Abstracts

HEART UK 26th Annual Conference
Civic Centre, Newcastle upon Tyne, UK
Abstracts of Free Communications
Wednesday 27 – Friday 29 June 2012
(1) GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLAEMIA IN WALE: IDENTIFICATION OF RECURRENT AND NOVEL VARIANTS

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FH is caused by variants in genes of the LDL receptor pathway. More than 1200 different variants in LDLR gene have been identified. Detection of a pathogenic variant gives an unequivocal diagnosis of FH and allows cascade testing.

The Wales FH cascade service was launched in December 2010. Lipid clinic patients with a diagnosis of FH or possible FH on clinical grounds are offered genetic testing.

A sequence variant was identified in 209/526 FH index patients (41%). 89% occurred in the LDLR gene, 10% in APOB and 1% in PCSK9. A total of 99 different variants have been identified in these 3 genes. 29 distinct variants (7% of patients) are currently classified as variants of uncertain significance (VUS). The five most common variants identified are: APOB-c.10580G>A (8%), LDLR-c.301G>A (5%), LDLR-c.1048C>T (4%), LDLR-c.301T>G>A (4%), LDLR-Del C7 (4%). One patient was homozygous (LDLR-c.1279G>C) and one a compound heterozygote (LDLR-c.1285G>A, c.932A>C).

To the best of our knowledge, the following 13 variants have not been previously described: APOB-c.10571A>C, LDLR-c.1909T>G, LDLR-c.1216DelC, LDLR-c.1702DelC, LDLR-c.1726T>A, LDLR-c.2164DelC, LDLR-c.694+3.694+19del, LDLR-c.918_919delinsCC, LDLR-c.967G>T, LDLR-Dup e16, LDLR-Del promoter-e1, LDLR-Del e3-8, LDLR-Dup e12-2.

Our experience of genotyping patients with a clinical diagnosis of FH in Wales has demonstrated 13 new genetic variants.

(2) THE ROLE OF IMPROVED PHENOTYPIC CONFIRMATION OF FAMILIAL HYPERCHOLESTEROLAEMIA IN ENHANCING THE USE OF GENETIC TESTING

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Genetic diagnosis of familial hypercholesterolaemia (FH) allows definitive identification of the index case and cascade testing of their family. However the decreasing population rates of coronary artery disease (CAD), and a reduction in the incidence of tendon xanthomata, suggests that existing clinical criteria for phenotypic identification of FH index cases, such as Simon Broome and Dutch Lipid Clinic Network, may be becoming less reliable.

We retrospectively examined the records of 235 patients who had attended our lipid clinic and undergone genetic testing for FH. The patients were 44% male, average age was 55±14 years, average total cholesterol was 8.4±2.2 mmol/L; average LDL-cholesterol was 6.06±2.01 mmol/L; tendon xanthomata were present in 34%, 41% met the Simon Broome criteria for family history of myocardial infarction, and 17% the Dutch Lipid Clinic Network criteria for premature CAD in the patient. A mutation was found in 46% on comprehensive genetic testing. To investigate the potential improvement in phenotypic confirmation of index cases achieved by adding in a personal history of CAD, a carotid intima-media thickness >75th centile, detectable plaque on duplex ultrasound or a coronary artery calcium score >75th centile, we re-analysed the dataset using these indications of increased atheroma burden. Our initial analyses had shown a Receiver Operator Area-Under-Curve (AUC) of 0.643 for Simon Broome criteria comprising an AUC of 0.641 for xanthoma(+) and 0.501 for xanthoma(-) patients and 0.72 for the Dutch criteria (score >5) in the whole cohort. Adding CAD or imaging to index case assessment increased the AUC for Simon Broome to 0.714 and Dutch score to 0.816. Imaging re-classified 25% of patients as lower risk and 38% as higher risk (p = 0.01). In FH gene(−) cases the prevalence of lipoprotein(a) > 0.5 g/L was 48% vs. 33% in FH gene(+) cases (P = 0.001).

Our study shows that the presence of CAD, and imaging index cases that present as CAD(−) who are suspected of having FH, increases the yield from genetic testing and hence the cost effectiveness of screening. Lipoprotein(a) levels should be measured in all these patients and are of particular clinical use for explaining the basis of familial premature CAD in those testing negative for an FH mutation.

(3) DEVELOPMENT OF A SINGLE COMPREHENSIVE GENE SCREENING TEST FOR FAMILIAL HYPERCHOLESTEROLAEMIA USING NEXT GENERATION SEQUENCING

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Bristol Genetics Laboratory provides a comprehensive gene testing service for Familial Hypercholesterolemia (FH) using: level 1 FH2O ARMS for 20 common mutations; level 2 MLPA and Sanger sequencing of LDLR; level 3 Sanger sequencing of PCSK9 and the APOB exon 26 mutation hotspot. Over a three year period of service provision a variant was identified in 93/232 index cases (40%). The ARMS methodology detected 48% of positive cases, LDLR sequencing 46% and the MLPA assay 6%. To reduce test costs, reduce turn around times and increase throughput we are developing a single assay for FH to detect all point mutations and copy number variation in LDLR, APOB and PCSK9. The coverage of APOB has increased to include all coding exons and an additional gene, LDLRAP1, associated with autosomal recessive hypercholesterolaemia, is included in the panel.

