

# **Comprehensive analysis of MMR mutations in CRC**

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# MMR mutation analyses

- Lines of evidence suggested that a high frequency of Amsterdam I HNPCC families may harbor large genomic deletions in *MLH1* or *MSH2* MMR genes

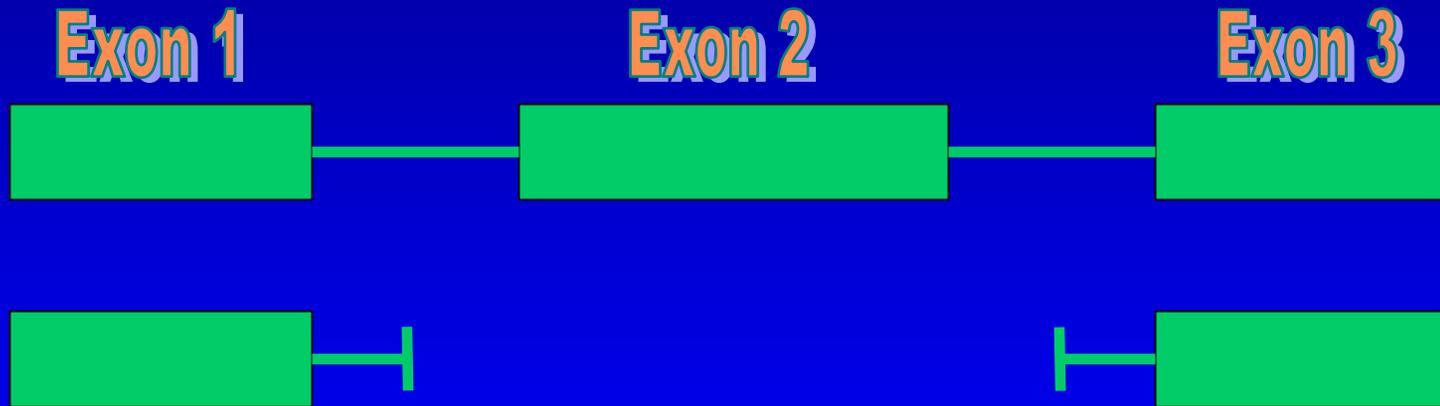
Yan et al. *Nature*. 2000

Nakagawa et al. *Cancer Res*. 2002

Wagner et al. *AJHG*. 2003

Vogelstein (pers. comm.)

