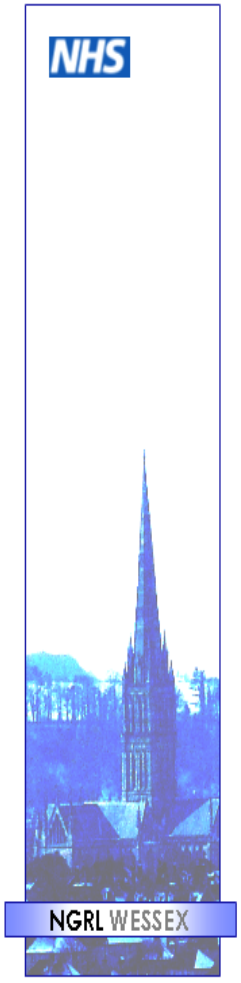
Salisbury NHS
NHS Foundation Trust

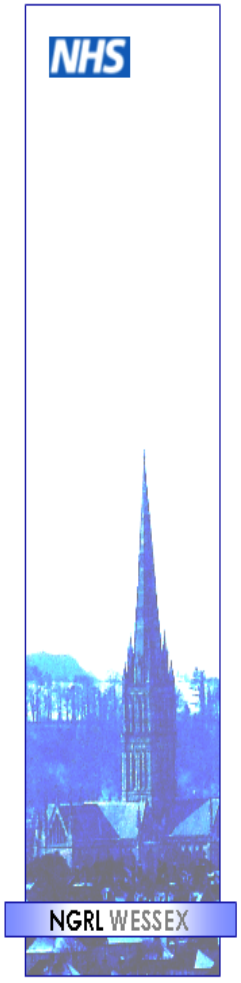


RNA Analysis

Helen White, NGRL (Wessex)