The assay uses a targeted capture next generation sequencing approach (Haloplex PCR, Illumina MiSeq) and data analysed using bioinformatics pipelines. The assay is currently being validated using 32 known positive control samples comprising: 23 samples with point mutations and indels, and 7 samples with exon duplications/deletions. Data from the first 16 patients indicates that 11/11 known pathogenic point mutations and indels were successfully identified and shows a high (>1000 fold) average read-depth with 92% of targeted bases covered at >30 × read depth. To explore the benefits of extended screening we are analysing a further cohort of 16 patients with a high scoring clinical/biochemical index for FH who have previously had a negative genetic test. A comprehensive high throughput assay at reduced cost should facilitate extended uptake and commissioning of FH testing in the UK.

(4) ROUTINE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA WITH NEXT GENERATION SEQUENCING

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Next Generation Sequencing methodology is revolutionizing the genetic diagnostic field, making it cheaper and faster. Benchtop devices, such as the GS junior system, have been recently introduced and their lower yield is appropriate to the throughput of clinical diagnostic laboratories.

To date Familial Hypercholesterolemia (FH) mutation detection is usually carried out by Sanger sequencing, or MLPA, since Copy Number Variations (CNVs) account for 5–10% of LDLR mutations. In order to replace these tedious and long analyses, we have designed multiplex PCRs to be sequenced in the GS Junior device, allowing the simultaneous detection of point mutation in the LDLR, PCSK9, LDLRAP1 exons and in APOB exon 26 and 29, as well as CNVs in the LDLR gene. During development, samples already genotyped by Sanger sequencing and MLPA, considered as gold standards for genetic diagnosis, were used.
The generation of proprietary algorithm based on the alignment provided by the Roche Software allowed us to get reliable variant detection for 20 to 24 FH patients per run, with accuracy similar to Sanger sequencing and MLPA. This algorithm has been fully integrated in a software that automatically launch the Roche Software, names the variants according to the HGVS nomenclature and perform a search in our mutation database, providing a pathogenicity status. With this work we show the feasibility of such an approach which serve as a proof of principle for the development of genetic diagnostic tests for any other diseases, either cardiovascular or not.

(5) HIGH PARAOXONASE-1 HDL IS MORE EFFECTIVE THAN LOW PARAOXONASE-1 HDL IN IMPEDING LDL OXIDATION IN VITRO

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Background: In tissue culture LDL must undergo chemical modification, for example oxidation, before its uptake is rapid enough for foam cell formation. HDL is known to protect LDL against oxidative modification. Experiments with transgenic mice indicated that paraoxonase-1 (PON1) located on HDL is involved in this antioxidative effect of HDL, but the epidemiology is equivocal.

Methods: 12 healthy volunteers were recruited and divided into two groups according to their median serum PON1 activity. LDL was incubated with Cu²⁺ in the presence of low and high PON1 HDL and with HDL enriched with recombinant PON1 (rPON1).

Results: Lipid peroxide (LPO) formation was least when high PON1 HDL was incubated with Cu²⁺. When standard low HDL was used, LPO formation was 88 nmol/ml/3 hours [35–116] with high PON1 HDL median [range] vs. 178 nmol/ml/3 hours [94–227]; (P < 0.05) with low PON1 HDL. Enriching HDL with rPON1 did not improve its antioxidative capacity in vitro although a significant increase in PON1 activity was observed and most of the rPON1 was distributed to HDL.

Conclusions: High PON1 HDL is more effective in protecting LDL from Cu²⁺-induced oxidation in vitro. However, rPON1 does not have the same capacity. Therefore PON1 may maintain the antioxidative capacity of HDL without itself being directly involved.

(6) IN STATIN TREATED PATIENTS, HIGH HDL PARAOXONASE-1 ACTIVITY PROTECTS LDL AND HDL FROM OXIDATION IN VITRO

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Background: LDL oxidation plays an important role in initiation and progression of atherosclerosis. HDL has antioxidative capacity and there is evidence to suggest paraoxonase-1 (PON1) plays an important role in this. We investigated the influence of serum PON1 activity on in vitro LDL antioxidative capacity, susceptibility of LDL to oxidation and the protection offered by HDL to LDL in statin-treated dyslipidaemic patients.

Methods: LDL and HDL were isolated by sequential ultracentrifugation in 43 statin treated dyslipidaemic patients. LDL was incubated with and without HDL, in the presence of Cu²⁺. Lipid peroxides (LPO) generated were measured over 3 hours. Similar in vitro experiments were carried out on HDL alone. Patients were divided into two groups based on median PON1 (108 nmol/ml/min). The low and high PON1 groups were compared.

Results: The LPO generated when HDL was incubated alone and with LDL were significantly lower with the high PON1 HDL compared to low PON HDL (11 vs 29 nmol/ml, P < 0.05 and 27 vs 50 nmol/ml, P < 0.01 respectively). This difference was not observed on incubating LDL alone. LPO generated after incubating LDL alone or LDL with HDL showed a significant negative correlation with PON1 activity (r = –0.359, P < 0.02 and r = –0.394, P < 0.01 respectively).

Conclusion: In statin-treated patients HDL protects LDL from oxidation in vitro. The capacity of HDL to protect itself and LDL from oxidation in vitro is significantly better in individuals with higher serum PON1 activity.