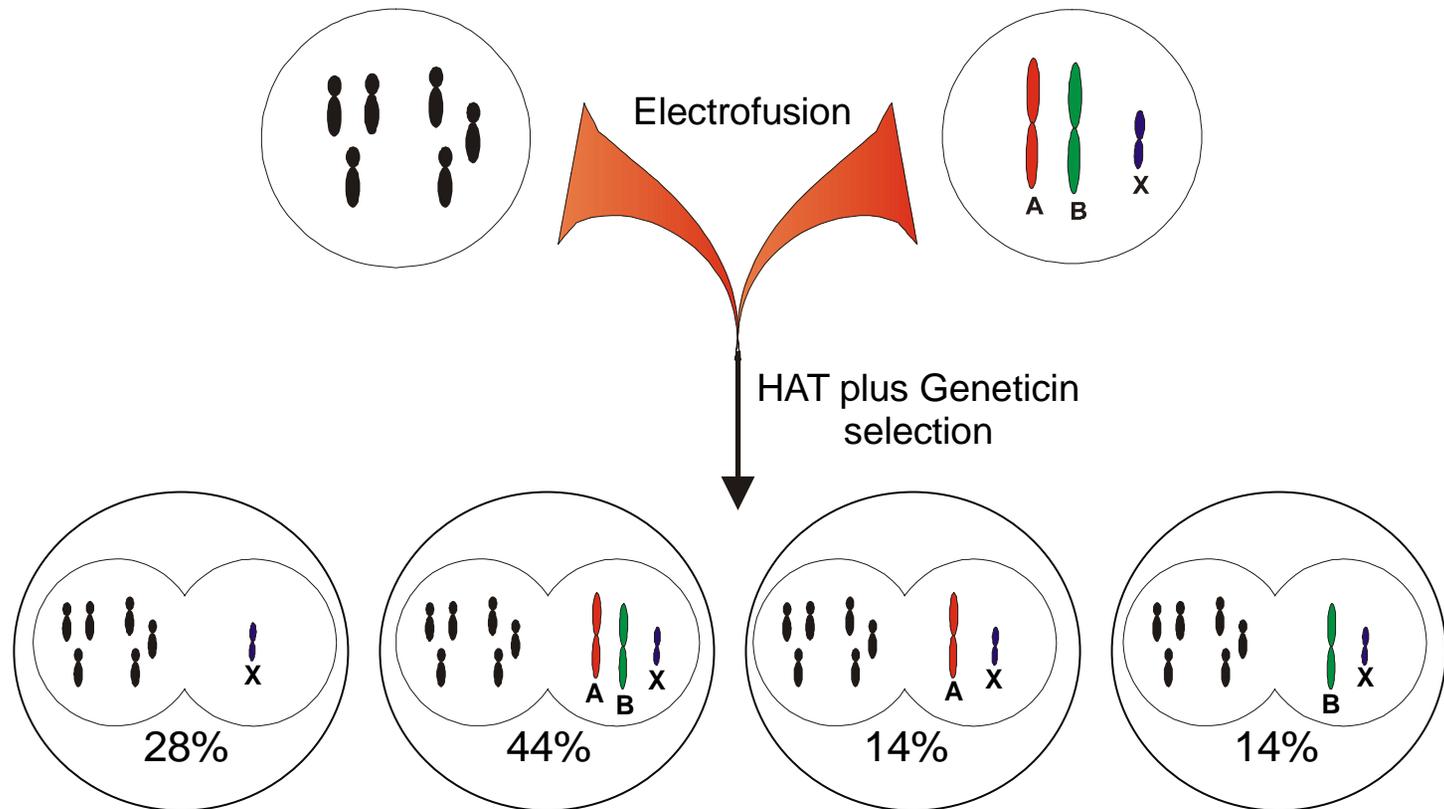
# Large genomic deletion mutations



# What is conversion analysis?

Mouse E2 (Recipient)  
HAT sensitive  
Geneticin resistant

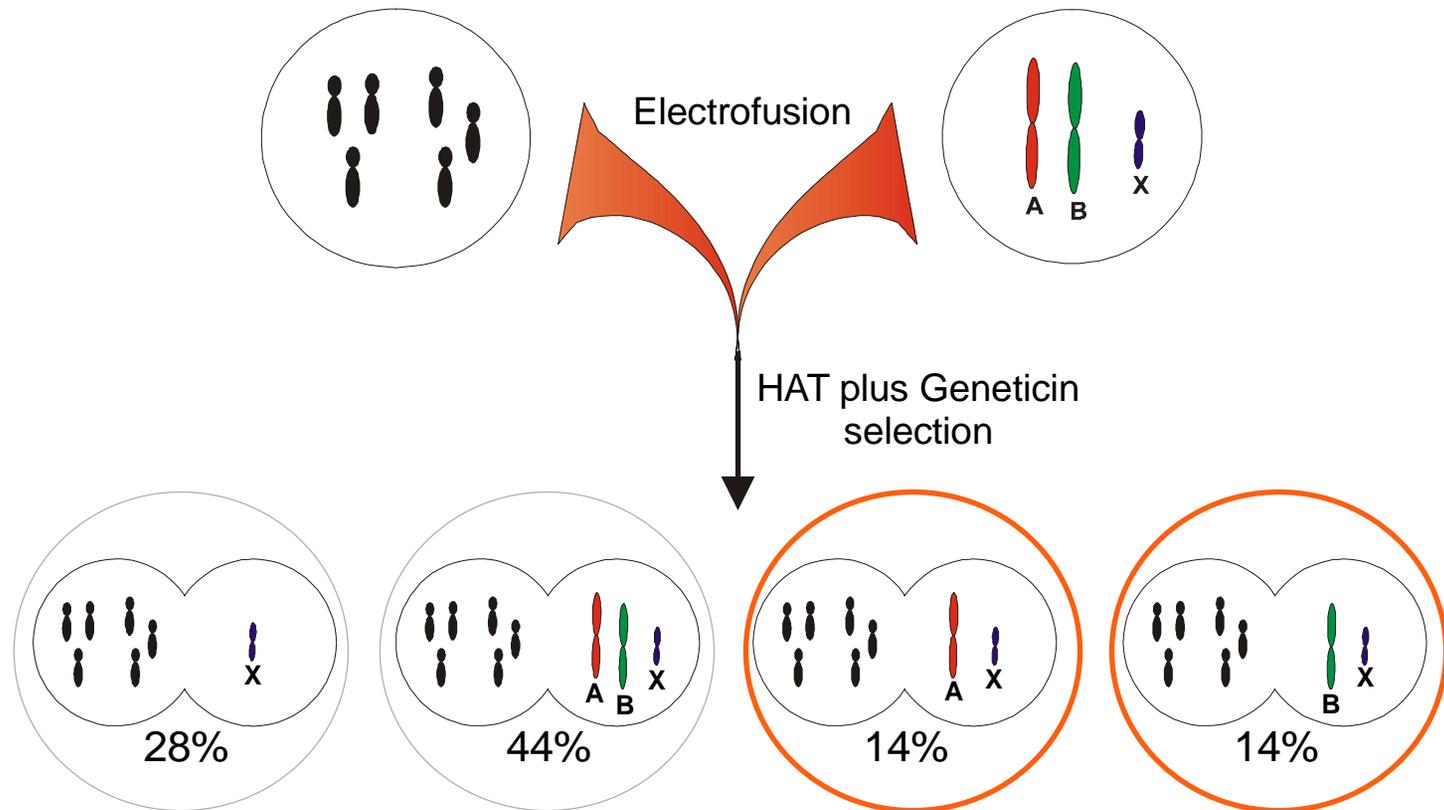
Lymphocytes (Donor)  
HAT resistant  
Geneticin sensitive



