

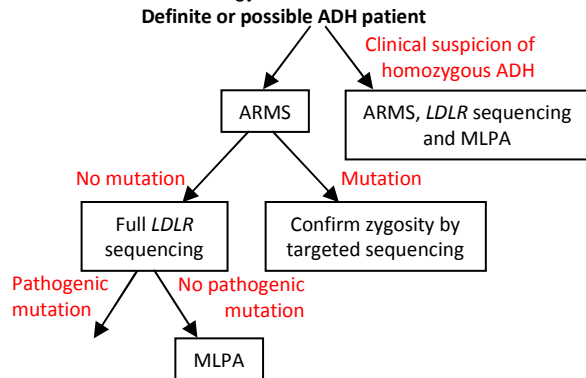


1. Introduction

Autosomal dominant hypercholesterolaemia (ADH) encompasses monogenic conditions characterised by elevated low density lipoprotein cholesterol (LDL-C) resulting in increased risk of coronary heart disease and shortened life expectancy if untreated. Within the UK this condition is 75-85% undiagnosed. The genes mutated in ADH are *LDLR* (79.1%), *APOB* (5.5%) and *PCSK9* (1.5%). In 2008 the National Institute for Clinical Excellence (NICE) recommended that genetic testing be performed as part of diagnostic and cascade screening for ADH. This study sought to evaluate a new microarray, LIPOchip® V.10 (Progenika), for use in ADH genetic testing.

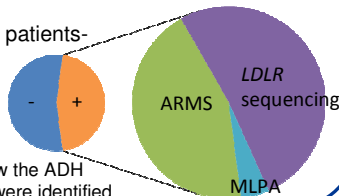
2. Existing diagnostic testing for ADH

1. Current Bristol strategy



2. Mutations identified in 64/139 patients-

- 28 identified by ARMS
- 33 identified by sequencing
- 3 identified by MLPA



Pie chart shows how the ADH causing mutations were identified

4. Method

36 samples from possible or definite ADH patients previously tested by ARMS/*LDLR* sequencing/MLPA were used in this evaluation. The cohort of samples consisted of:

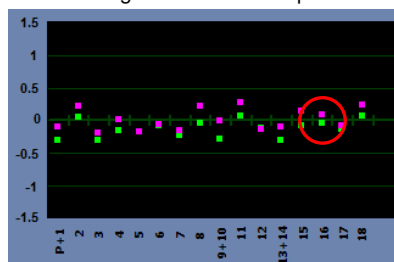
- 21 samples with point mutations detectable by LIPOchip®
- 5 samples with whole exon duplications
- 2 samples with whole exon deletions
- 6 samples with no mutations detected by ARMS/*LDLR* sequencing/MLPA
- 2 samples with mutations similar but not identical to those detectable by the LIPOchip® (c.2093G>T where LIPOchip® detects c.2093G>A and c.11G>A where LIPOchip® detects c.12G>A)

6. Results- Exon copy number changes

Exon 7-18 deletion correctly identified



False negative- exon 16 duplication



- Only 2/7 exon copy number changes were correctly identified, therefore it appears that the LIPOchip® cannot fully replace MLPA testing.
- Both of the correctly identified exon copy number changes were deletions.

7. Results- Cost analysis (calculated from current reagent quotes and staff time costs)

- The cost of diagnostic testing of the cohort of 139 patients by the current screening method was approximately £33K.
- Testing the same patients using a LIPOchip®/*LDLR* sequencing/MLPA strategy would cost approximately £46K.
- As >50% of ADH referrals are mutation negative (see boxes 2&3), the majority of patients require all diagnostic tests.

8. Conclusions

- LIPOchip® appears to be effective at detecting point mutations, however the detection of variation in exon copy number was not satisfactory.
- LIPOchip® could potentially replace ARMS as a first line screen for diagnostic screening but MLPA would still be required.
- A LIPOchip®/*LDLR* sequencing/MLPA strategy appears less cost effective than the current screening protocol based on current quotes.

3. LIPOchip® and diagnostic testing

LIPOchip® was designed to allow detection of 189 ADH causing point mutations (182 in *LDLR*, 3 in *APOB* and 4 in *PCSK9*) which is claimed to represent 80.5% of UK ADH-causing mutations.

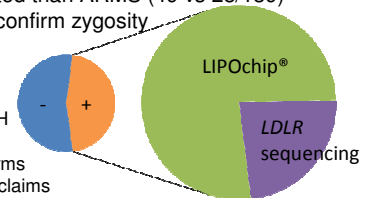
Point mutation detection uses the equation below to determine mutation status and zygosity. Ratio of 1 indicates no mutation, 0.5 indicates heterozygote and 0.1 indicates a homozygote.

$$\text{ratio} = \frac{\text{signal from normal probe}}{\text{signal from normal probe} + \text{signal from mutant probe}}$$

LIPOchip® also allows detection of exon copy number changes in *LDLR* (around 5% of ADH).

Potential advantages of LIPOchip® as a first line test:

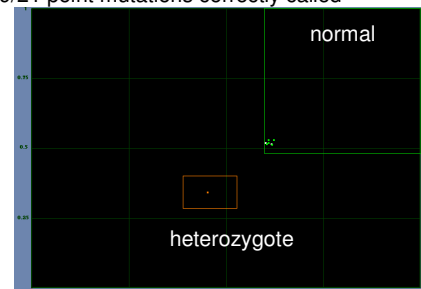
- More point mutations detected than ARMS (49 vs 28/139)
- No need for sequencing to confirm zygosity
- No need for MLPA



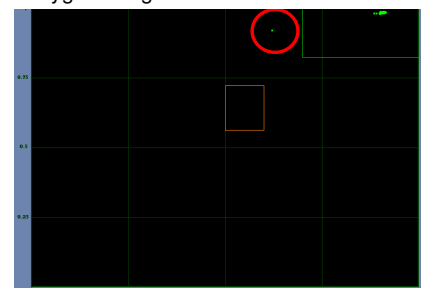
Pie chart shows how the ADH causing mutations would be identified if LIPOchip® performs according to manufacturer's claims

5. Results- Point mutations

- 19/21 point mutations correctly called



- 2/21 point mutations required sequencing to confirm, as results fell outside both the normal and heterozygote ranges.



- No point mutations missed and no false positives
- c.2093G>T could also be detected (sequencing was required for confirmation).