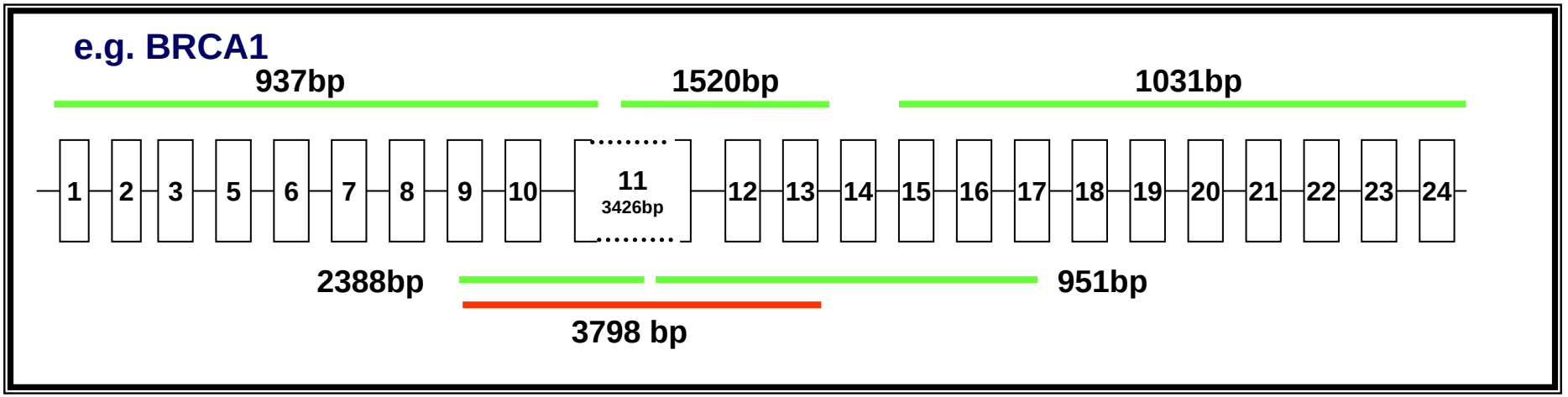
DH Department of Health

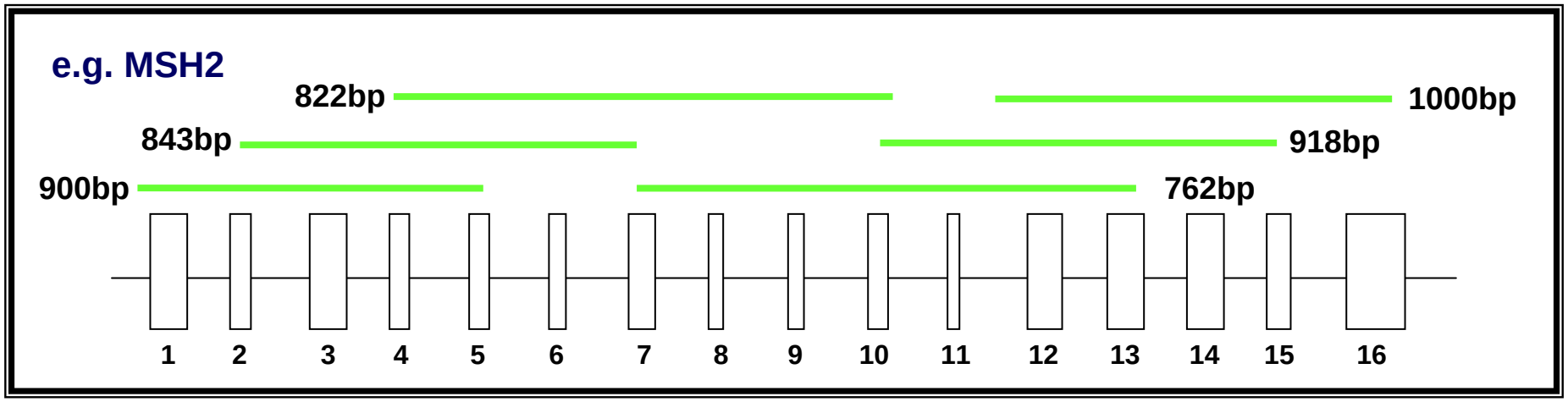
Outline of talk



- Case report
- Interpretation
- Stability of alleles

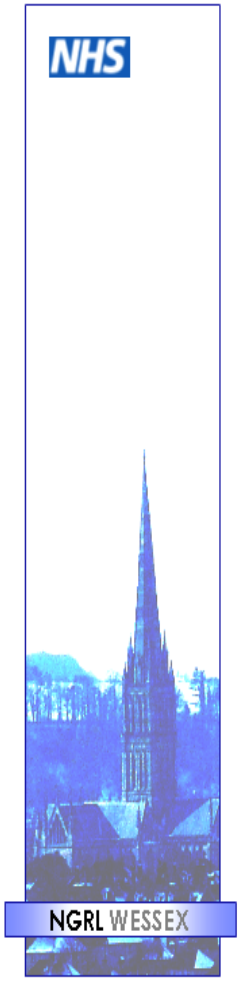
Overlapping RT-PCR for BRCA1, BRCA2, MSH2 & MLH1





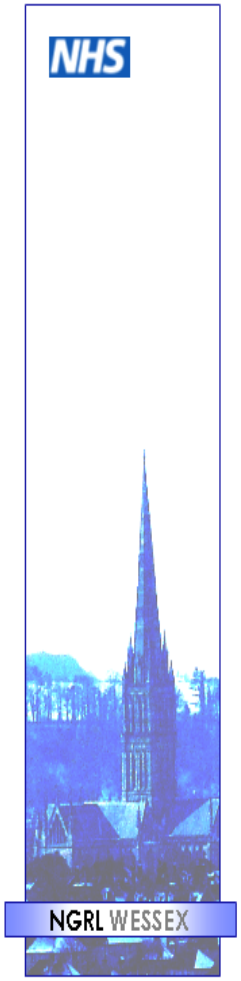
cDNA samples from peripheral blood are amplified and products digested to detect abnormal splice variants

Cases analysed to date



Gene	Exon	Mutation	Protein
BRCA1	3	c.122 A>G	p.His41Arg
BRCA2	18	c.7988A>T	p.Glu2663Val
BRCA1	15	c.4644G>A	p.Thr1548Thr
BRCA1	17	c.4999 A>G	p.Lys1667Glu
BRCA2	Intron 2	c.68-7T>A	N/A
BRCA2	15	c.7565C>T	p.Ser2522Phe
BRCA2	11	c.3698C>T	p.Ala1233Val
BRCA2	23	c.9098C>T	p.Thr3033Ile
BRCA2	20	8567A>C	p.Glu2856Ala
BRCA1+2	N/A	Very strong family history	N/A
BRCA1	7	441+39T>C, 441+41T>C,?551+44 t>C	Intronic
BRCA1	11	c.855 T>G	p.Leu246Val
BRCA1	10	671-2A>G	Intronic
BRCA1+2	N/A	Very strong family history	N/A
MSH2	11	c.1667T>G	p.Leu556Trp
MSH2	8	c.1355A>T	p.Glu452Val
hMSH2	5	G>A@817 & T>A@818	
hMLH1	16		p.Lys618Ala
hMLH1	2	G>A@199	p.Gly67Arg
hMLH1	10	C>T793	

Case report



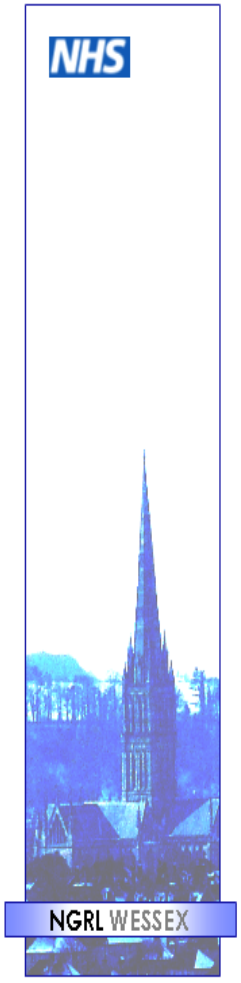
Patient JT

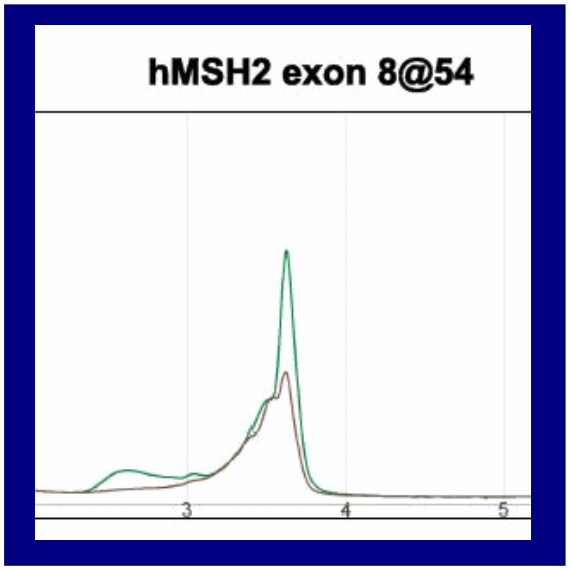
- 55 year old male
- Referred from Cardiff for mutation testing for HNPCC

