

Searching for mutations using whole gene sequencing and MLPA

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Introduction

- MUTYH*-associated polyposis (MAP) is an autosomal recessive disorder characterised by multiple adenomas and colorectal cancer.
- The *MUTYH* gene encodes a DNA glycosylase (protein MYH (hMYH alpha1)) involved in oxidative DNA damage repair. It is made up of 16 exons spanning ~11 kb of genomic DNA.
- Two common mutations (p.Gly396Asp and p.Tyr179Cys) have been estimated to account for 85% of mutations in the Northern European population and p.Glu480X is the most common mutation in the Gujarati population.
- Initial *MUTYH* screening in the laboratory has historically involved testing only for the above three mutations. Sequencing of the entire *MUTYH* coding region was introduced in September 2009 and MLPA in November 2010 (Kit by MRC Holland-P378).

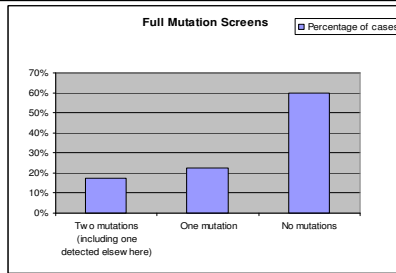


Figure 1: Proportion of affected/ non affected cases received to date

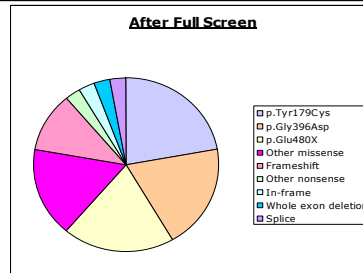


Figure 2: Types of mutation detected in affected cases

Interesting Findings

Patient 1 had a diagnosis of colorectal polyposis and was initially found to be heterozygous for p.Tyr179Cys mutation.

- MLPA revealed an apparent heterozygous deletion of exon 9.
- To investigate the presence of a SNP under the ligation site sequencing analysis using primers flanking exons 8 and 9 (as intron 8 is small) was performed.
- Agarose gel electrophoresis showed two products (Figure 4).
- Sequencing (Figure 5) confirmed that exon 9 and some surrounding intronic sequence had been deleted.

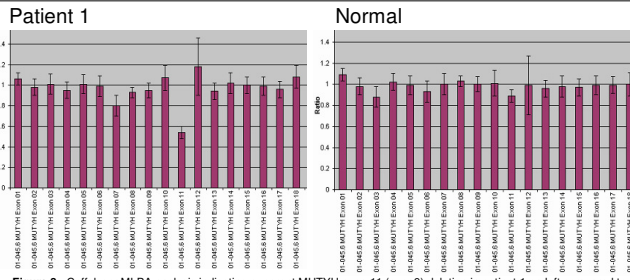


Figure 3: Coflyser MLPA analysis indicating apparent *MUTYH* exon 11 (exon9) deletion in patient 1 on left compared to normal control on right

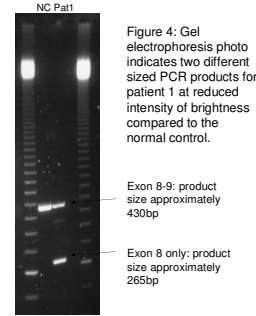


Figure 4: Gel electrophoresis photo indicates two different sized PCR products for patient 1 at reduced intensity of brightness compared to the normal control.

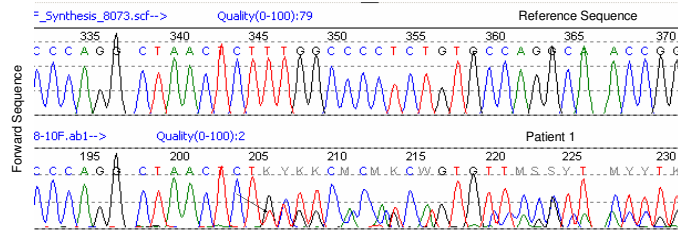


Figure 5 Arrows indicate the breakpoints between exon 8 and 10 for patient 1.

Patient 2 had a diagnosis of colorectal polyposis. A common mutation screen found a single copy of the mutation p.Gly396Asp. MLPA analysis found an apparent duplication of *MUTYH* exon 10 (Figure 6). PCR using 9F and 11R showed no obvious second product, reducing the likelihood of a simple duplication (scenario 1, figure 7).

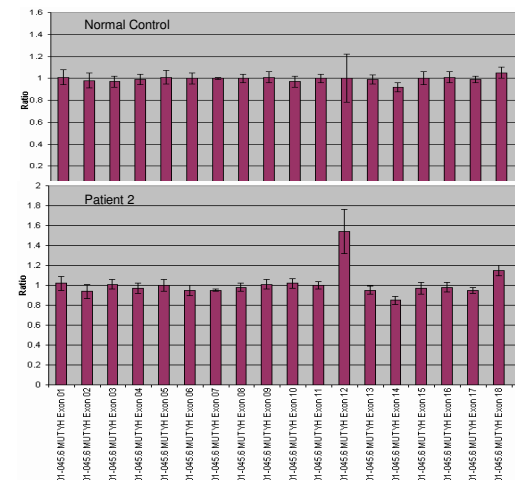


Figure 6: An apparent duplication of *MUTYH* exon 12 in Patient 2 compared to a normal control

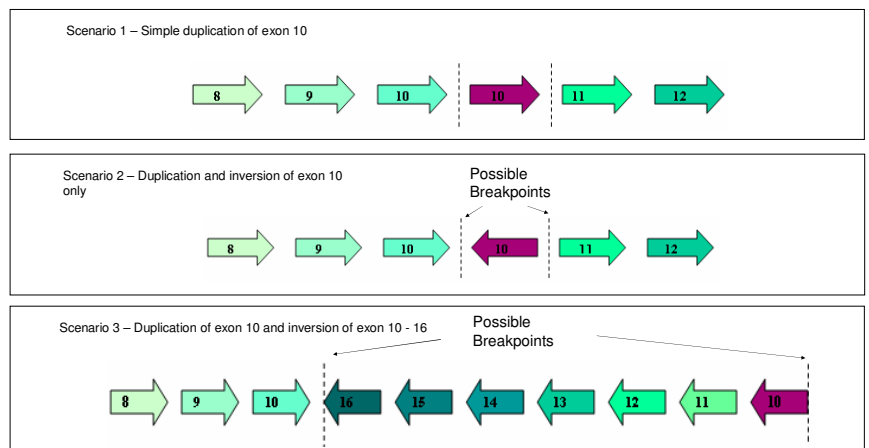


Figure 7: Possible hypothesis for location and orientation of the exon 10 duplication

Conclusions/Further Work

MLPA is now used routinely as part of *MUTYH* full gene analysis. Full gene analysis and MLPA can also be performed for patients with a strong clinical diagnosis or a partner affected with *MUTYH*. Further investigations are continuing on patient 2 to determine if the MLPA result is caused by scenario 2 or 3 in figure 7 or an alternative cause.