Abstracts Presented in the Medical & Scientific Programme

FAMILIAL HYPERCHOLESTEROLAEMIA (FH) IN WALES IS GENETICALLY HETEROGENEOUS

K. Haralambos 1*; S.D. Whatley 1; B.N. Datta 2; D. Townsend 3; R. Gingell 3; R. Edwards 1; P. Lansberg 4; C. Graham 5; L. Palacios 6; P. Dean 7; I.F.W. McDowell 2

1 Cardiff University, Cardiff, UK; 2 University Hospital of Wales, Cardiff and Vale Health Board, Cardiff, UK; 3 All Wales FH Cascade Testing Service, All Wales Medical Genetics Service, Cardiff, UK; 4 Academic Medical Centre, Amsterdam, The Netherlands; 5 Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, UK; 6 Progenika Biopharma SA, Spain; 7 Bristol Genetics Laboratory, North Bristol NHS Trust, UK

* Corresponding author.

A clinical scoring system has been developed for the Wales FH service as a guide for clinicians to determine whether lipid clinic patients with a clinical diagnosis of possible FH are suitable for FH genetic testing. This is based on a modification of the Dutch Lipid Clinic Network scoring criteria. The scoring system was applied prospectively to index patients who underwent genotyping. A sequence variant was identified in 270/780 FH index patients (35%). In patients who scored 13 or more a pathogenic variant was identified in 81%. This decreased progressively with lower scores in those who scored 5 or less. With scores of 6, 7–8, 9–10, 11–12 the pickup rate was 9%, 16%, 38%, and 51% respectively. A total of 120 different variants have been identified in 3 genes – 88% in LDLR gene, 11% in APOB, 1% in PCSK9. One patient was homozygous and 3 were found to be compound heterozygotes. 42 distinct variants (8% of variant positive patients) are currently classified as variants of uncertain significance (VUS). Studies are ongoing with the aim of further refining the clinical significance of these variants.

The five most common genetic variants accounted for 25% of variant positive patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Identifier</th>
<th>Protein Identifier</th>
<th>% of variant positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOB</td>
<td>c.10580C&gt;A</td>
<td>p.Arg3527Gln</td>
<td>9%</td>
</tr>
<tr>
<td>LDLR</td>
<td>c.301G&gt;A</td>
<td>p.Glu101Lys</td>
<td>5%</td>
</tr>
<tr>
<td>LDLR</td>
<td>c.1816G&gt;T</td>
<td>p.Ala606Ser</td>
<td>4%</td>
</tr>
<tr>
<td>LDLR</td>
<td>c.2042G&gt;C</td>
<td>p.Cys681Ser</td>
<td>4%</td>
</tr>
<tr>
<td>LDLR</td>
<td>c.3133G&gt;T</td>
<td>–</td>
<td>3%</td>
</tr>
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</table>

A total of 25 variants have been reported for the first time from this Wales cohort of which 15 have been published previously and the remaining 10 are listed as follows: APOB: c.10251C>T; LDLR: c.68-35G>A; LDLR: c.418G>C; LDLR: c.887G>T, LDLR: c.888C>G, LDLR: c.1156G>T, LDLR: c.1823C>A, LDLR: c.1524C>C, LDLR: c.220G>A, LDLR: c.2301G>A.

The genotyping scoring system is a useful tool to help predict the likelihood of identification of an FH-causing variant. In Wales FH is a genetically heterogeneous condition.

