Leitão**

**<sup>1</sup>Molecular Pathobiology Investigation Center (CIPM); <sup>2</sup>Gastroenterology Department -  
Portuguese Institute of Oncology, Lisbon - Portugal**



**InSiGHT  
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**Colorectal tumours associated to Lynch syndrome (LS):**

- found in proximal colon (70%)
- MSI-H (defect in the MMR system)

**Mutation in genes from WNT pathway had been previously associated to FAP and MSS sporadic colorectal cancer (CRC)**

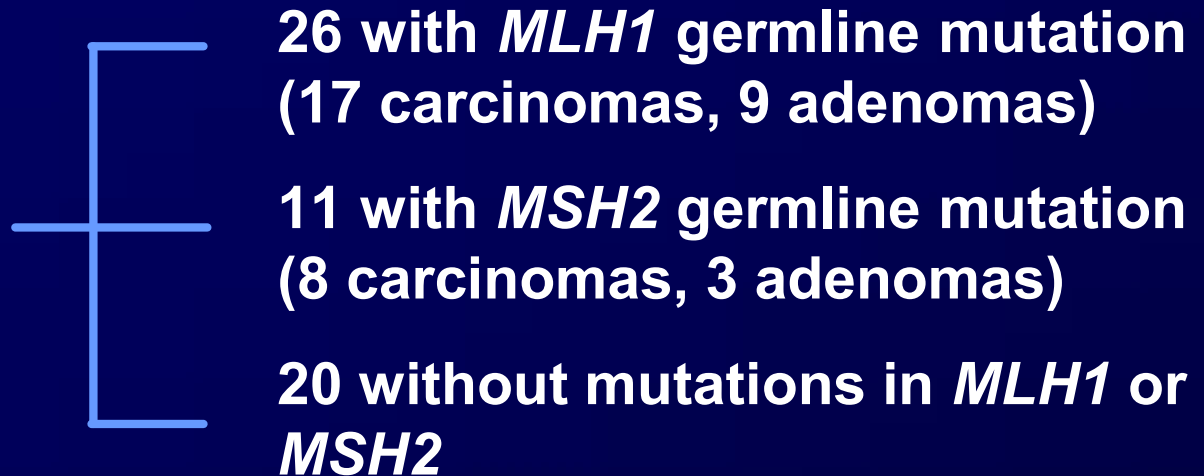
**LS colorectal tumours have been found to present mutations in genes from WNT pathway  *APC,  $\beta$ -catenin, Axin1 and Axin2***

### **$\beta$ -catenin mutations**

- ❖ MSI-H tumours and proximal colon (sporadic and LS tumours)
- ❖ more frequent in LS colorectal tumours

**Evaluation of  $\beta$ -catenin mutations in Lynch syndrome colorectal tumours and correlation with the localisation of the tumours, MSI and the presence of germline MMR mutations**

57 LS colorectal  
tumours

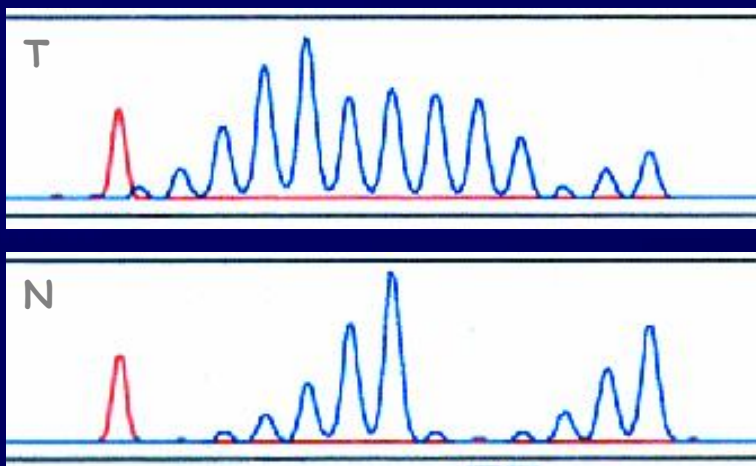


- Somatic mutational analysis in  $\beta$ -catenin - exon 3 (SSCP)