(7) COMPARISON OF SERUM LIPOPROTEIN(a) CONCENTRATION AND TWO SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH ELEVATED LIPOPROTEIN(a) TO KRINGLE REPEAT NUMBER DETERMINED FROM GENOMIC DNA

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Elevated lipoprotein(a) [Lp(a)] levels are an independent marker for cardiovascular disease (CVD) risk. Lp(a) is extremely heterogeneous in size due to a common copy number variation in the LPA gene leading to a variable number of kringle-IV type 2 (KIV2)-like domains in the major component of Lp(a), apolipoprotein (a) [apo(a)]. The genetically determined KIV2 repeat size affects the final size of the apo(a) protein, with small isoforms of apo(a) associated with an increased risk CVD. In addition, small isoforms of apo(a) are associated with two single-nucleotide polymorphisms (rs10455872 and rs3798220) in the LPA locus. We have developed a real-time PCR (qPCR) method for the rapid determination of KIV-2 repeat number from genomic DNA. DNA was obtained from a cohort of 300 patients attending the lipid clinic in Birmingham, UK. KIV-2 repeat number was compared to Lp(a) concentration and the two SNPs in the LPA gene.

Numbers of KIV2 repeats in our cohort ranged from 19 – 73 with KIV-2 repeats negatively correlated with plasma Lp(a) concentration (R² = 0.22, P < .001). Mean Lp(a) levels were 84, 74, 36 & 20 mg/dL for the first, second, third and fourth quartiles of the KIV2 repeats (test for trend P < .001).

Patients who were heterozygous for either one of the Lp(a) mutations, rs10455872 or rs3798220, were associated with significantly lower KIV2 repeat values when compared to wild-type (P values of <.001 and 0.015 respectively).

In conclusion, this demonstrates proof-of-concept of a qPCR method for the rapid evaluation of apo(a) KIV2 repeat number from genomic DNA. Due to the difficulty in accurate measurement of Lp(a) in serum because of particle heterogeneity, this KIV2 assay could potentially be used as an adjunct to the determination of plasma Lp(a) concentration to assess CVD risk. However, more work is needed to confirm whether patients with a smaller apo(a) isoform are of greater CVD risk in our cohort.

(8) DISTRIBUTION AND CONCENTRATION OF APOLIPROTEIN E IN HEALTHY VOLUNTEERS

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Background: Apolipoprotein E (apoE) is an important component of plasma lipoproteins and plays a vital role in lipoprotein metabolism. The influence of age and gender on apoE concentration and distribution across lipoproteins is not clear.
We present the case of a 29 year old female with no family history of hyperlipidaemia. Manipulation of diet is an essential aspect of treating any hyperlipidaemia which are established risk factors for cardiovascular disease.

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Results:

Methods:

ApoE and glycated apoE distributions in different lipoproteins were investigated. Total apoE concentration (mean ± SEM) was 48.2 ± 2.4 mg/L. Of this 13.3 ± 2.5 mg/L (27.6%) was in VLDL, 10.6 ± 2.2 mg/L (22.0%) in LDL, 14.4 ± 1.2 mg/L (29.0%) in HDL and 9.9 ± 2.0 mg/L (20.5%) in the density range >1.21 g/ml. ApoE concentration in VLDL was strongly correlated with VLDL-apoB and VLDL-TG. There was no significant difference in total apoE between men and women. However, in men VLDL-apoE was significantly higher (15.6 ± 1.4 mg/L vs. 10.3 ± 1.4 mg/L, p < 0.01) and HDL-apoE was significantly lower (11.7 ± 1.0 vs. 17.9 ± 2.1 p < 0.001) compared with women. Total apoE and HDL apoE concentrations increased from the time of the menopause in women, but age had no effect in men. Glycated apoE concentration and the percentage of apoE glycated were highest in VLDL (0.69 ± 0.08 mg/L, 1.48%), followed by LDL (0.14 ± 0.03 mg/L, 0.29%). Only (0.05 ± 0.006 mg/L, 0.10%) apoE was in HDL and (0.02 ± 0.003 mg/L, 0.04%) in d > 1.21 g/ml were glycated.

Conclusions: The apoE concentration and lipoproteins distribution are influenced by age and gender. VLDL and LDL apoE are more susceptible to glycation in vivo.

Key words: ApoE, Glycated ApoE, Concentration, Distribution, Age, Gender

(9) EVALUATION OF GENOTYPE SCORING CRITERIA FOR FAMILIAL HYPERCHOLESTEROLAEMIA (FH) IN WALES

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The Wales FH cascade service was launched in December 2010 with the introduction of a diagnostic service for FH, combined with family cascade testing. An FH genotyping scoring system was developed to guide whether lipid clinic patients with a clinical diagnosis of FH are suitable for FH genetic testing. This was based on a modification of the Dutch Lipid Clinic Network scoring criteria. A numeric score is derived from measures of family history, clinical signs, cardiovascular clinical history and lipid concentrations. A provisional threshold of 6 was applied such that if a patient scores 6 or above then they would be regarded as eligible for genetic testing, and below this only eligible if there are special clinical circumstances. Lipid clinic clinicians and FH nurses applied these criteria prospectively to 398 consecutive index patients who underwent genotyping. In patients who scored 5 or less a pathogenic mutation was identified in 6% increasing to 85% in those who scored 13 or more. Scores of 6, 7–8, 9–10, 11–12 are associated with likelihoods of 15%, 20%, 44%, and 44% respectively. The criteria scoring system is a useful tool to help predict the likelihood of identification of an FH-causing mutation.

