

THE IMPACT OF ROUTINE NEXT GENERATION SEQUENCING TESTING FOR FAMILIAL HYPERCHOLESTEROLAEMIA – 5 MONTHS SERVICE EXPERIENCE

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NICE recommends comprehensive genetic testing in all patients clinically diagnosed with FH and genetic cascade testing of at-risk relatives, however, the cost of FH genetic testing still remains a barrier to commissioning.

Bristol Genetics Laboratory (BGL) has developed a comprehensive, high throughput diagnostic genetic test for FH using next generation sequencing. The custom-designed targeted capture assay (HaloPlex, Agilent) sequences 4 FH genes; *LDLR*, *PCSK9*, *APOB* and *LDLRAP1* and the *SLCO1B1* variants (*rs2306283* and *rs4149056*) associated with statin-induced myopathy. A control gene is included to aid copy number (deletion/duplication) detection. Data analysis uses an open-source pipeline; alignment (bwa), variant calling (GATK), variant annotation (Geneticist Assistant, SoftGenetics), and copy number analysis (CONTRA/bespoke CNV tool).

BGL has provided FH testing since 2008 and our patient cohort now exceeds 900. The NGS service was launched in October 2013, with parallel MLPA testing to further validate copy number detection. To date, 130 patients have been reported with 33 distinct mutations and a 30% (39/130) positive detection rate, the most common mutations being *APOB* c.10580G>A and *LDLR* c.313+1G>A. NGS copy number analysis successfully detected 5 *LDLR* deletions and generated a reportable copy number result in 71% of cases. MLPA will now be used as a reflex test where NGS data is equivocal, generating cost savings.

A further 9% (12/130) of patients have variants of unknown significance (VUS) with 11 of these found in *APOB*. Comprehensive *APOB* screening was precluded prior to NGS due to the large size of the gene. Recent literature evidence and our service data suggest that there are clinically significant variants outside of the exon 26 hotspot region supporting a comprehensive screening approach.

Current cost of NGS diagnostic testing is £250, which may reduce further with increased throughput. We report on our service experience, and future prospects illustrated by interesting case studies.

GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) IN FAMILIAL HYPERCHOLESTEROLAEMIA (FH): CAN FAMILY BASED ASSOCIATION STUDIES HELP DETERMINE PATHOGENICITY?

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Genetic testing of 1191 index patients from lipid clinics in the Wales FH service revealed a pathogenic mutation in 23% and a genetic variant of uncertain significance (VUS) in 8% (102 individuals, 67 variants). A VUS is a DNA sequence variant for which there is insufficient laboratory or clinical evidence to designate the variant as causative or not. Finding a VUS leaves the diagnosis uncertain for patients and cannot be used for family cascade testing.

In this study family members are tested for LDL-cholesterol and VUS with the aim of assessing whether the variant tracks with LDL-C. Specialist

genetic association statistical analysis can be used to quantify the likelihood of pathogenicity based on the family relationship, number of subjects, and LDL-C (with adjustment for age and gender), allowing for the genetic relatedness between family members. Data from families who share the same VUS can be combined. Data from 51 family members with 11 different variants has been collected to date, concentrating first on those with more extensive families available for testing.

All available members have been tested in three families, with each family having a different VUS in the *LDLR* gene. Analysis showed the VUS to be significantly associated with LDL-C in two families (c.2087G>A p=0.007) (c.1073G>A; p=0.002), but not in the other (c.2098G>A; p=0.14) indicating that in two families the variant is likely to be pathogenic whereas in the third it is not.

This study demonstrates the feasibility and value of a quantitative statistical approach to family studies compared to qualitative segregation studies which do not take into account the concentration of LDL-C.

This approach provides useful additional evidence for the genetic diagnostic laboratory which can be shared with other centres using anonymous genetic data bases for FH. The information helps clinicians provide more clarity for their patients and families.

THE IMPORTANCE OF CONSIDERING LOW-DENSITY LIPOPROTEIN CHOLESTEROL RESPONSE AS WELL AS CARDIOVASCULAR RISK IN DECIDING WHO CAN BENEFIT FROM STATIN THERAPY.

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Introduction: Statins are in many countries the most frequently prescribed class of medication. In clinical trials statins achieved a mean reduction in low density lipoprotein cholesterol (LDL-C) of 1.07mmol/l and decreased atherosclerotic cardiovascular disease (CVD) incidence by 24% relative to placebo. Guidelines seeking to deploy statin treatment optimally rely heavily on the use of estimates of absolute CVD risk as an arbiter of who should receive statins. **Aim:** To demonstrate that this is not an effective strategy unless the LDL cholesterol response to statin treatment, which is determined by the choice of statin, its dose and the pre-treatment LDL cholesterol level, is taken into account.

Method: The formula for calculating NNT to prevent one CVD event with statin therapy, taking into account the decrease in LDL cholesterol achieved, is derived as follows:-

Events prevented per 100 people (ε) = Absolute CVD risk* x LDL cholesterol decrease** x 0.22

$NNT = 100 \div \epsilon$

$NNT = 100 \div (\text{absolute CVD risk} \times \text{LDL cholesterol decrease} \times 0.22)$

*% over next 10 years. s**mmol/l.

Results: We show that this is easily achieved and can be integrated to cardiovascular risk software. Application of this evidence reveals that many people with high LDL cholesterol levels can benefit more than people currently receiving statin treatment solely on the basis of their absolute CVD risk, whereas others at higher CVD risk, but with lower LDL cholesterol, will derive little benefit.

Conclusion: Taking pre-treatment LDL cholesterol, absolute risk and number need to treat in consideration when deciding who should receive statin therapy will lead to a better patient selection.

GENDER REASSIGNMENT AND CARDIOVASCULAR RISK

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Background: Gender reassignment involves psychological, hormonal and surgical interventions to achieve transition to the opposite gender.