# What is conversion analysis?

Mouse E2 (Recipient)  
HAT sensitive  
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# Analysis of MMR mutations in CRC

## Subjects:

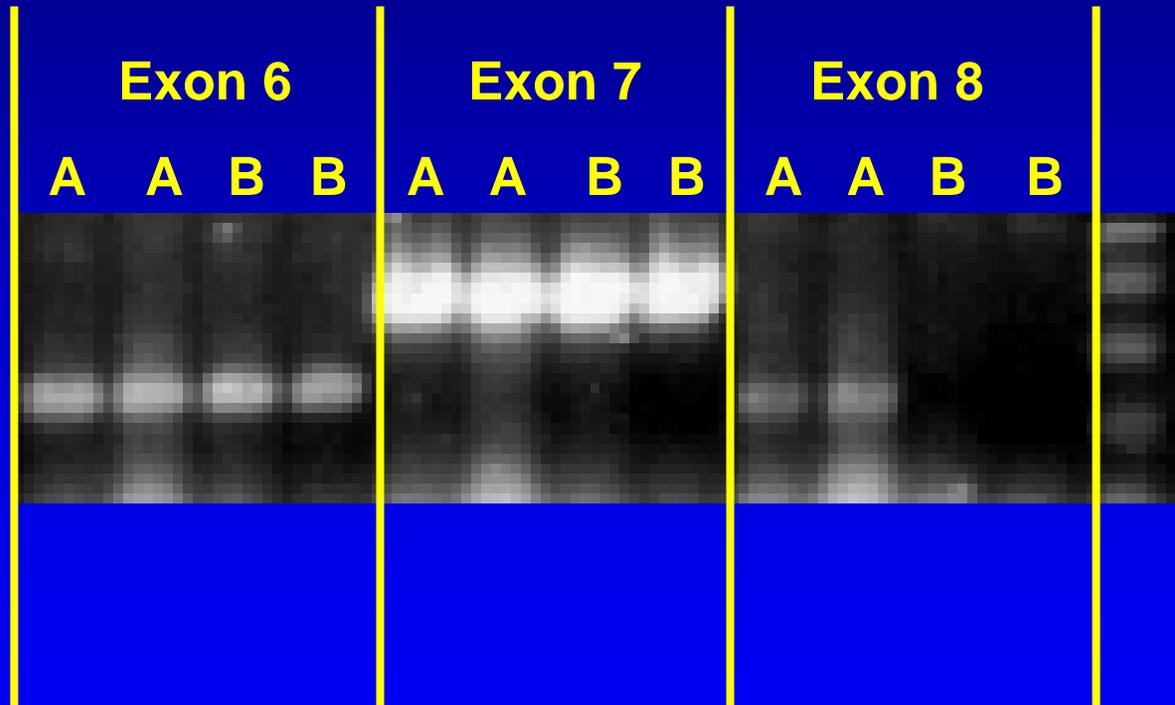
64	HNPCC (Amsterdam I criteria)
8	HNPCC-like
17	< 50 years CRC

- 93% (83/89) of cases were either MSI-high and/or stained IHC negative for *MLH1* or *MSH2*