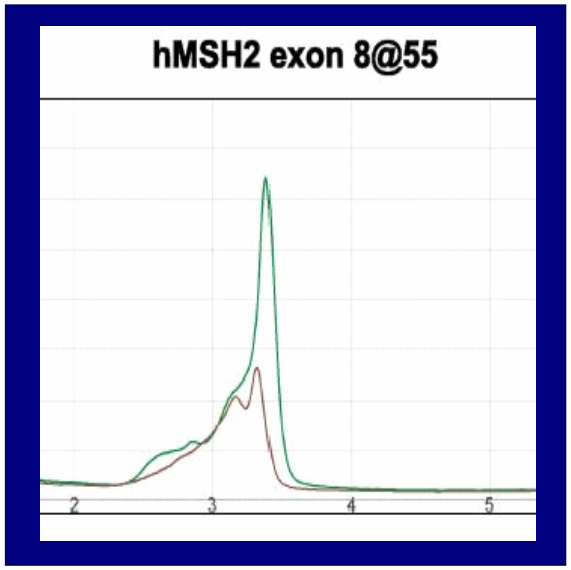
DNA Analysis

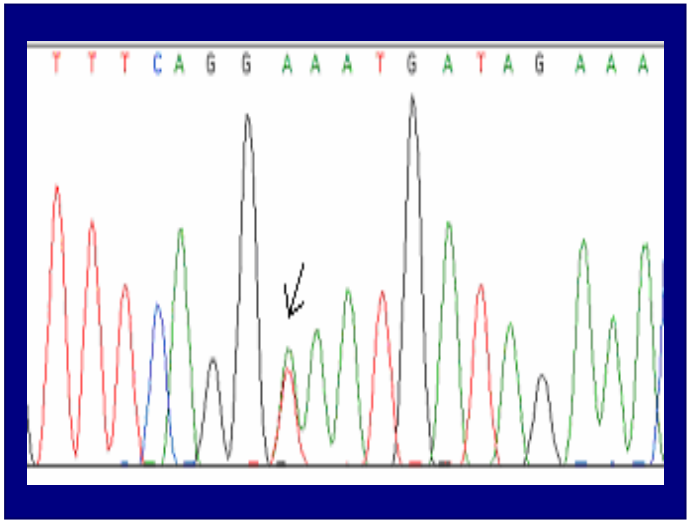
- dHPLC screening of hMLH1 and hMSH2

dHPLC and Sequencing







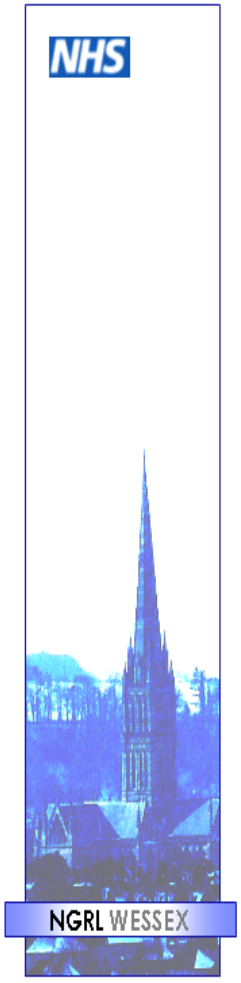


hMSH2 exon 8 missense mutation

c.1355A>T, p.Glu452Val

unknown significance

http://www.fruitfly.org/seq_tools/splice.html



NORMAL

Donor site predictions

