

Characterisation of the deletion breakpoints in Familial Hypercholesterolemia families with a common deletion of exons 2-6 of the LDLR gene

Frances White¹, Julie Sibbring¹, Alison Taylor³, Janice Harland² and Roger Mountford¹

¹Merseyside and Cheshire Regional Molecular Genetics Service, Liverpool Women's Hospital, Crown Street, Liverpool. L8 7SS.

²School of Pharmacy and Biomolecular Sciences, Byrom Street, Liverpool, L3 3AF

³N.E Thames Regional Genetics Service, Great Ormond Street Hospital, London, WC1N 3JH

BACKGROUND

Familial Hypercholesterolemia (FH) is an autosomal dominant condition resulting in high serum cholesterol levels and a significantly increased risk of early onset cardiovascular disease. It has an incidence of 1 in 500 and is principally caused by mutations in the Low-Density Lipoprotein Receptor (LDLR) gene.

- Initial analysis of a cohort of 15 FH patients using the Elucigene FH20 kit followed by sequence analysis together with detection of large scale rearrangements of the LDLR gene using MLPA, identified two families with a heterozygous deletion of exons 2-6.
 - In total five affected members from the two families had the deletion
 - Three more cases were found to have the same exons deleted when a further 52 patients were tested.
 - This deletion is the most common mutation in the LDLR gene detected to date in the North-West Region of the UK.
- An additional 4 cases with this deletion were referred by the North East Thames Regional Genetics Service, for breakpoint analysis.

METHODS

Multiplex Ligation-dependent Probe Amplification-MLPA

Stored DNA samples were tested to confirm the deletion.

Long-Range Polymerase Chain Reaction

A Long-Range PCR approach was used to amplify across the breakpoint to determine the approximate size of the deletion.

Intron 1 is >10Kb in size and intron 6 is approx. 2Kb in size as shown below in figure 1.

Forward and reverse primers were designed from the respective introns (2 & 6) and used in different combinations to identify as small a sized product as possible that spanned the breakpoint.

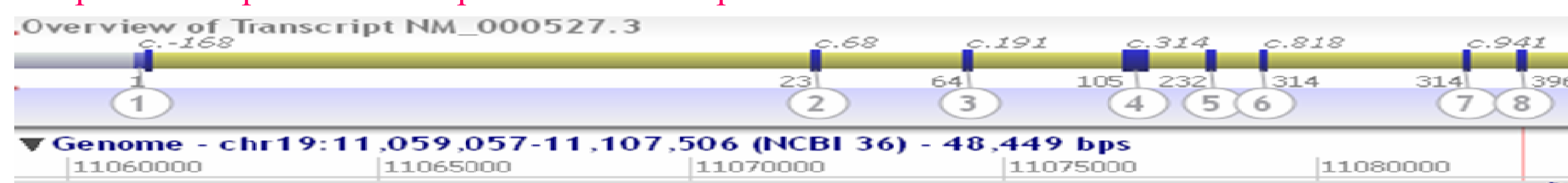


Figure 1.(taken from Alamut). Blue boxes—Exons Yellow boxes—Introns

Sequencing

Purified PCR product was sequenced with internal primers across the breakpoint.

RESULTS

MLPA – Confirmed deletion of exons 2-6 in LDLR gene in all 12 patients.



Figure 2 MLPA results: a) Normal patient

b) patient with LDLR exon 2-6 deletion

Long-Range PCR - PCR products of approx. 10Kb, 2.5Kb and 300bp were obtained. All 12 patients had the same sized products

