



AUCKLAND, NEW ZEALAND

INTERNATIONAL SOCIETY FOR  
GASTROINTESTINAL HEREDITARY  
TUMOURS (InSiGHT)

20 - 23 March 2019 | [www.insight2019.com](http://www.insight2019.com)



New Zealand Society of  
Gastroenterology

# Highly sensitive *MLH1* methylation analysis in blood identifies a cancer patient with low-level mosaic *MLH1* epimutation

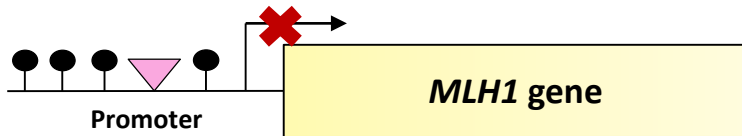
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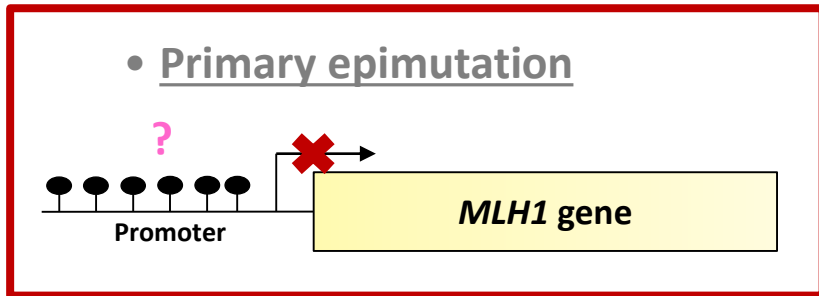
# Constitutional *MLH1* epimutations as a cause of Lynch syndrome

Constitutional *MLH1* epimutations:

- Secondary epimutation



- Primary epimutation



## Epimutation carriers



**10-50%**  
75 cases

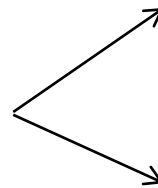


**≤ 10%**  
7 cases



## Healthy controls



**≤ 10%**  
(78%) (Auclair *et al.*, 2011)



Which is the true **prevalence** of mosaic *MLH1* epimutations?

 Genetic variant  
 Methylation

# Patients and methods

## Identification of mosaic *MLH1* epimutation carriers:



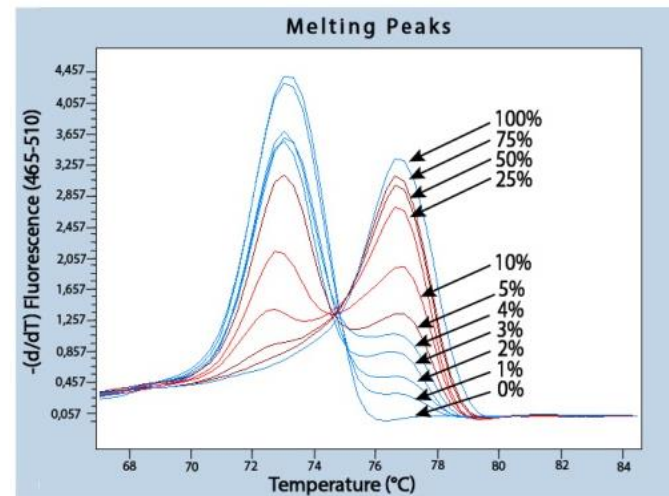
**18 patients**

- *MLH1* methylated CRC  $\leq$  50 years or multiple tumors  $\leq$  60 years
- Blood methylation levels by MS-MLPA  $\leq$  5%  
(sensitivity 10%)