GENETIC TESTING OF FAMILIAL HYPERCHOLESTEROLAEMIA AT BGL – A FOUR YEAR AUDIT

L. Yarram 1; M. Greenslade 1; G. Bayly 2; M. Balasubramani 2; A. Taylor 3; A. Day 4; S. Whatley 1; I. McDowell 2; M. Williams 1

1 Bristol Genetics Laboratory, Pathology Sciences, Southmead Hospital, Bristol, United Kingdom; 2 Clinical Biochemistry, Bristol Royal Infirmary, Bristol, United Kingdom; 3 Diabetes and Endocrinology, Royal United Hospital, Bath, United Kingdom; 4 Chemical Pathology, Weston General Hospital, Weston-super-Mare, Somerset, United Kingdom; 5 University Hospital of Wales, Cardiff, United Kingdom

The Bristol Genetics Laboratory has provided a comprehensive genetic testing service for Familial Hypercholesterolaemia for four years. Cases are referred through lipid clinics; genetic testing is offered to dFH/pFH cases (Simon Broome criteria). Level 1 testing is sequencing of LDLR, ApoB exon 26 mutation hotspot (codons 3385–3570), PCSK9 exon 7 and MLPA of LDLR for copy number changes. Level 2 sequencing includes full sequencing of PCSK9 available to patients meeting Simon Broome criteria that are negative following Level 1 analysis. In 2013 BGL will be replacing level 1 and 2 diagnostic testing with a comprehensive test using next generation sequencing.

Over a four year period 654 index cases were tested; a variant was identified in 217 of these (33%). 123 distinct variants were identified; this includes 35 novel variants (not previously reported to the LDLR locus specific database http://www.ucl.ac.uk/ldlr/Current/). 7 of these novel variants are large duplications/deletions or frameshift variants and are therefore classified as pathogenic. The clinical significance of the 28 remaining novel variants were assessed using bioinformatic web-based tools: SIFT, PolyPhen and AGVGD (functional studies are not performed) and by the use of segregation analysis. Data on the outcome of these investigations will be presented.

A lower than expected referral rate for cascade testing has been observed, with only 151 cascade tests being performed, this may reflect the lack of available commissioning funding in the South West of England. We will present an audit of four years of genetic testing for FH at BGL, illustrated by interesting cases.

THE USE OF NEXT GENERATION SEQUENCING FOR DETECTION OF MUTATIONS IN FAMILIAL HYPERCHOLESTEROLAEMIA

Jana Vandrovcova 1; Penny J. Norsworthy 1; Ellen R.A. Thomas 1; Jennifer Biggs 1; Clare Neuwhirn 1; Yvonne Tan 1; Laurence Game 1; Archie Campbell 2; Shona M. Kerr 2; Blair H. Smith 3; Anna Dominiczak 4; David Porteous 2; Andrew Morris 1; Anne Soutar 1; Timothy J. Altman 1

1 MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College London, UK; 2 Medical Genetics Section, University of Edinburgh, UK; 3 Biomedical Research Institute, University of Dundee, UK; 4 College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Familial hypercholesterolaemia (FH) is a common autosomal dominant condition characterised by raised plasma cholesterol levels and increased risk of premature coronary heart disease. Treatment with statins is highly effective, but a minority of cases have a formal molecular diagnosis and receive appropriate treatment. The high cost and low accessibility of conventional DNA testing contributes to low numbers of molecularly diagnosed FH patients. This study tested a PCR-based next generation sequencing (NGS) protocol for FH mutation detection.

Amplicons were designed to cover the whole LDLR gene and mutation hot spots in PCSK9 and APOB genes. After amplification and barcodeing using the Access Array system (Fluidigm) 48 to 96 samples were pooled and sequenced using the MiSeq platform (Illumina). In the first stage the assay was tested on 75 previously characterised FH patients and was further extended to 150 prospective patients. In addition, 400 individuals from the Generation Scotland population cohort with either high cholesterol or on statin treatment were screened for mutations. Large deletions and duplications were screened using multiplex ligation-dependent probe amplification (MLPA, MRC Holland). In the first stage of the study the sensitivity for short variant detection was 98% (47/48) and specificity 100%. In the prospective group of patients studied so far (n = 84), pathogenic mutations were found in 67% of cases of clinically definite FH, 23% of patients with possible FH and 8% of hypercholesterolaemic patients not meeting clinical criteria for FH. The