(10) AN EGGSTREME DIET; THE EFFECT OF EGG INTAKE ON THE LIPID PROFILE

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Dietary cholesterol intake has been shown to increase total and LDL cholesterol which are established risk factors for cardiovascular disease. Manipulation of diet is an essential aspect of treating any hyperlipidaemia and modifying cardiovascular risk. We present the case of a 29 year old female with no family history of cardiovascular disease who was referred to the lipid clinic with a severe hypercholesterolaemia (total cholesterol = 8.8mmol/L). Repeat clinical fasting lipid profile revealed total cholesterol = 7.4mmol/L, triglyceride = 0.9mmol/L, HDL-C = 2.9mmol/L and LDL-C = 4.1mmol/L. She suffered from epilepsy, asthma and impaired fasting glycaemia. She abstained from alcohol and undertook very limited exercise. Her medication included carbamazepine, sodium valproate, clonazepam and a salbutamol inhaler. She had no stigma of hyperlipidaemia and renal, liver and thyroid function were normal. Her BMI was 20.9kg/m². She had previously seen a dietician when diagnosed with impaired fasting glucose. However a dietary history in clinic revealed a very high intake of eggs, on average 42 per week as part of a restrictive diet, high in salt and low in fibre. At initial presentation daily total fat intake was calculated at 101g with 1.4g of cholesterol. Previous guidelines have suggested that an intake of 0.3g per day or less of cholesterol is recommended with each egg on average containing around 0.2g of cholesterol. Dietary and lifestyle advice aimed to reduce saturated fat intake, increase lean protein and decrease salt intake. No cholesterol lowering medication was commenced. At follow up a year later she had reduced her egg intake to 6 per week whilst her weight remained static and her lipid profile showed an improvement; total cholesterol = 4.9mmol/L, triglyceride = 1.1mmol/L, HDL-C = 2.1mmol/L, LDL-C = 3.2mmol/L.

Dietary manipulation in this patient led to substantial improvement in her lipid profile, avoided the need for drug therapy, highlighting the importance of a thorough dietary history.

(11) PRE-DIABETES GP BASED GROUP EDUCATION AND LIFESTYLE SUPPORT

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Introduction: Pre-diabetes is characterised by impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or both. Glucose results are higher than the normal range but not high enough to be diagnosed as diabetes.

Method: In order to reduce the number of pre-diabetic patients who go on to develop diabetes, three General Practices in Sandwell developed and piloted a group education intervention and health trainer lifestyle support programme. This aimed to support pre diabetic patients to reduce their risk of developing diabetes, develop and evaluate group health education provision combined with health trainer delivered lifestyle support, and discover what support a GP Practice needed to follow up pre diabetic patients in the long term. Patients were invited to attend a practice based group education session delivered by a dietician. Patients were then offered 1:1 lifestyle support delivered by a Health Trainer. Physiological parameters were measured and repeated at one year.

Results: The patient uptake was 50% for group education. Of those 76% elected to receive health trainer support. Outcome at a year with some results yet to report. 14% routine care achieved better glycaemic control, 33% education only achieved better glycaemic control and 50% education plus Heath Trainer achieved better glycaemic control.

Conclusion: Patients with pre-diabetes who attend the group session and receive health trainer support are more likely to have a measurable reduction in progression to diabetes. They are also more likely to return for annual review. The pilot has initiated expansion of this service across the borough.

(12) VERY-LOW CALORIE DIETS AND MORBIDITY: A SYSTEMATIC REVIEW OF LONGER-TERM EVIDENCE

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Evidence from the literature supports the safe use of very-low-calorie diets for up to 3-months in supervised conditions for patients who fail to meet a target weight loss using a standard low-fat, reduced-calorie approach. There is, however, a need for longer-term outcomes on obesity and associated morbidity following a very-low-calorie diet. This systematic review aims to investigate longer term outcomes from studies using very-low-calorie diets, with a minimum duration of
12-months, published between January 2000 and December 2010. Studies conducted in both children and adults, with mean/median body mass index of \( \geq 28 \) kg/m\(^2\) were included. PubMed, MEDLINE, Web of Science and Science Direct were searched. Reference lists of studies and reviews were manually searched. Weight loss or prevention of weight gain and morbidities were the main outcomes assessed.

A total of 32 out of 894 articles met the inclusion criteria. The duration of the studies ranged from 12 months to 5 years. Periods of VLCD ranged from 25 days to 9 months. Several studies incorporated aspects of behaviour therapy, exercise, low fat diets, low carbohydrate diet or medication. Current evidence demonstrates significant weight loss and improvements in blood pressure, waist circumference and lipid profile in the longer-term following a very-low-calorie diet. Interpretation of the results, however, was restricted and conclusions with which to guide best practice are limited due to heterogeneity between the studies.

This review clearly identifies the need for more evidence and standardised studies to assess the longer-term benefits from weight loss achieved using very-low-calorie diets.

**Conflicts of interest:** Professor Iain Broom is the medical director for LighterLife Ltd. Funding was provided by LighterLife Ltd for this research.

### (13) GENETIC VARIANT OF CD36 GENE IS NOT ASSOCIATED WITH CARDIOVASCULAR RISK FACTORS IN SOUTH ASIANS

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**Introduction:** In the UK South Asian populations have an increased risk of CVD, the pathophysiology of which is multifactorial and explained in part by a susceptibility to diabetes and its associated metabolic defects. The CD36 gene encodes a glycoprotein membrane receptor implicated in fatty acid transport, which provides a putative link between diabetogenesis and atherogenesis. Polymorphisms in the CD36 gene are common to some ethnic groups and in turn are associated with CVD risk factors. Our aim was to measure the prevalence of the CD36 36498C \( \rightarrow \) T polymorphism in UK South Asians, its association with soluble CD36 levels and its contribution to CVD risk in this population.