Start	End	Score	Exon	Intron
172	186	0.99	ggatcag	gtatgcaa

MUTANT

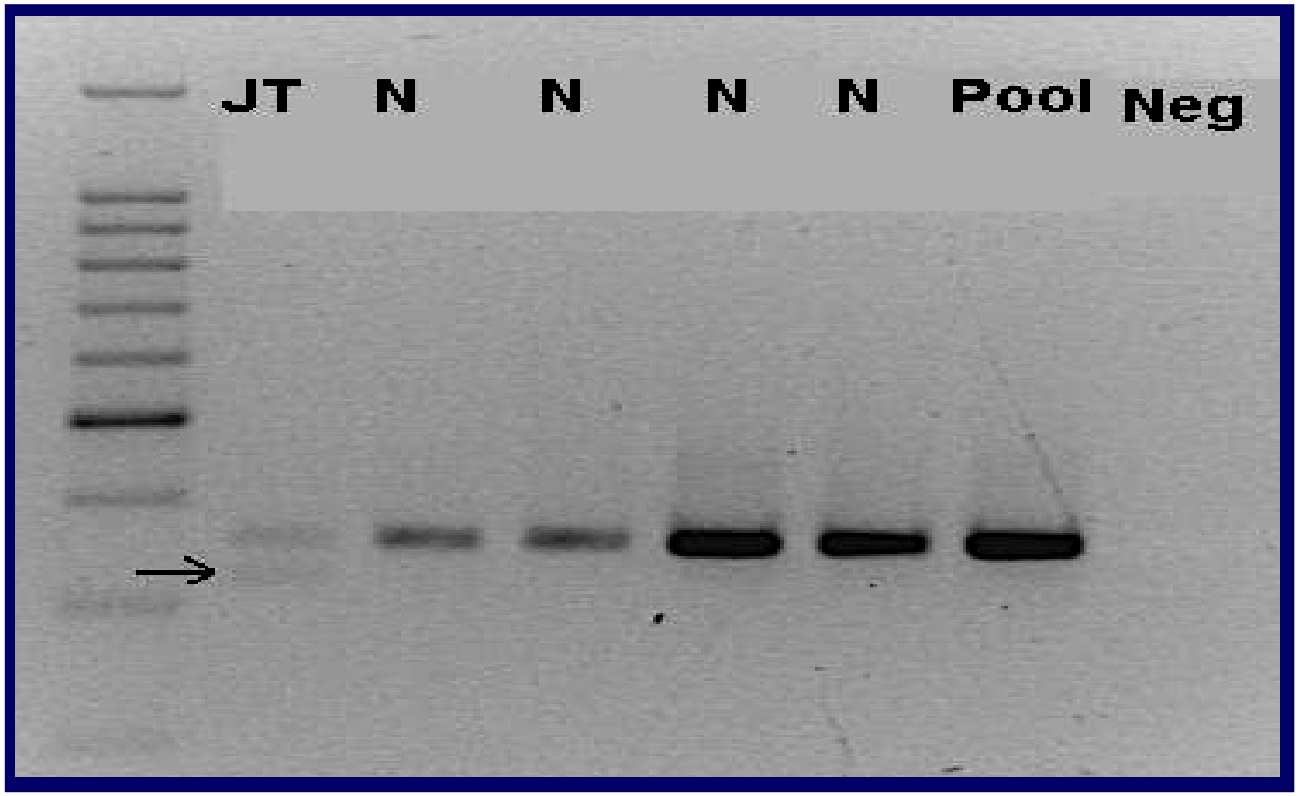
Donor site predictions

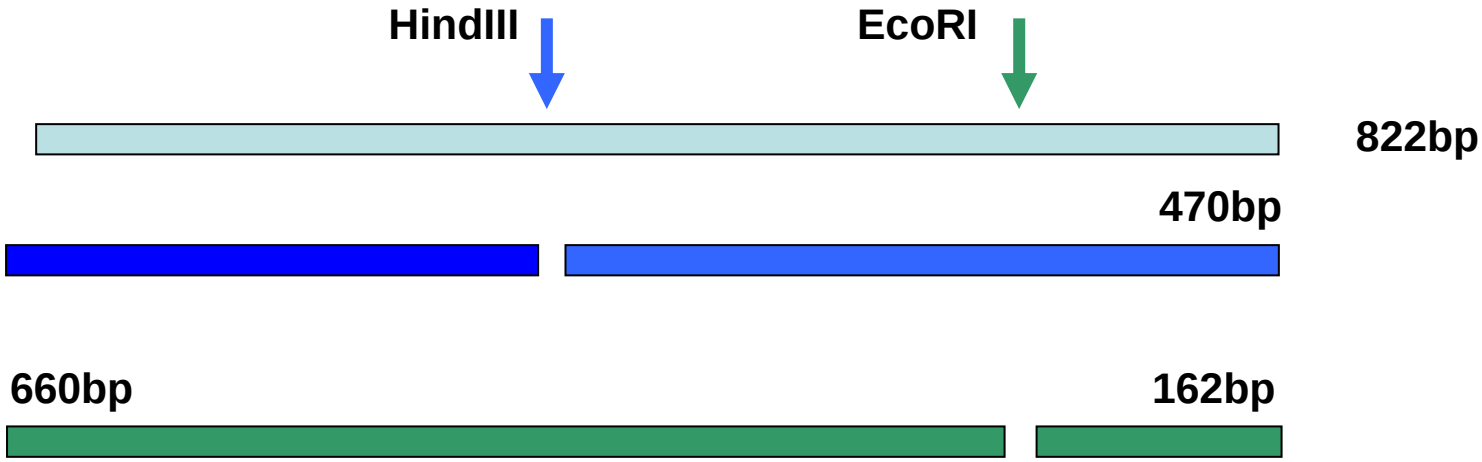
Start	End	Score	Exon	Intron
139	153	0.95	gtttcag	gtaatgat
172	186	0.99	ggatcag	gtatgcaa

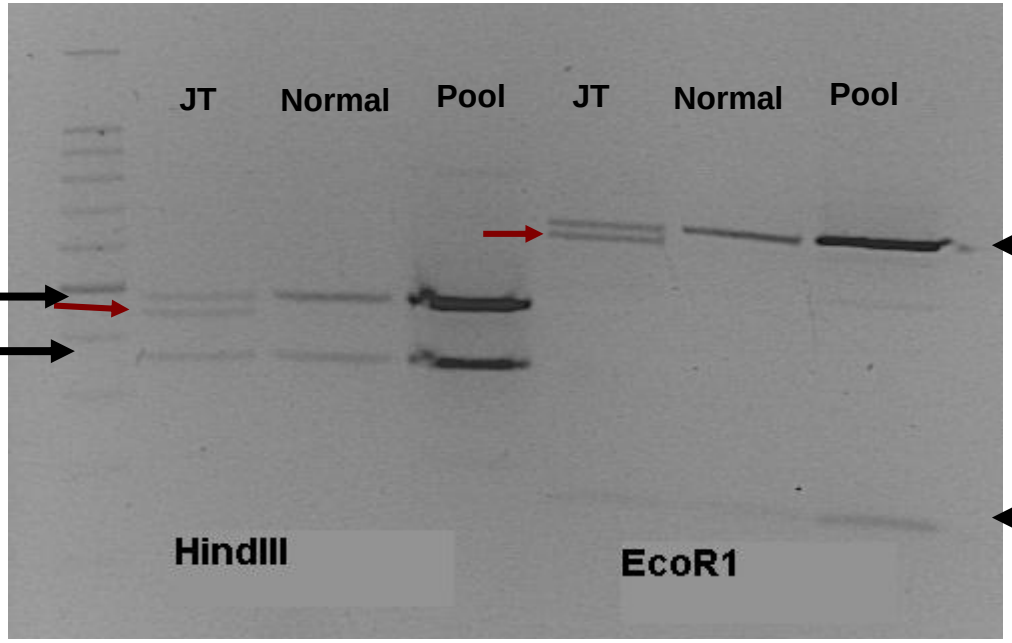
Cryptic splice donor site may be activated by this mutation