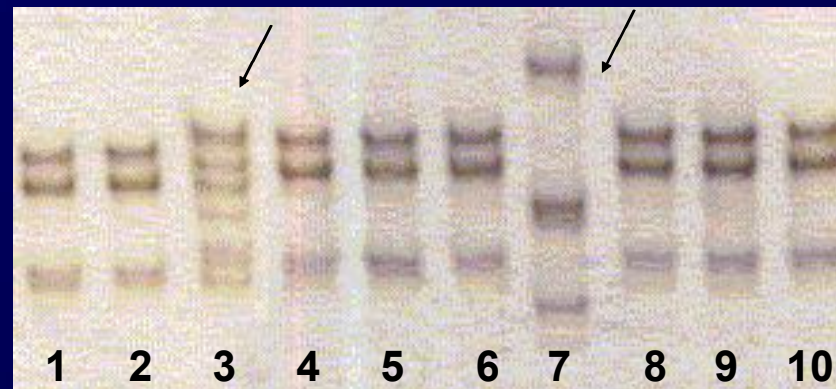


Direct sequencing

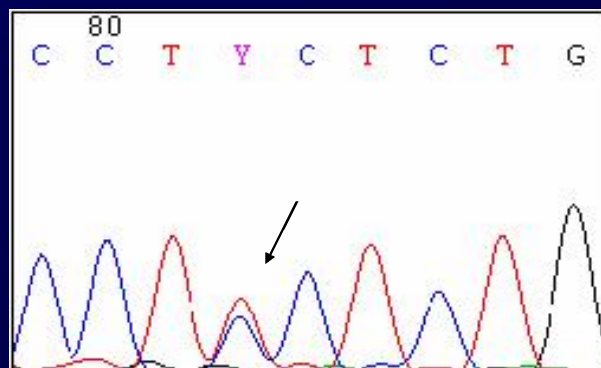
- Microsatellite instability analysis (D2S123, D5S346, D17S250, BAT-25 and BAT-26) – GeneScan ABI Prism 310 (Applied Biosystems)



GeneScan: D5S123



8% polyacrylamide gel: exon 3  $\beta$ -catenin



Missense mutation S45P: TCT → CCT

MMR Germline mutation (tumours)	MSI analysis		Tumour localisation		β-catenin mutations		
					All tumours	MSI-H tumours	Proximal tumours
<i>MLH1</i> (n =26)	MSI-H	23/26 (88%)	Proximal	23/26 (88%)	11/26 (42%)	11/23 (48%)	11/23 (48%)
	MSI-L	2/26 (8%)	Distal	3/26 (12%)			
	MSS	1/26 (4%)	Left Rectum/Sigmoid	1/3 2/3			
<i>MSH2</i> (n=11)	MSI-H	10/11 (91%)	Proximal	6/11 (54%)	0/11 (0)	0/10 (0)	0/6 (0)
	MSI-L	0/11 (0)	Distal	5/11 (46%)			
	MSS	1/11 (9%)	Left Rectum/Sigmoid	1/5 4/5			
<i>MLH1</i> and <i>MSH2</i> mutation negative (n=20)	MSI-H	5/20 (25%)	Proximal	3/20 (15%)	1/20 (5%)	1/5 (20%)	1/3 (33%)
	MSI-L	0/20 (0)	Distal	17/20 (85%)			
	MSS	15/20 (75%)	Left Rectum/Sigmoid	1/20 16/20			

**P= 0,000014 (Fisher's exact test)**

**P= 0,00057 (Fisher's exact test)**

MMR Germline mutation (tumours)	MSI analysis		Tumour localisation		β-catenin mutations		
					All tumours	MSI-H tumours	Proximal tumours
<i>MLH1</i> (n =26)	MSI-H	23/26 (88%)	Proximal	23/26 (88%)	11/26 (42%)	11/23 (48%)	11/23 (48%)
	MSI-L	2/26 (8%)	Distal	3/26 (12%)			
	MSS	1/26 (4%)	Left	1/3			
<i>MSH2</i> (n=11)	MSI-H	10/11 (91%)	Proximal	6/11 (54%)	0/11 (0)	0/10 (0)	0/6 (0)
	MSI-L	0/11 (0)	Distal	5/11 (46%)			
	MSS	1/11 (9%)	Left	1/5			
<i>MLH1</i> and <i>MSH2</i> mutation negative (n=20)	MSI-H	5/20 (25%)	Proximal	3/20 (15%)	1/20 (5%)	1/5 (20%)	1/3 (33%)
	MSI-L	0/20 (0)	Distal	17/20 (85%)			
	MSS	15/20 (75%)	Left	1/20			