**Methods:** DNA was extracted from blood samples collected from 449 South Asians (mean age 61 years, 53% male) recruited through local population research in Birmingham (UK). The CD36 36498C \( \rightarrow \) T polymorphism was analysed by using TaqMan SNP genotyping. Soluble CD36 was measured using ELISA.

**Results:** The minor allele (T) frequency was 25\% (95\% CI 22–28) and did not impact on the magnitude of CVD risk factor (table 1). Levels of soluble CD36 had an average value of 85.38 ng/ml (IQR 0.00–261.32 ng/ml) and was not associated with any CVD risk factors.

**Conclusions:** In UK South Asians, the frequency of CD36 36498C \( \rightarrow \) T polymorphism was similar to that reported in populations of African Caribbean origin but more than twice that reported in White populations.

### (14) AGAROSE ELECTROPHORESIS ASSAY FOR THE QUANTITATIVE DETERMINATION OF LIPOPROTEIN-X USING NOVEL ENZYMATIC STAINING FOR TOTAL AND FREE CHOLESTEROL

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**Background:** Lipoprotein X (Lp-X) is an abnormal lipoprotein associated with cholestatic liver disease. Lp-X displays a unique cathodal mobility on agarose gel electrophoresis, however it overlaps with low density lipoprotein (LDL-C). In view of its diagnostic significance it is important to distinguish Lp-X from LDL-C, however currently there are no commercial methods for its detection and quantification. As Lp-X cholesterol is almost entirely unesterified, we adapted an established commercial electrophoretic method for simultaneous estimation of total and free cholesterol enabling quantification of Lp-X in dyslipidaemic samples.

**Methods:** An enzymatic reagent containing both cholesterol esterase and cholesterol dehydrogenase (Helena Biosciences Europe) was modified in order to measure both Total and Free cholesterol on the same gel. Electrophoresis of duplicate samples using the SAS-MX HDL Agarose gel produced separation of lipoproteins. Simultaneous enzymatic staining was performed on 2 halves of the gel, using a custom template to separate the total and free cholesterol reagents and the ratio measured between the paired lanes. The area under the curve for the total and free cholesterol was quantified in non-HDL-C zones using densitometry. The ratio between measured total and free cholesterol and Lp-X in non-HDL-C was calculated.

**Results:** To establish a normal range for total:free cholesterol ratio, 54 patient samples were analysed after exclusion of secondary dyslipidaemia and values outlying the 3 SD range from the mean. An abnormal ratio in combination with cathodal electrophoretic mobility was used to identify Lp-X in samples from a patient with biliary obstruction pre- and post-treatment. The concentrations of total and free cholesterol, cholesterol ester and Lp-X were calculated.

Precision was calculated using repeated samples giving a between gel CV of the total:free cholesterol ratio of 14\% (n = 18) and within gel CV of 7\% (n = 6). The method was linear to 6.5 mmol/L of non-HDL-C.

**Conclusion:** Agarose electrophoresis with simultaneous enzymatic staining for total and free cholesterol permits accurate identification and quantification of Lp-X. This method may prove useful in diagnosis and monitoring of patients with cholestatic liver disease.

### (15) MANAGEMENT OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA IN PREGNANCY

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**Abstract** Homozygous familial hypercholesterolaemia (FH), including compound heterozygotes, occurs in approximately one in a million births. If untreated, it can lead to the development of severe atherosclerosis in childhood, so early lowering of LDL cholesterol (LDL-c) is essential. The LDL receptors in this condition are minimally functional, if at all, and high doses of statins, often in combination with other medications, are only moderately effective in improving lipid levels. If drug therapy is unsuccessful at reducing LDL-c, lipoprotein apheresis should be used and started before 7 years of age. Pregnancy provides an additional complicating factor in patient management as most lipid-lowering agents are contraindicated in pregnancy and breast feeding, leaving apheresis as the predominant treatment. The physiological state of pregnancy itself also elevates lipid levels. This combination of factors often results in a significant rise in cholesterol for several months. As these patients commonly display

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CC (n − 255)</th>
<th>CT or TT (n = 194)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Male (n)</td>
<td>52.9 (135)</td>
<td>59.3 (115)</td>
<td>0.100</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>61.2</td>
<td>60.36</td>
<td>0.450</td>
</tr>
<tr>
<td>Mean body mass index (kg/m(^2))</td>
<td>27.3</td>
<td>27.1</td>
<td>0.960</td>
</tr>
<tr>
<td>% with diabetes (n)</td>
<td>33.3 (85)</td>
<td>39.3 (76)</td>
<td>0.680</td>
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<tr>
<td>Mean systolic BP (mmHg)</td>
<td>118.3 (30)</td>
<td>155.5</td>
<td>0.400</td>
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<tr>
<td>Mean diastolic BP (mmHg)</td>
<td>141</td>
<td>140</td>
<td>0.788</td>
</tr>
<tr>
<td>Mean serum cholesterol (mmol/L)</td>
<td>82.0</td>
<td>82.9</td>
<td>0.467</td>
</tr>
<tr>
<td>Mean HDL cholesterol (mmol/L)</td>
<td>4.27</td>
<td>4.30</td>
<td>0.796</td>
</tr>
</tbody>
</table>

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cardiovascular abnormalities secondary to aorto-coronary atherosclerosis, there is a substantial risk of cardiac decompensation. Management in pregnancy therefore requires close collaboration between lipidologists, the apheresis nursing team, obstetricians, cardiologists and anaesthetists. We describe two cases of homozygous FH and the multidisciplinary approach taken to manage these patients in the tertiary lipid clinic at Hammersmith Hospital since childhood.