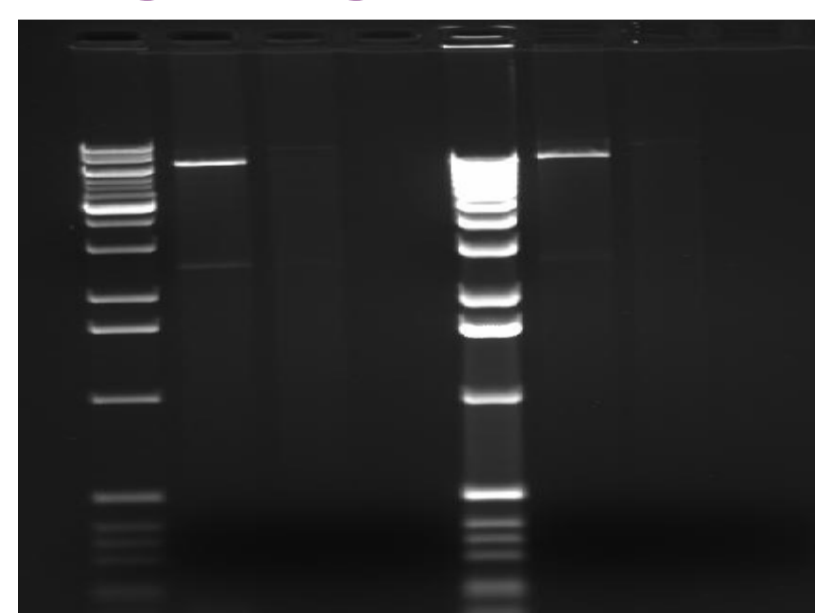


Figure 3. >10Kb PCR product

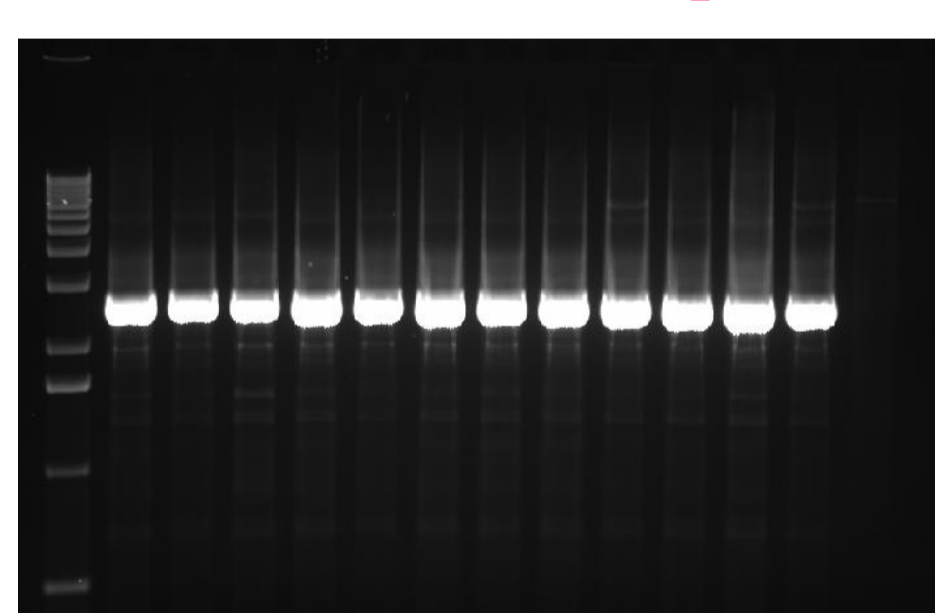


Figure 4. ~2.5Kb PCR product

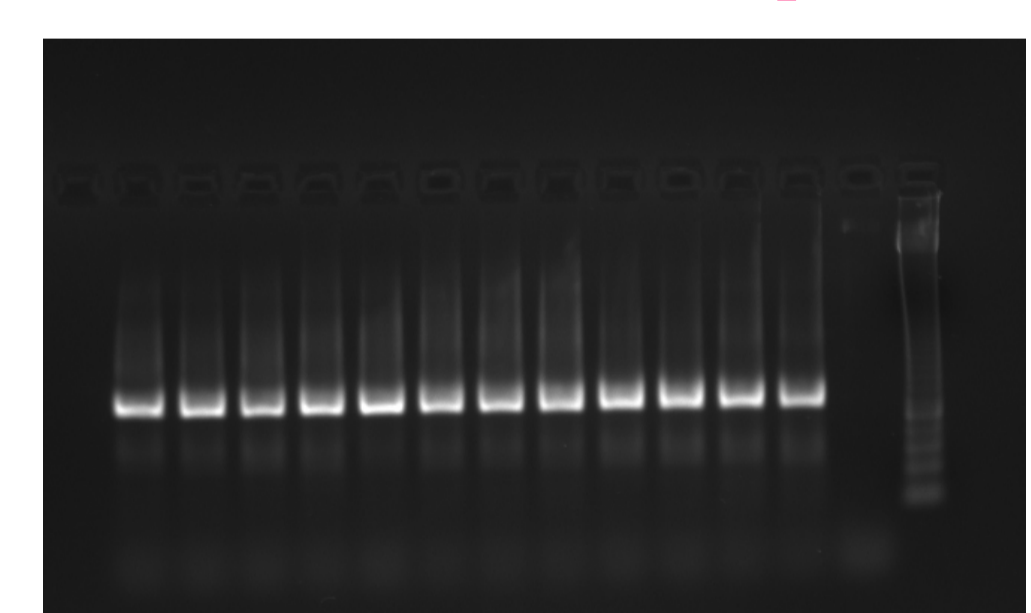
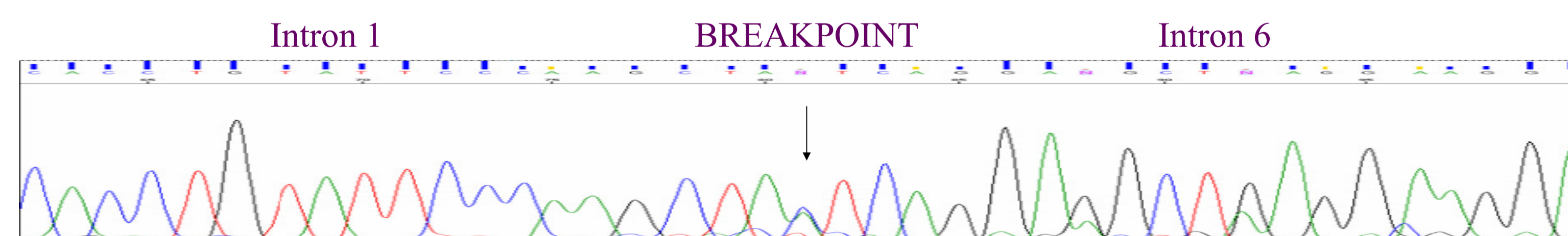


Figure 5. ~300bp PCR product

Sequencing - Breakpoint was identified from sequencing 300bp purified PCR product at c.68-2467_940+1229del10,999.



DISCUSSION AND CONCLUSIONS

- All 12 patients have exactly the same breakpoint.
- The breakpoint occurs in a region of highly repetitive DNA sequence.
- Further work is ongoing looking at polymorphic loci flanking the breakpoint to determine whether all 12 cases represent a single ancestral mutation or whether the region is prone to produce the same deletion.
- The aim now is to design an assay that allows other family members a simple and cost effective test as part of cascade screening in FH rather than use MLPA. It may also be possible to integrate a test for this deletion into the initial screen for “common” mutations given its high frequency in our population.