$P < 10^{-6}$  (Fisher's exact test)

$P = 0,026$  (Fisher's exact test)

# Results

MMR Germline mutation (tumours)	MSI analysis		Tumour localisation		β-catenin mutations		
					All tumours	MSI-H tumours	Proximal tumours
<i>MLH1</i> (n =26)	MSI-H	23/26 (88%)	Proximal	23/26 (88%)	11/26 (42%)	11/23 (48%)	11/23 (48%)
	MSI-L	2/26 (8%)	Distal	3/26 (12%)			
	MSS	1/26 (4%)	Left Rectum/Sigmoid	1/3 2/3			
<i>MSH2</i> (n=11)	MSI-H	10/11 (91%)	Proximal	6/11 (54%)	0/11 (0)	0/10 (0)	0/6 (0)
	MSI-L	0/11 (0)	Distal	5/11 (46%)			
	MSS	1/11 (9%)	Left Rectum/Sigmoid	1/5 4/5			
<i>MLH1</i> and <i>MSH2</i> mutation negative (n=20)	MSI-H	5/20 (25%)	Proximal	3/20 (15%)	1/20 (5%)	1/5 (20%)	1/3 (33%)
	MSI-L	0/20 (0)	Distal	17/20 (85%)			
	MSS	15/20 (75%)	Left Rectum/Sigmoid	1/20 16/20			

**P= 0,007 (Fisher's exact test)**

**P= 0,039 (Fisher's exact test)**

## Results

	Histology	MMR germline mutation	$\beta$ -catenin mutation	MSI analysis	Tumour localisation
439	Ca, wd	MLH1 exon 17, c.659	T41A	MSI-H	Right colon
429P1	TA, LGD	MLH1 exon 12, c.354	T41A	MSI-H	Transverse colon
429P2	TA, LGD	MLH1 exon 12 c.354	T41A	MSI-H	Transverse colon
443	Ca, md, muc	MLH1 exon 13, c.487	S45F	MSI-H	Caecum
443P	VA, LGD	MLH1 exon 13, c.487	T41A	MSI-H	Right colon
565	Ca, ?, muc	MLH1 del exons 4-6	T41A	MSI-H	Right colon
647	Ca, md	MLH1 exon 14, c.540	S45P	MSI-H	Right colon
529	Ca, ?	MLH1 exon 1, c.26	S45F	MSI-H	Right colon
101	Ca, pd	MLH1 exon 1, c.26	S45F	MSI-H	Transverse colon
371	Ca, wd, muc	MLH1 exon 17, c.648	S45F	MSI-H	Right colon
T7	Ca, md	MLH1 IVS-11 T->A	S45P	MSI-H	Right colon
287T	Ca, md	No mutation identified	S45F	MSI-H	Transverse colon

**8/11 (73%)  $\beta$ -catenin positive cases found in tumours with  
MLH1 mutation localised upstream exon 13**

**P= 0,01**  
*(Fisher's exact test)*

### ***$\beta$ -catenin* mutations**

- ↪ more frequent in LS proximal tumours with *MLH1* germline mutation
- ↪ seem to be associated to the localisation of the *MLH1* mutation (upstream exon 13)

***$\beta$ -catenin* mutations appear to be indicative of a germline mutation in the *MLH1* gene**

**Mutation analysis of  *$\beta$ -catenin* exon 3 in LS colorectal tumours should be used as a fast pre-screening tool to direct germline mutational analysis in LS families (first exons of the gene)**

**MLH1 protein cleaved by caspase-3 at Asp418 (5' half)**

 **pro-apoptotic C-terminal product targeting the apoptotic machinery**

(Chen F *et al*, JBC, 2004)

**Short truncated proteins resulting from mutations at the 5' half of the gene can not be cleaved**



**Cells with short truncated proteins will be more resistant to apoptosis**

Activating *β-catenin* mutations and the consequent overexpression of this protein could induce apoptosis



*β-catenin* mutations are more frequent in LS colorectal tumours probably due to *MLH1* germline mutation

*β-catenin* mutations may be selected based on the level of resistance to apoptosis



- region of the colon
- localisation of *MLH1* germline mutation