**20 healthy controls**

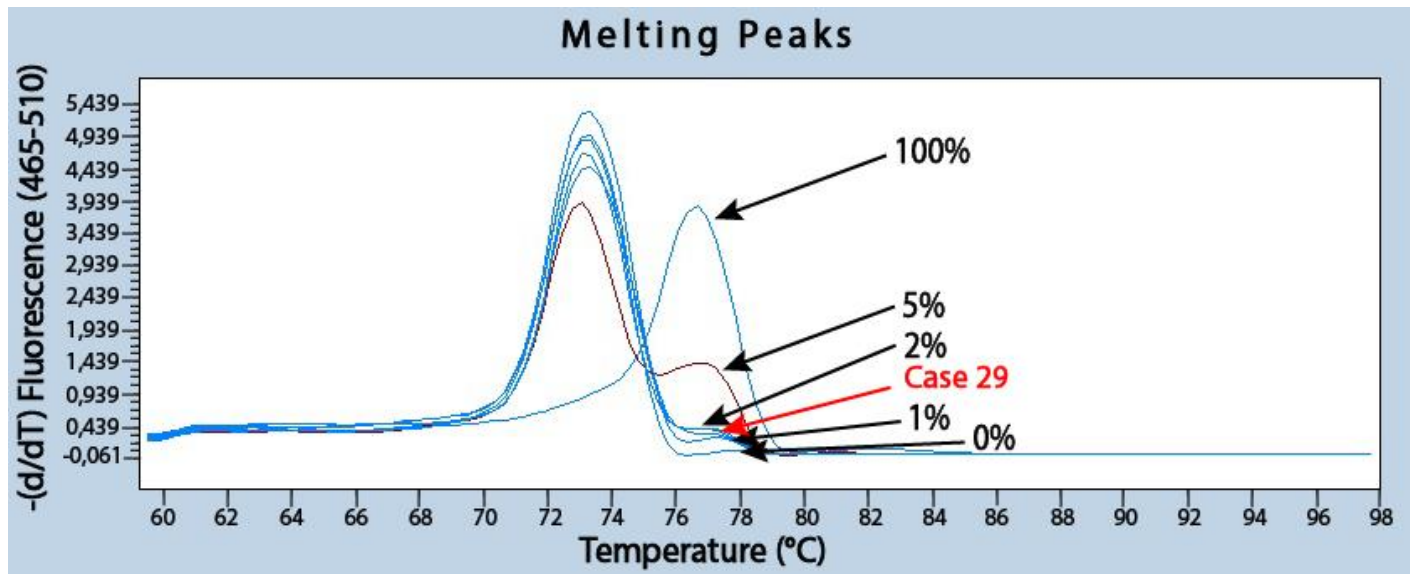
MS-MCA of *MLH1* promoter C region



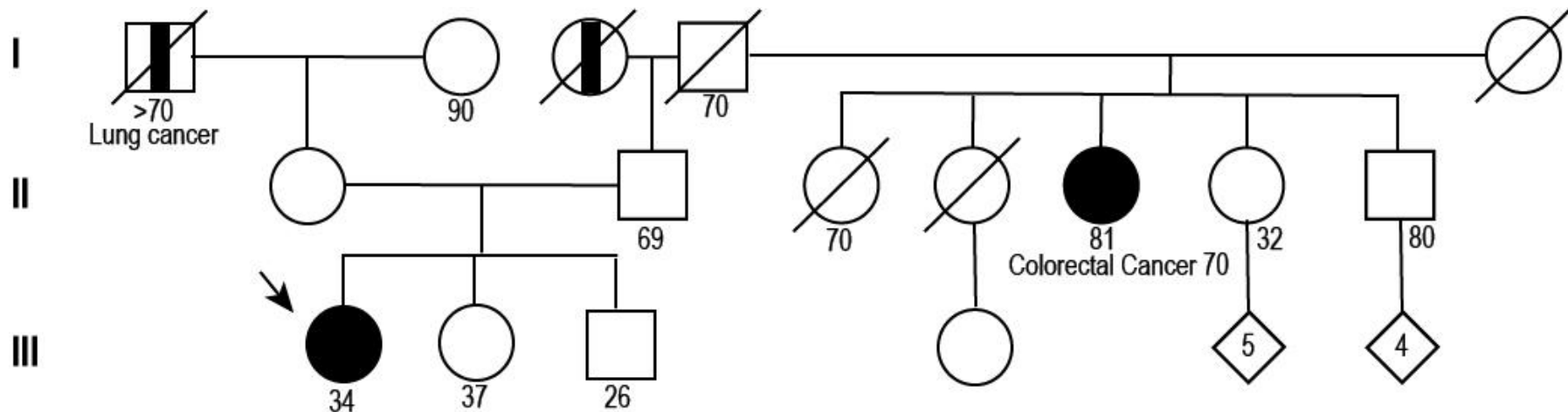
## Confirmation and characterization of *MLH1* epimutation

# One patient showed methylation at a level of 1-2% in PBL

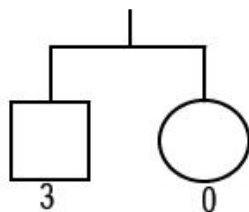
Highly sensitive *MLH1* methylation screening by MS-MCA (C region)