(16) COMPUTER-ASSISTED VISUAL ASSESSMENT FOR MALNUTRITION: LOWER ABSOLUTE ERROR IN ASSESSMENTS OF FOOD CONSUMPTION USING NOVEL SOFTWARE APPLICATION

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40% of older adults are at risk of malnutrition developing or becoming worse on admission to hospital. The Care Quality Commission Dignity and Nutrition Inspection Programme report identified inadequate monitoring of food intake in hospitals as a key concern. We present the mappmal software application for computer-assisted visual assessment of food consumption in hospitals. The application presents users with an image of standardised hospital foods and drinks on a touch screen tablet computer. The user erases the amount consumed corresponding to a patient’s meal. The application calculates the percentage of each food item consumed, from which nutritional information is automatically derived. An evaluation study, comparing application use to best possible practice was carried out, employing three conditions: trained Nutritionists using the application (TrApp, n = 3), untrained individuals using the application (UnApp, n = 4), and trained Nutritionists carrying out an unaided visual assessment (VisApp, n = 2). Ten portions of seven meals were assessed. All food items were weighed for standardised comparison. Results suggested that significantly lower absolute error [F(2, 1077) = 57,426, p = 0.000] is achieved through use of the application (VisApp: avg = 18 g, TrApp: avg = 9 g, UnApp: avg = 8 g). This suggests that the application outperforms best possible practice and is suitable for monitoring food intake in hospitals.

(17) EVALUATION OF AN AUTOMATED METHOD TO MEASURE SMALL DENSE LDL CHOLESTEROL

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Introduction: Small dense low density lipoprotein (sdLDL) is highly atherogenic and has been recognised as an emerging risk factor. Methods used to measure sdLDL include density gradient ultracentrifugation, (DGU), gradient gel electrophoresis (GGE) and nuclear magnetic resonance spectroscopy but none are suitable for use in routine clinical laboratories as they are labour intensive and require specialised techniques and equipment. We compared sd-LDL cholesterol levels in both fresh and frozen plasma using a newly available one step fully automated homogeneous assay (sdlLDL-C, Denka Seiken, Japan) with DGU. Results: 137 plasma samples (95M 42F aged 40–80) were analysed within 72 hrs of collection for sd-LDL-C by DGU. After storage at –70°C for 5–10 yrs they were defrosted and analysed using the automated assay on an ILab 600 clinical chemistry analyser. In addition 47 samples (23 M and 24 F aged 30–60) were analysed within 72 hrs of collection by both methods. Results: On regression analysis the frozen samples gave a Pearson’s Correlation r = 0.69 (p = 0.0001) Mean value (SD) were 39.8 mgs/dL, (18.8) automated and 58.7 mgs/dL(38.1) DGU. The fresh samples gave a Pearson’s Correlation Coefficient r = 0.80. (p = 0.0001) mean value (SD) 32.6 mgs/dL(15.4) automated and 32.8 mgs/dL(25.0), Discussion: The new automated homogenous assay shows a significant relationship with values obtained by density gradient ultracentrifugation in both fresh and frozen plasma. This is a robust assay with good inter and intra batch precision and could be easily adopted by routine clinical laboratories.

(18) FENRETTINIDE TREATMENT FOR HIGH FAT DIET-INDUCED OBESITY AND INSULIN SENSITIVITY

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Introduction: With the obesity epidemic ever increasing, commonly associated pathologies, loosely termed the metabolic syndrome, are escalating at almost the same rate. Higher caloric intake and decreased activity levels contribute to both the development of obesity and its complications: e.g. hyperglycaemia, insulin resistance and hyperlipidaemia all contribute to cardiovascular disease. The synthetic retinoid, Fenretinide, has previously been demonstrated to slow obesity progression and improve insulin sensitivity in male mice without affecting food intake.

Aim: To investigate the effects of Fenretinide, in high fat diet-induced obesity and insulin sensitivity in female mice.

Methods: Nulliparous and parous female C57BL6 mice were studied on Chow, high fat (either 45% or 55%) or 45% high fat diet with Fenretinide incorporated. Body composition, blood glucose and hepatic protein expression were also investigated.

Results: Nulliparous mice were shown to be protected against severe high fat diet-induced obesity. Strikingly, Fenretinide treatment caused parous mice to maintain a similar weight to those on a normal diet. Adiposity was found to be lower in all Fenretinide treated mice. Insulin sensitivity did not appear to be impaired in female mice however mice on 55% high fat diet did exhibit mild signs of insulin resistance. Molecular results suggested Fenretinide may function through modulation of the retinoic acid pathway.

Conclusion: Fenretinide treatment appears to protect against high fat diet-induced obesity in female mice. Insulin resistance was not present in obese females in contrast to previous obesity studies in male mice. The exact mechanism through which Fenretinide functions is still unknown.

(19) THE HYPERTRIGLYCERIDEMIC WAIST AND THE METABOLIC TRIAD: A WEIGHT LOSS STUDY IN CLINICALLY OBESE CHILDREN

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Objective: The metabolic triad is characterised by increased intra-abdominal adipose tissue and insulin resistance. The main objective was to examine the associations between components of the HyperTG waist (TG and waist cut-points derived from two metabolic syndrome definitions) with a continuous metabolic triad score (MTS) calculated as a cumulative z score of fasting insulin, adiponectin and HDL peak particle density in obese youth.