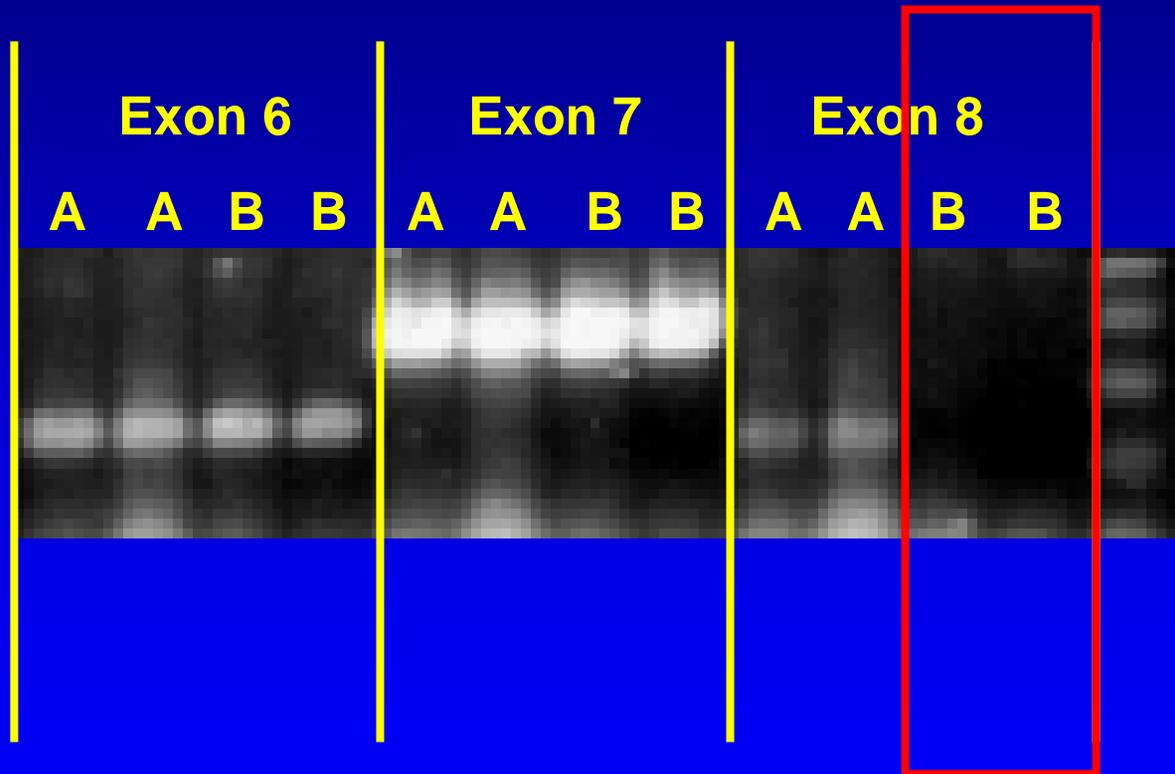
## Strategy:

All cases analyzed by DNA sequencing (CoH) and Conversion Analysis (GMP) for *MLH1* or *MSH2* mutations. Mutation negative cases were then analyzed for *MSH6* mutations using both methods.

# Genomic deletion of exon 8 in *MSH2*



# Genomic deletion of exon 8 in *MSH2*



# Summary of MMR Mutations

HNPCC Amsterdam I with defective MMR (n = 64)

	Gene and mutation type			
	MLH1	MSH2	MSH6	Total
Genomic rearrangements/large genomic deletions	1 (0)	7 (0)	0	8/64 (13%)
Frameshift/nonsense/splice mutations	21 (9)	20 (14)	0	41/64 (64%)
Loss of Expression	0	0	0	0/64 (0%)
<b>Subtotal:</b>	22/64 (34%)	27/64 (42%)	0/64 (0%)	<b>49/64 (77%)</b>

# Summary of MMR Mutations

## HNPCC-like with defective MMR (n = 8)

	Gene and mutation type			
	MLH1	MSH2	MSH6	Total
Genomic rearrangements/ large genomic deletions	0	2 (0)	0	2/8 (25%)
Frameshift/nonsense/splice mutations	1 (1)	4 (2)	0	5/8 (63%)
Loss of Expression	0	0	0	0/8 (0%)
<b>Subtotal</b>	1/8 (13%)	6/8 (75%)	0/8 (0%)	<b>7/8 (88%)</b>

# Summary of MMR Mutations

<50 years with defective MMR (n = 17)

	Gene and mutation type			
	MLH1	MSH2	MSH6	Total
Genomic rearrangements/ large genomic deletions	1 (0)	2 (0)	0	3/17 (18%)
Frameshift/nonsense/splice mutations	0	3 (1)	1 (1)	4/17 (24%)
Loss of Expression	0	0	0	0/17 (0%)
<b>Subtotal</b>	1/17 (6%)	5/17 (29%)	1/17 (6%)	<b>7/17 (41%)</b>

# Summary

- Conversion analysis provided an increase of 33% (14/42) over DNA sequencing alone in the identification of deleterious MMR mutations.
- Conversion analysis clarified the effect of some missense and splice site mutations, and increased the diagnostic yield by 56% (35/63) over DNA sequencing alone.