RNA Analysis

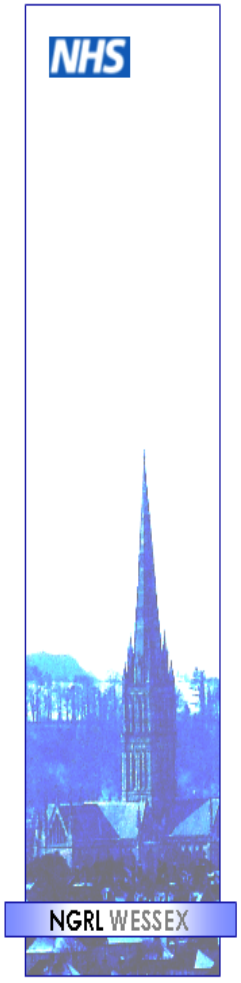
- Fresh EDTA blood sample received
- RNA extracted
- RT-PCR of Exon 7 -10




HindIII
EcoRI
822bp
470bp
660bp
162bp


JT
Normal
Pool
JT
Normal
Pool
470bp
352bp
660bp
162bp
HindIII
EcoR1

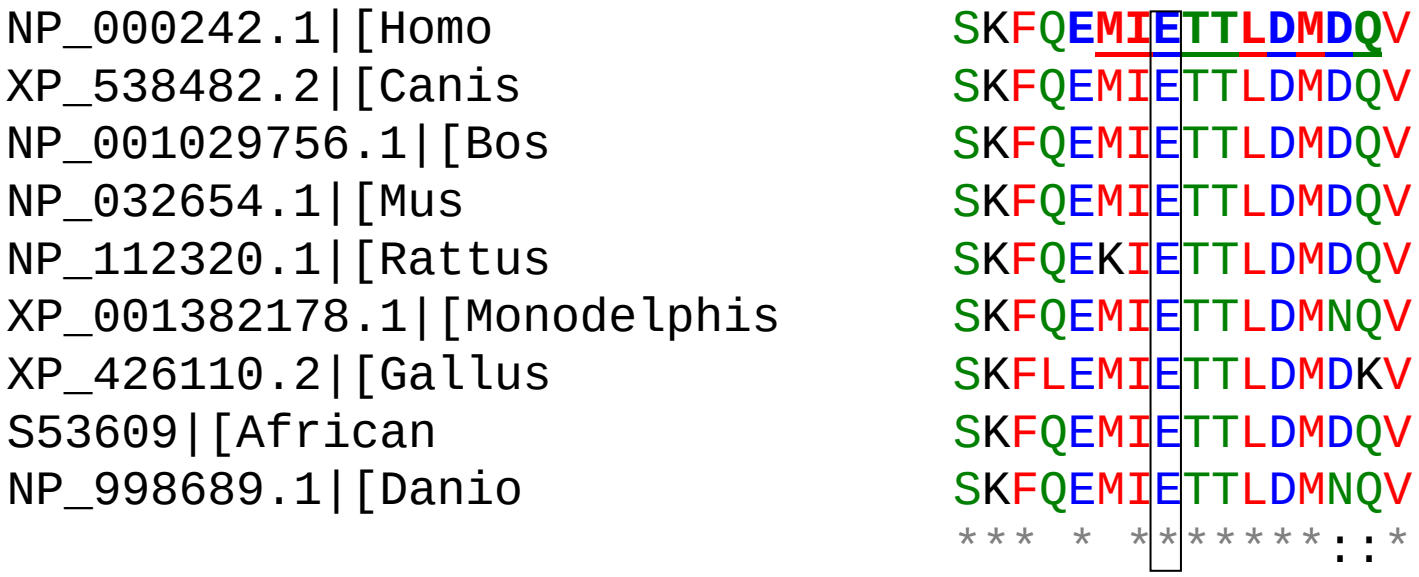
Cloning and sequencing



- **The whole PCR product was cloned**
- **Sequencing of ~30 clones carried out**
- ***c.*50% clones had a 33bp in-frame deletion of the last 11 amino acids of exon 8, p.Glu452_Gln462del**
- **Cryptic donor splice site is being used**
- ***c.*50% clones showed normal splicing, none of these had mutation**

Species conservation and protein structure

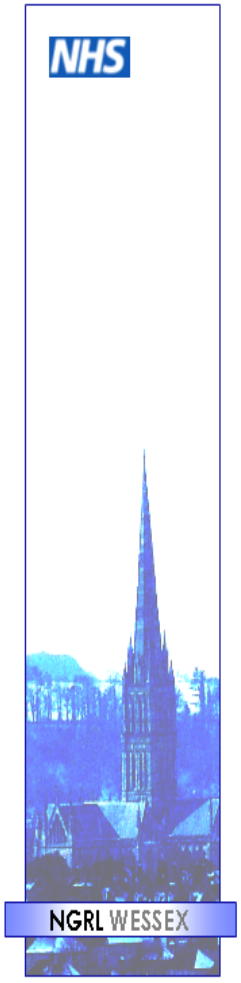
- 11 deleted amino acids highly conserved across species



- Predicted to be within a MutS DNA binding domain, vital for function of the hMSH2 protein as a mismatch repair protein, therefore predicted to be pathogenic