# Case 29 was diagnosed with three gastrointestinal hypermethylated tumors



Colorectal cancer 22  
 Small bowel cancer 24  
 Gastric cancer 25



MSI-high  
 MLH1/PMS2 - +  
 MLH1 methylated

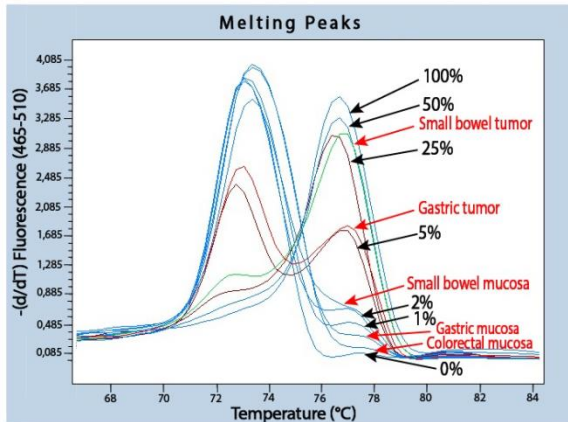
KRAS G12D  
 CK7 -  
 CK20 -  
 CDX2 +  
 MUC1,2,5 +

**MLH1 methylation by MS-MLPA:**

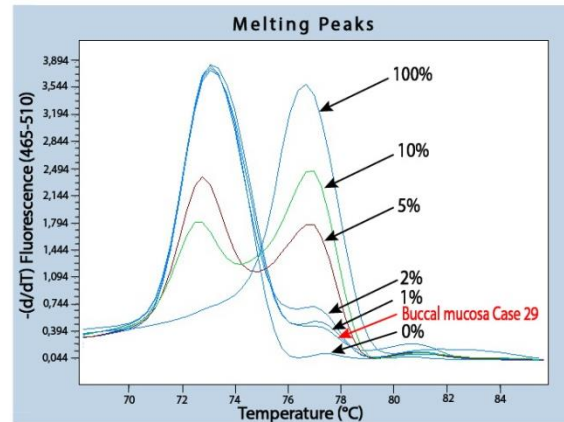
- Blood: <4%
- Normal GI tissues: <10%

# The presence of methylation was confirmed in all embryonic layers

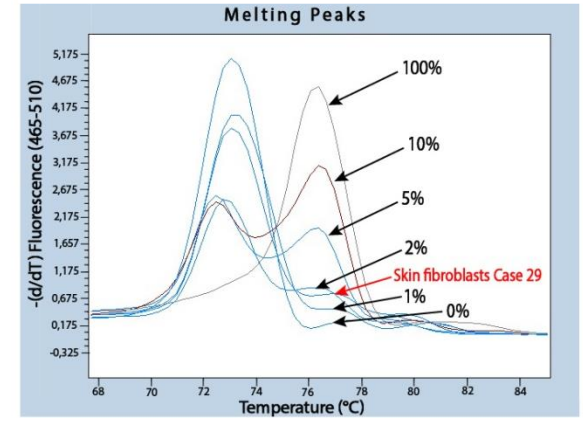
## MLH1 methylation analysis by MS- MCA (C region):



Gastrointestinal tissues  
(endoderm)

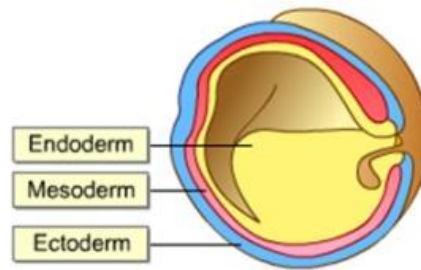


Buccal mucosa



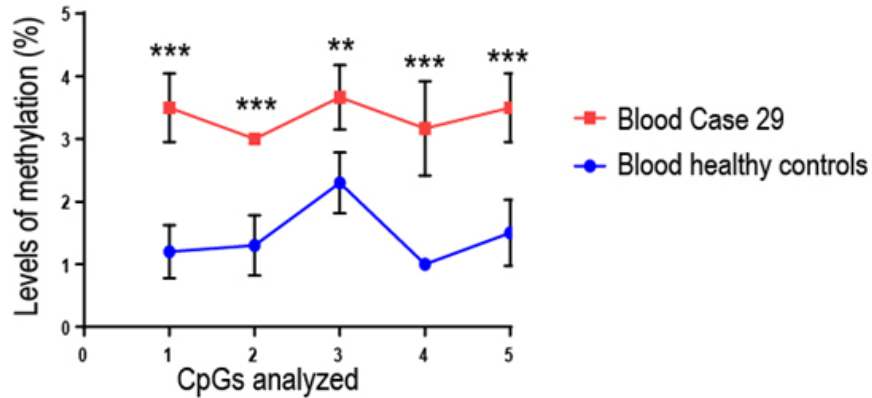
Skin fibroblasts

(ectoderm)



