EFFECT OF BARIATRIC SURGERY ON HDL QUANTITY AND QUALITY

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Background: High-density lipoprotein (HDL) particles have an important role in atheroprotection, with properties including antioxidation, antiglycation and promotion of cholesterol efflux. Obese patients have increased systemic inflammation and thus higher risk of vascular disease. Little is known of the changes in HDL particles and HDL functionality associated with obesity. We aimed to assess the effects of obesity itself and bariatric surgery-induced weight loss on both HDL quantity and quality.

Methods: We measured HDL-C, oxidised HDL (Ox-HDL), apolipoprotein AI (ApoAI), apolipoprotein AII (ApoAII), apolipoprotein M (ApoM), Myeloperoxidase (MPO) mass, HDL-apolipoprotein E (apoE), serum paraoxonase-1 (PON1) activity and capacity of HDL to promote cholesterol efflux *in vitro* for 37 obese patients at baseline and at 6 and 12 months following bariatric surgery. We compared HDL functionality in obese patients with age and sex matched controls.
Results: At baseline, HDL-C, ApoAI and cholesterol efflux capacity were significantly lower among obese patients than healthy controls. Six months after bariatric surgery, although ApoAII and HDL-ApoE were significantly decreased (both $p < 0.05$), HDL-C, ApoA1, MPO mass, PON1 activity and cholesterol efflux were unchanged compared to baseline. At 12 months after bariatric surgery HDL quantity and quality were considerably improved. HDL-C and cholesterol efflux capacity were significantly increased compared with their baseline (both $p < 0.001$), as were PON1 activity and HDL-apoE concentration (both $p < 0.01$). MPO mass decreased and a further reduction in ApoAII was observed (both $p < 0.01$). ApoAI, ApoM and ox-HDL were unchanged. We used biological systems analysis to identify factors influencing HDL functionality.
Conclusions: Bariatric surgery significantly increases circulating HDL cholesterol levels and improves HDL quality, but effects are not apparent until 12 months after treatment.

IDENTIFICATION OF TWO RARE APOE MUTATIONS ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLAEMIA IN THE NORTH OF ENGLAND

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Mutations in three different genes (*LDLR, APOB and PCSK9*) affecting the LDL-receptor pathway are known to cause Familial hypercholesterolaemia (FH). However in approximately 20% of probands with a definite clinical diagnosis of FH no mutations are detected suggesting that there are FH causing mutations located in other genes. We report the finding of pathogenic *APOE* mutations in two probands from the North East of England with the FH phenotype.

Methods: DNA samples from probands were analysed by next generation sequencing (NGS) of the *LDLR, PCSK9 and APOE* genes and a selected region of the *APOB* gene. Variants were confirmed by Sanger sequencing.
Results: Proband A was a 37 year old female with a family history of hypercholesterolaemia and elevated levels of total and LDL-cholesterol (11.7 and 8.8 mmol/L respectively) with normal levels of HDL-cholesterol and triglycerides (2.4 and 1.2 mmol/L respectively). NGS results showed that she was heterozygous for a mutation in exon 4 of the *APOE* gene (c.500_502delTCC; p.Leu167del) previously reported in French and Italian FH families. She was also heterozygous for the *LDLR* variant c148G > T (p.Ala50Ser) previously described as both pathogenic and benign. Proband B was a 6 year old boy with a family history of premature myocardial infarction and elevated levels of total and LDL-cholesterol (8.5 and 6.4 mmol/L respectively) with normal levels of HDL-cholesterol and triglycerides (1.3 and 1.9 mmol/L respectively). NGS results showed that he was heterozygous for a previously unreported mutation in exon 4 of the *APOE* gene (c.492_493delinsCT; p.Lys164_Arg165delinsAsnTrp). Family studies are ongoing but the *APOE* mutations appear to co-segregate with hyperlipidaemia in both families, while the *LDLR* variant did not.
Conclusions: Screening of the *APOE* gene, in addition to the *LDLR, APOB* and *PCSK9* genes, is warranted in the setting of molecular diagnosis of FH in the UK.

WEB BASED TOOLS TO ASSESS ELIGIBILITY FOR GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLAEMIA (FH)

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Selection of patients who are appropriate for genetic testing for FH is a balance between diagnostic yield and cost. The Wales FH service has implemented a clinical scoring system to guide the selection of patients based on lipid levels, personal and family history of cardiovascular disease plus physical signs. In an evaluation of 623 patients referred to lipid clinics, the proportion of patients with a mutation ranged from 4% in those scoring 5 or less to 85% in those scoring >15.