Methods: 75 clinically obese boys and girls (standardized body mass index (BMI) 3.09 ± 0.6 and 3.05 ± 0.59 kg m², respectively), aged 8–18 years were assessed after acute weight loss, comprising of diet and exercise.

Results: There was no association of waist with the MTS, using the MetS waist cut-offs of deFerranti (2004) or Zimmet (2007). Log TG concentration (p < 0.0001) and waist circumference (p = 0.008) were both independent predictors of the MTS, together accounting for 30.6% total variance in the MTS. Waist, TG and all other cardio-metabolic risk factors were significantly improved following acute weight loss. Log TG change was the only predictor of MTS change (\(r^2 = 24.7\%\), p < 0.0001).

Conclusion: TG was the strongest independent predictor of the MTS in obese youth in the cross sectional study. The change in TG (but not waist change) is particularly important to assess the change in the MTS, a surrogate for increased intra-abdominal adipose tissue and insulin resistance in obese youth.
(20) IMPROVED CARDIOVASCULAR OUTCOMES WITH DPP-4 INHIBITORS IN DIABETES – A META-ANALYSIS

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Dipeptidyl-peptidase-4 is a ubiquitous endothelial enzyme whose substrates include GLP-1 and the endothelial repair factor SDF-1. Phase 2-3 cardiovascular outcome trial data from short-term studies (mostly 26–54 weeks) have been published for five agents which are potent inhibitors of the enzyme, four of which (linagliptin, saxagliptin, sitagliptin, vildagliptin) are recently licensed glucose-lowering drugs. In the present meta-analysis hazard ratio point estimates and confidence intervals have been subjected to meta-analysis and forest plots using standard software. Numbers exposed were 21709 for DPP-4 inhibitors, and 14073 for placebo/active controls combined. Studies with linagliptin and saxagliptin individually exposed were 21709 for DPP-4 inhibitors, and 14073 for placebo/active controls combined. Studies with linagliptin and saxagliptin individually showed hazard ratios for MACE events with upper confidence intervals below 1.00, while the other studies all had point estimates for HR < 0.85, although not statistically significant. Forest plots suggest that drugs with more cardiovascular events tend to have hazard ratio point estimates closer to 0.80. Meta-analysis of the data from all five drugs gives fixed effects HR of 0.67 (95% CI 0.54, 0.84), < 0.001, with similar results for a random effects model or when only using the licensed medications. These preliminary data are encouraging and suggest need for formal cardiovascular outcome studies (four are in progress) and investigation of local endothelial mechanisms.

(21) HEART RATE VARIABILITY MEASURED BY A NOVEL DEVICE IN PARTICIPANTS WITH HEART FAILURE

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Background: Heart rate variability (HRV) is a non-invasive measure that examines autonomic influences on the heart, indicating how the body is trying to preserve its equilibrium. Thus HRV has the potential to monitor the autonomic response to exercise volume on a daily basis. A reduced level of HRV has been demonstrated to be a significant predictor of cardiac event and death.

Purpose: The aim of this study was to determine the feasibility of HRV measurements using a new personal heart rate variability monitor (Ithlete) while estimating the normal pattern for HRV in participants with heart failure.

Methods: Eighty-five participants were tested, of which 65 were male and 20 were female. Each participant received a device for daily measurement of HRV and heart rate (HR) in the seated position for at least 28 days. Participants were also asked to fill in a daily exercise diary which included exercise intensity and duration.

Results: Almost half of the 75 participants took sufficient correct measurements while they had the Ithlete device. The average HRV measurement found in this study was 48.6 ms (rMSSD). The relationship between HRV and HR was found to be negatively correlated.

Discussion: At rest HRV (rMSSD) was lower than that recorded in the literature for a healthy population. Participants with heart failure varied in their level of use of the device. Some found the device very easy to use while others cited problems. The relationship between HRV and HR was similar to the findings from other studies.

Conclusion: This study is the first to look at measurements of HRV in heart failure patients using the Ithlete device. This study could be the basis for more research in to what HRV measurements are to be expected in this population and how they can be used to benefit the health and care of heart failure patients.

(22) CHANGES IN CD36 EXPRESSION AND LIPID METABOLISM IN RESPONSE TO AN ORAL GLUCOSE CHALLENGE IN SOUTH ASIANS

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Changes in CD36 expression and lipid metabolism in response to an oral glucose challenge in South Asians

Objectives: To investigate lipid and lipoprotein metabolism and its relationship with the expression of the fatty acid translocase CD36 on monocytes in South Asians, an ethnic group in whom there is a higher risk of diabetes mellitus, and an earlier and more advanced progression of micro- and macro-vascular complications.

Research design and methods: This was a preliminary, “proof-of-concept” study in 29 healthy South Asian participants (mean age 34.6 (8.9) years, 76.2% male, mean body-mass index 25.0 (5.2) kg/m²) recruited from an inner city area of Birmingham (UK). The main outcome measures were post-prandial (30 min) and post-absorptive (120 min) changes from fasting (0 min) in circulating lipids, lipoproteins and hormones, and monocyte expression of CD36 following the administration of a 75 g oral glucose challenge. We also analysed the determinants of variations of monocyte CD36 expression.

Results: There were marked changes in monocyte CD36 expression following the glucose challenge (P < 0.001). NEFA levels progressively decreased during the challenge (P < 0.001), while cholesterol (but not triglyceride) concentrations within very low density lipoprotein (VLDL) and low density lipoprotein (LDL) sub-fractions increased (P < 0.001). Levels of serum triglycerides, glucose and HDL cholesterol were largely unchanged. Variations of monocyte CD36 were negatively (r = −0.47, P = 0.04) associated with dietary fat and positively to dietary carbohydrate (r = 0.65, P < 0.001).