# Confirmation of the mosaic constitutional *MLH1* epimutation by clonal bisulfite sequencing

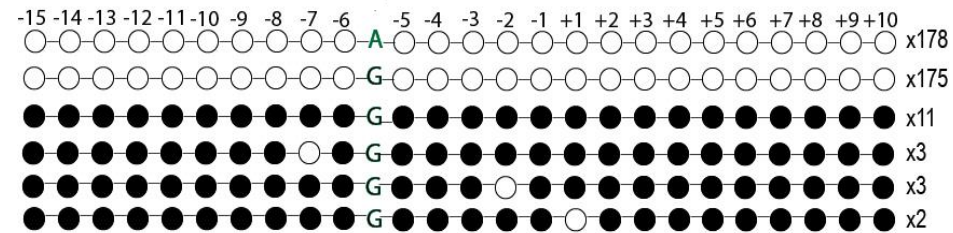
## Pyrosequencing (C region)



Increased methylation levels  
below the limit of detection (5%)

## Clonal bisulfite sequencing (C-D regions)

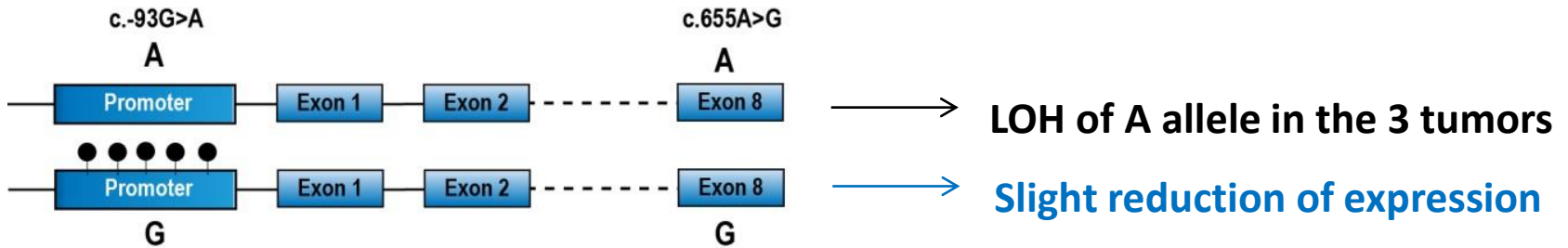
○ CpG non methylated  
● CpG methylated



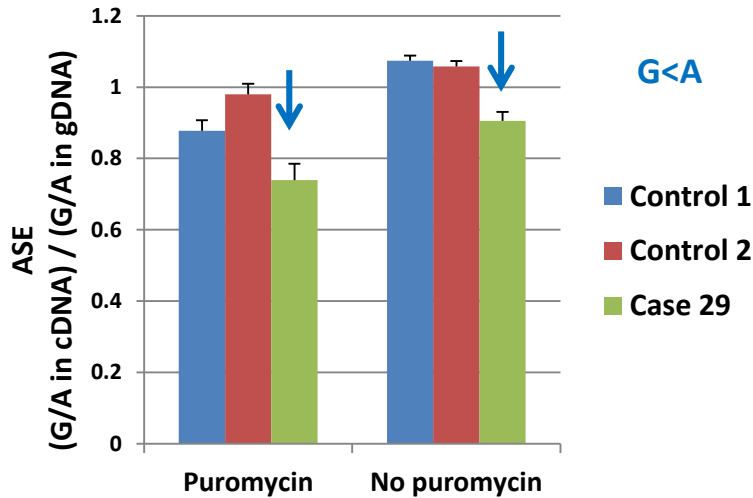
19/372 methylated clones  
5% methylation; 95% CI 0,01–0,05

c.-93G allele  
(rs18000734)

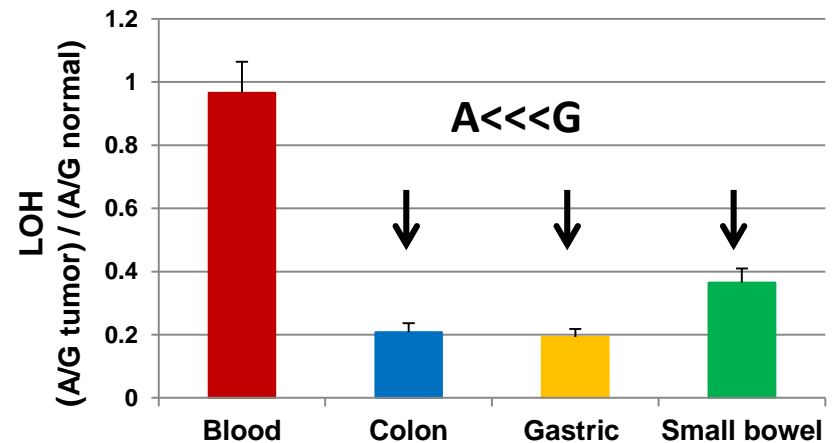
# Reduced expression of the methylated allele and LOH of the opposite one in tumors