- ICH showed loss of hMSH2 expression in JT's tumours

Conclusions



- c.1355A>T is highly likely to be pathogenic
- Mutation activates a cryptic donor site in exon 8 of hMSH2 leading to a 33bp in frame deletion within a conserved functional domain
- This mutation is likely to account for the loss of expression of hMSH2 seen by ICH (Cardiff)
- DNA/ RNA from other family members would be useful for co-segregation studies

Interpretation problems: Example 1

hMLH1 Exons 1-10 & Exons 11-16:

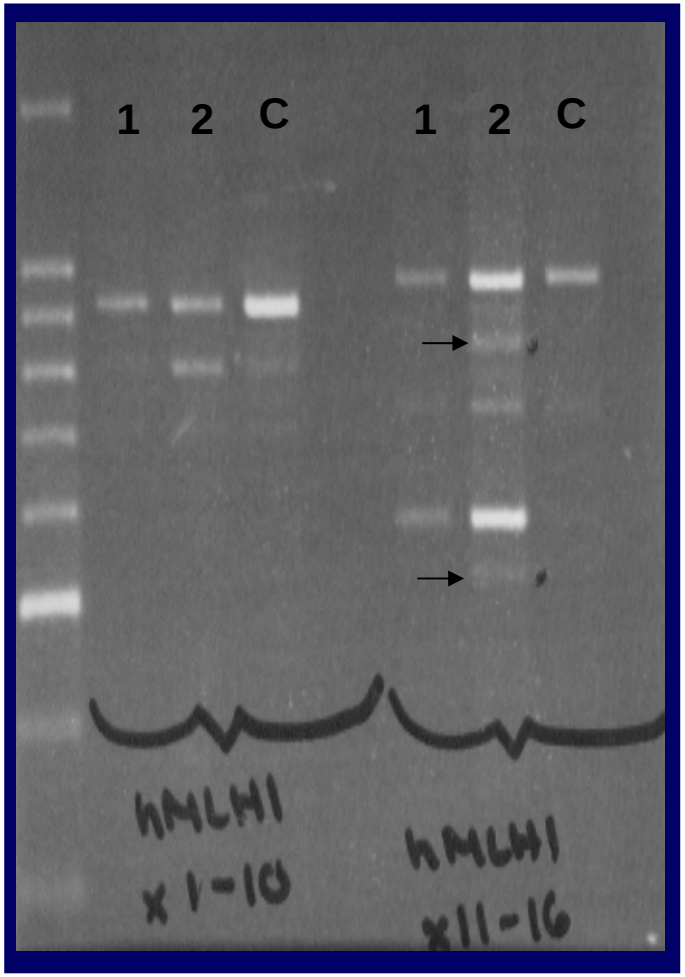
Patient 1 (hMLH1 Exon 16 mutation)

Identical sized transcripts as controls for both PCRs

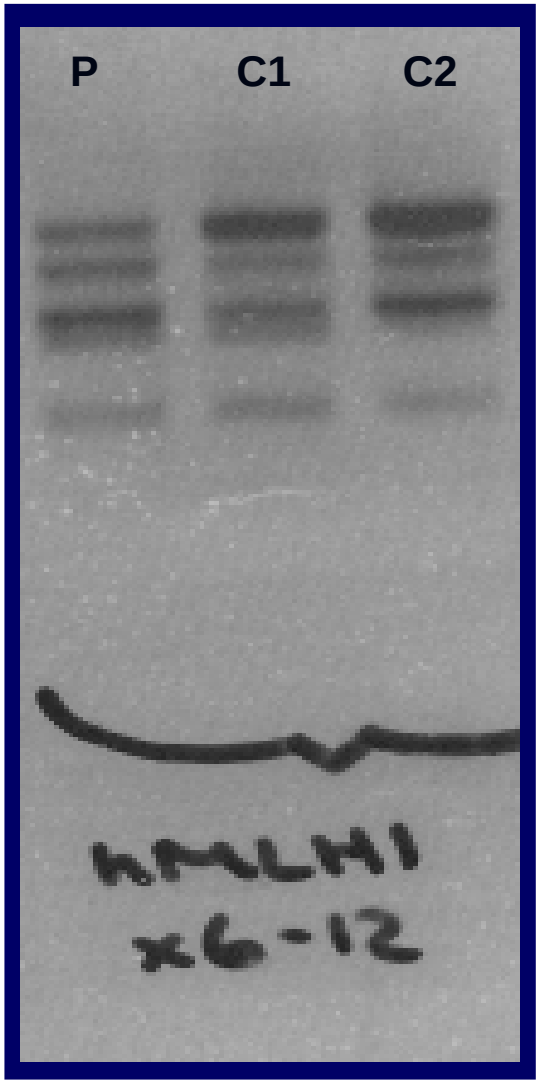
Patient 2 (hMLH1 Exon 2 mutation c.199 G>A)

Same sized transcripts as normal control (C) but different levels of expression for Exons 1 - 10.

But, patient 2 shows 2 differently sized transcripts in the Exon 11-16 PCR even though the mutation is in exon 2. How should you interpret this?