We have developed two web tools to increase the utility of these FH scoring criteria.
The **Pre-treatment LDL-C Estimator** uses current LDL-C concentrations and current lipid lowering treatment to estimate pre-treatment cholesterol levels. It can also be used in reverse i.e. to estimate the level of LDL-C lowering that can be expected from prescribing a specific lipid lowering treatment. Treatments include dosages of all common single and combination lipid treatments (eg statin plus ezetimibe).
The **FH Genetic Testing Assistant (The Welsh Scoring Criteria)** is a five question, multiple choice tool that incorporates clinical signs, personal and family history plus lipid levels (based on the pre-treatment LDL-C estimator) to provide a genotyping score and indication of whether or not to proceed with a genotyping referral.
Qualitative evaluation of the tools has been used to refine these for use in the clinical setting. Both tools have been shown to be easy to use and interpret. The tools also have potential for further development to include more complex calculators such as age and gender adjusted LDL-C. It is anticipated that they will help general practitioners and secondary care clinicians to decide on which patients to refer for FH genotyping.

DEVELOPMENT OF AN E-LEARNING PROGRAM ON FAMILIAL HYPERCHOLESTEROLAEMIA (FH) FOR PRIMARY CARE

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Raised cholesterol is common in healthcare settings, with over 8 million patients currently being treated with lipid lowering medications in the United Kingdom alone. It is important that individuals with Familial

Hypercholesterolaemia (FH) are specifically identified, because of the importance of diagnosing younger family members by cascade testing.

With only three FH Clinical Specialist Nurses in Wales working within the FH Cascade testing service, there is a limited opportunity to access all rural and remote primary care practices within Wales. To help address this challenge a FH e-learning program has been developed. E-learning has become integral to higher educational institutions, but remains a poorly resourced educational tool within clinical settings. Therefore, the aim of this initiative is to provide primary care with accessible information regarding FH. This will assist GP's / Practice Nurses towards identifying FH patients, whilst demonstrating learning activity for their appraisal and continuous development targets.
The FH E-Learning program has been completed, and approval granted for it to be made accessible via the intranet and internet on learning@nhs.wales.uk. The program consists of: anatomy and physiology of FH, the patient pathway through the service, and a patient and primary care perspective. Learning is assessed using Multiple Choice Questions and a certificate is issued following completion, which can be included as part of CPD portfolio. National and local guidance also forms an essential part of the e-learning package.
The program is currently being evaluated for its accessibility and ease of use by the stakeholders of the FH service and a selected group of FH naïve healthcare professionals. The results will be published by September 2015.
It is anticipated that this educational innovation, will improve the detection and referral rates of those with possible FH, and therefore reduce the premature cardiovascular death rates for those with the condition.

GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) IN FAMILIAL HYPERCHOLESTEROLAEMIA (FH) IN WALES: YEAR 2 UPDATE

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Genetic testing of 1570 hypercholesterolaemic index patients from lipid clinics in the Wales FH service found a pathogenic mutation in 22% and a genetic variant of uncertain significance (VUS) in 9% (136 individuals, 89 variants). The designation of a variant as VUS precludes its use for diagnostic cascade testing and causes uncertainty for patients and clinicians.

We report our 2 year experience of a project to reclassify VUS using family studies combined with a quantitative statistical analysis. Family members of VUS index patients are tested for LDL-cholesterol (LDL-C) and the VUS. Family based genetic association analysis is used to quantify the likelihood of pathogenicity based on the family relationship and LDL-C (with adjustment for age and gender). The degree of association is statistically quantified as a p-value.
Data from 126 family members from 34 families has been collected to date and statistical analysis has been completed on 14 of these families (9 different VUS). Based on this analysis the All Wales Medical Genetics Service has re-classified most as pathogenic or non-pathogenic (Table).
This project demonstrates the 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 databases for FH. The information helps clinicians provide more clarity for their patients and families.

Gene	Genetic identifier	Protein identifier	P value	Status
LDLR	c.2087G>A	p.(Cys696Tyr)	$p = 0.007$	Re-classified pathogenic
LDLR	c.1073G>A	p.(Cys358Tyr)	$p = 0.002$	Re-classified pathogenic
LDLR	c.1217G>C	p.(Arg406Pro)	$p < 0.001$	Re-classified pathogenic
LDLR	c.2098G>A	p.(Asp700Asn)	$p = 0.14$	Remain as VUS
APOB	c.10739A>G	p.(Asn3580Ser)	$p = 0.975$	Re-classified non-pathogenic
LDLR	c.2312-47G>A	n/a	$p = 0.894$	Re-classified non-pathogenic
APOB	c.10251C>T	p.(=)	$p = 0.978$	Re-classified non-pathogenic
LDLR	c.1328G>C	p.(Trp443Ser)	$p < 0.001$	Pathogenicity review pending
LDLR	c.887G>T	p.(Cys296Phe)	$p = 0.0074$	Pathogenicity review pending

ATHEROSCLEROSIS IN MONOGENIC FAMILIAL HYPERCHOLESTEROLAEMIA VERSUS POLYGENIC HYPERCHOLESTEROLAEMIA

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Introduction: Familial Hypercholesterolaemia (FH) is a common autosomal dominant disorder caused by mutations in three genes, LDLR, APOB and PCSK9. In practice, around 60% of patients with clinical diagnosis of FH do not have a detectable mutation in these genes, and in this group we have shown that a polygenic cause for their raised low density lipoprotein-cholesterol (LDL-C) level is most likely. The aim of this study was to assess