Allele-specific expression at c.655A>G



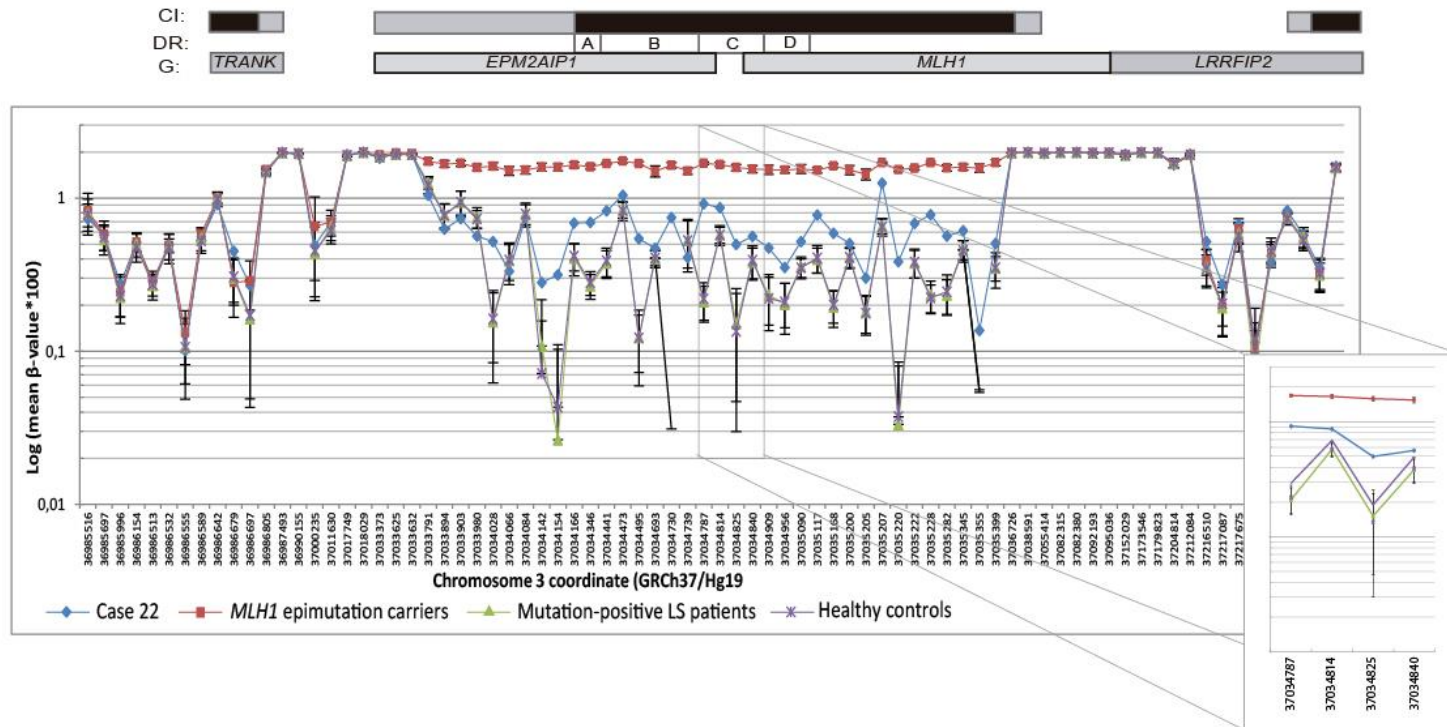
Loss of heterozygosity in tumors at c.655A>G





# The differentially methylated region is shared with constitutional *MLH1* epimutation carriers

## Global wide methylation profiling (Infinium Human MethyloMe 450K Beadchip)



# Conclusions

- We have identified a *bona fide* case of low-level *MLH1* epigenetic mosaicism by using highly sensitive *MLH1* methylation analysis
- We recommend the use of highly sensitive techniques for screening for constitutional *MLH1* methylation
- Special attention should be paid to patients diagnosed by early onset and/or multiple *MLH1*-methylated tumors



*ciberonc*



**cnag**

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