Interpretation problems: Example 2



hMLH1 Exons 6-12

- Control samples C1 and C2

Same sized alternative transcripts but each is apparently expressed at different levels in each control

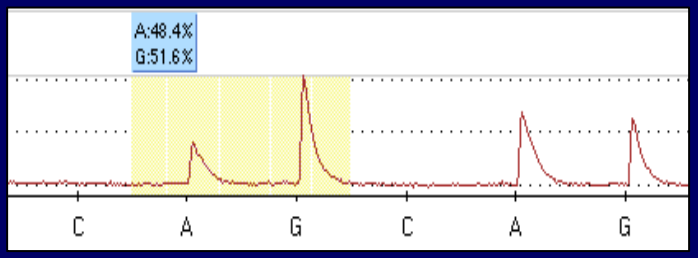
- Patient (P) (hMLH1 Ex10 mutation c.793 C>T)

Same sized transcripts as controls but again the levels of expression of each transcript appear to be different – is this significant, normal population variation or an artefact relating to RNA quality?

Quantification of Allelic Expression (NMD) by Pyrosequencing

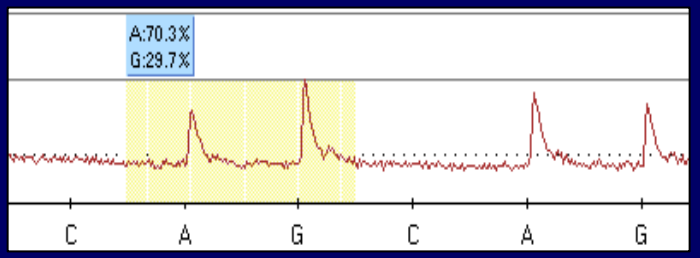
DNA sample

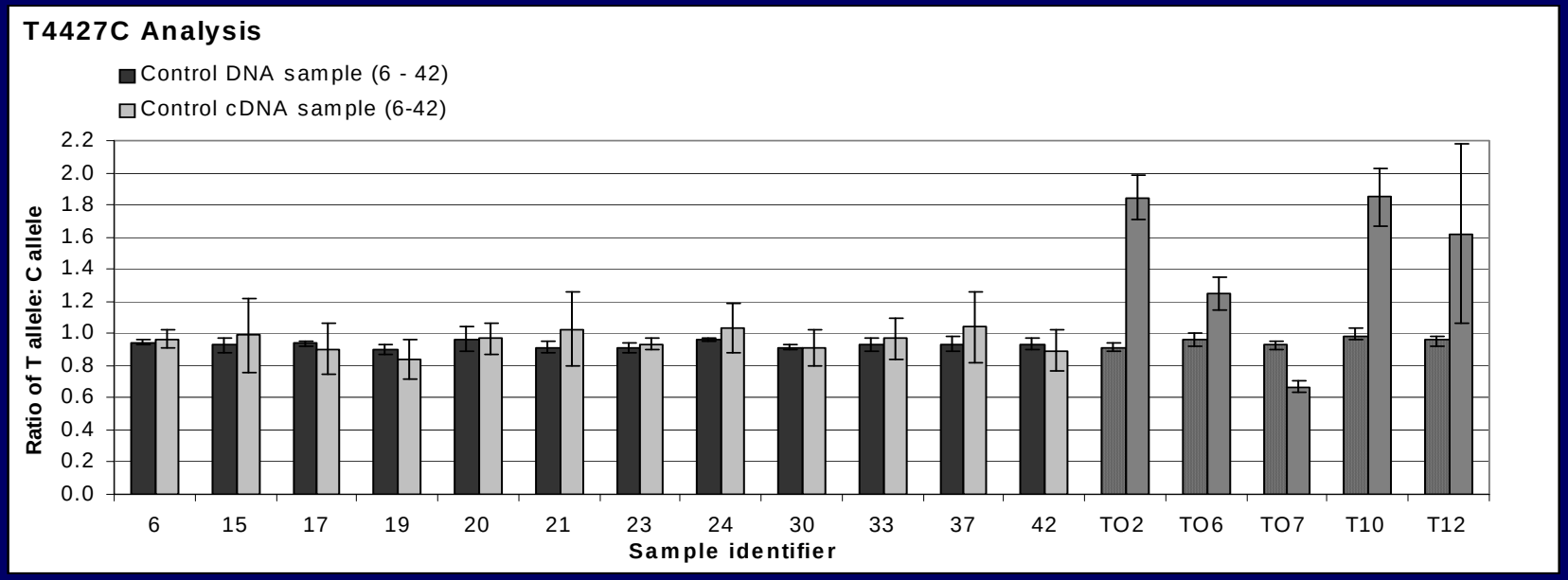
Ratio of C:T is 1:1



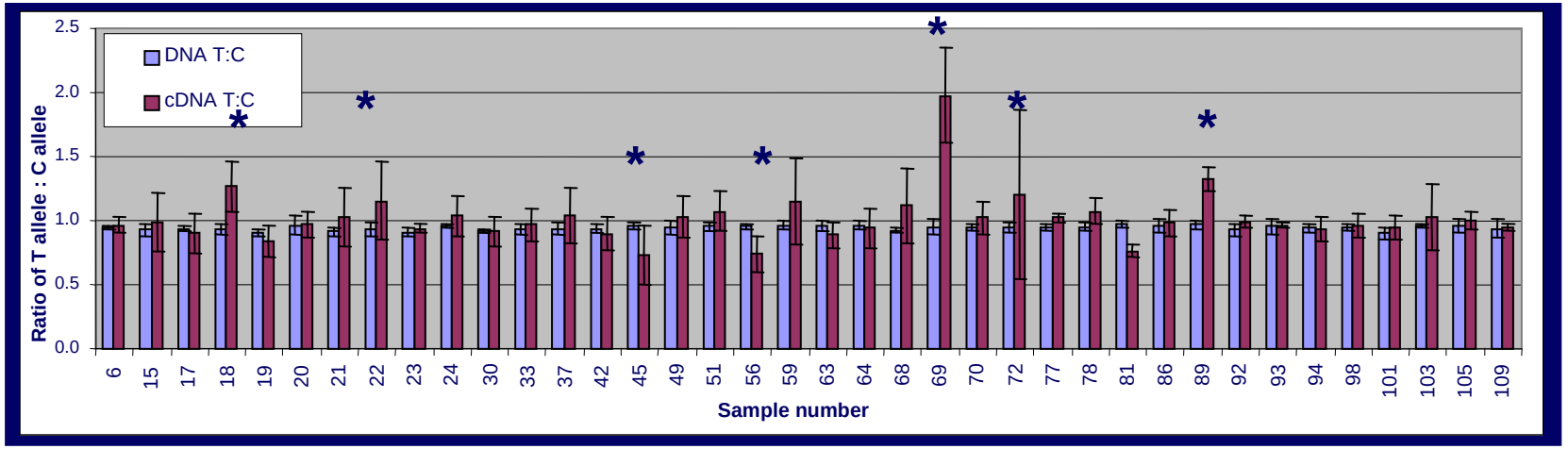
cDNA sample

2.3 fold imbalance

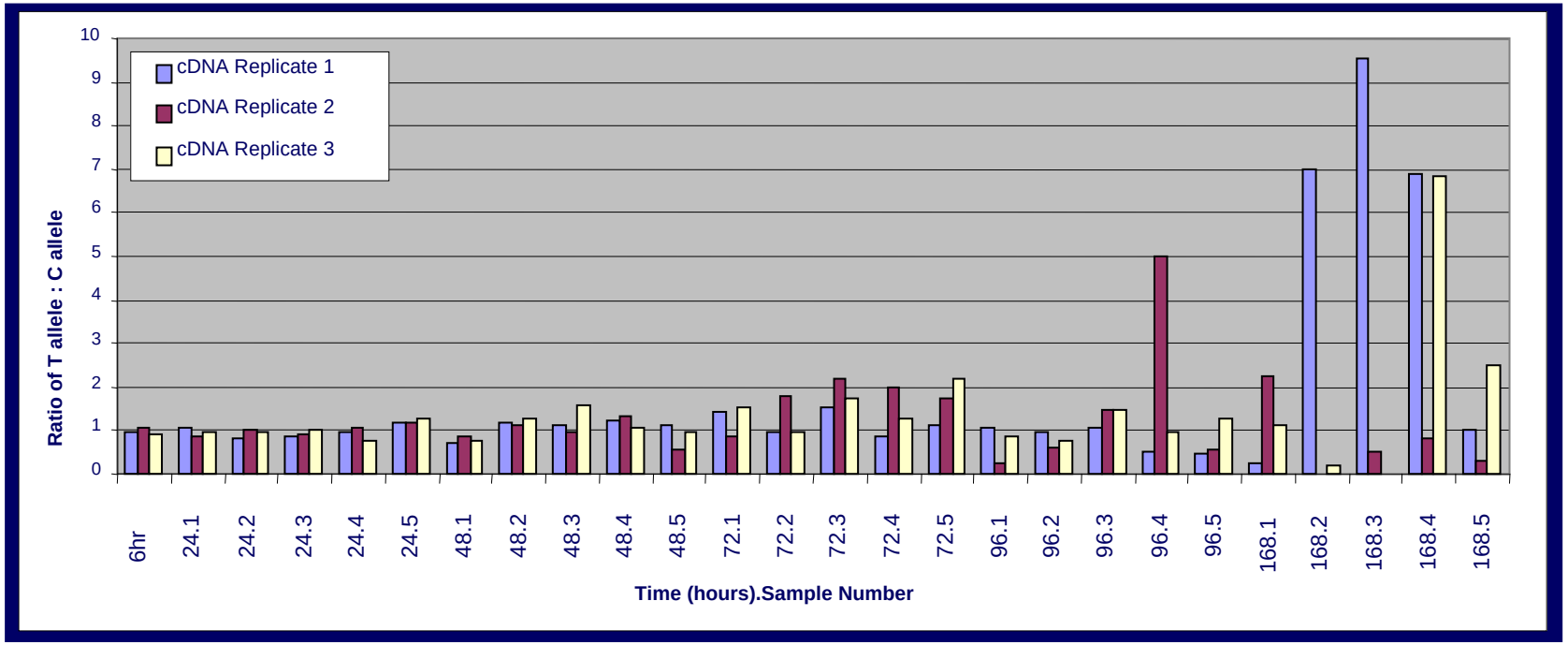




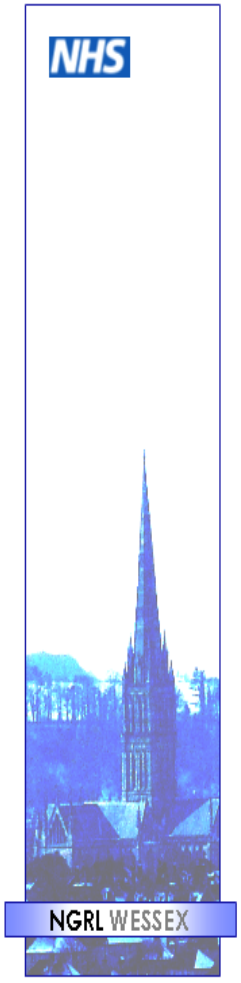
Analysis of normal controls (n=38)



Effect of transit time



Acknowledgements



- Wessex Regional Genetics
  Esta Cross
- National Genetics Reference Laboratory (Wessex)
  Vicky Hall
- Cardiff
  Ian Frayling