Conclusion: These data suggest that the generation of VLDL follows the ingestion of glucose within this population, and it is presumed that the sequestration of NEFA from these particles happens through the increased availability of CD36 receptors. While these are preliminary data, it would appear that lifestyle exposures moderate the expression of CD36.

(23) AN UNUSUAL CASE OF SECONDARY HYPERLIPOIDEMIA

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Secondary causes should be considered in case of severe mixed hyperlipidaemia. Amyloidosis is a systemic disorder which can affect multiple organs although in itself is rarely associated with dyslipidaemia. We report an unusual case of secondary dyslipidaemia which appeared to predate the symptoms of amyloidosis.

A previously normolipidaemic, non-diabetic 59 year old male developed Type 2 diabetes and severe combined hyperlipidaemia (total cholesterol 17.2 mmol/L, Triglycerides 9.2 mmol/L, HDL-C 0.4 mmol/L). HbA1C was 92 mmol/mol indicating poor glycaemic control but no
other secondary causes were found to explain his dyslipidaemia. Serum and urine electrophoresis was normal although kappa light chains were markedly elevated. Liver biopsy failed to stain for light chains typical of AL amyloidosis and the type of amyloid present in the tissue remains uncertain. Plasma lipoproteins were assessed by beta quantification and lipoprotein electrophoresis. This confirmed low HDL-cholesterol (0.44 mmol/L) and LDL-C was measured at 13.4 mmol/L, however a broad beta band and beta VLDL indicated the presence of remnant like lipoproteins. Total apolipoprotein B was measured at 4.2 g/L, and the apolipoprotein B/total cholesterol ratio of 0.25 was not in a range typically found in dysbetalipoproteinaemia (Type III). Apolipoprotein E was markedly elevated at 140 mg/L (23–63). Immunofixation of lipoprotein electrophoresis for light chains did not suggest abnormal binding of the lipoproteins. A new onset dysbetalipoproteinaemia with unusually elevated apolipoprotein B could highlight the presence of this, an amyloidosis associated lipid abnormality.

(24) EXPERIENCE FROM THE FIRST 10-YEARS OF A LIPOPROTEIN APERPHESIS UNIT IN A TERTIARY CARDIOTHORACIC CENTRE IN THE UK

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Abstract: The lipoprotein-apheresis service at Harefield Hospital was established in November 2000. The service is nurse-led and is run by a Nurse Consultant with a team of clinical nurse specialists. A Consultant Cardiologist who specialises in lipid management supervises the service. Initially there was one patient treated, increasing to three patients by the end of the second year. Patient numbers have increased with a total 27 patients (Male 14: Female 13, age range 22–69 years) receiving a total of 1629 treatments in the first 10 years. The number of treatments per patient ranged from 2 to 203 during this period.

Two patients died, one had a total of 71 treatment and the other 79 treatments. Lipoprotein-apheresis had been stopped for both patients several months prior to their death due to other medical problems. Six patients have stopped treatment either for personal reasons or due to problems during treatment. One of the patients treated is a German citizen who asked to receive treatment on two occasions whilst she was on holiday in the UK. Initial treatments used the Kaneka MA01 DL75 system (84 treatments – 5.16%). This was subsequently changed to the Life 18 immunoadsorption system (94 treatments – 5.77%). The majority of treatments, 1302, have been carried out using the Kaneka DX21 DL75 whole blood system (79.93%). The DAL1 whole blood system has been used in three patients (54 treatments – 3.31%). Kaneka MA03 LA15 in one patient (82 treatments – 5.03%) and double filtration in two patients (13 treatments – 0.80%). Access for treatment is via peripheral veins in 10 patients, an AV fistula in 12 patients and via a long-term central line in 5 patients. On average patients achieve a 45% reduction in total cholesterol and a 57% reduction in LDL cholesterol with each treatment. Treatments are generally carried out with few problems. Documented side effects of apheresis treatment include vaso-vagal episodes, nausea, chest pain and vasospasm.

The unit has continued to expand and a further six patients have started treatment since November 2009. Lipoprotein-apheresis is an important treatment option for patients whose cholesterol levels cannot be adequately controlled by lifestyle changes and medical therapy. Provision of this service in the UK remains poor.

(25) ACTION OF PHYTOCHEMICALS ON LIPID ACCUMULATION AND LITPOXICITY IN HEPG2 CELLS

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Introduction: Non-alcoholic fatty liver disease (NAFLD) has the potential to pose a significant health burden in developed countries due to its increasing prevalence. The spectrum of NAFLD ranges from excessive triglyceride accumulation (steatosis) to progressive non-alcoholic steatohepatitis (NASH). After steatosis develops, hepatocytes are vulnerable to fatty acid-induced oxidative insults (lipotoxicity) leading to the development of NASH. Current treatments are limited. However, studies have indicated that phytochemicals possess antioxidant properties that may be beneficial for treating NASH. The aim of this study was to determine the actions of four phytochemicals ((−)epigallocatechin gallate, quercetin, indole-3-carbinol and l-sulforaphane) on lipid accumulation and cell viability of HepG2 cells and to evaluate the robustness of the experimental NASH model.

Method: Oleate and palmitate are abundant fatty acids in livers with NASH. HepG2 cells were incubated with oleate and palmitate to produce a model for steatosis and lipotoxicity, respectively. Each phytochemical was added to individual models. Subsequently, cells were stained with Nile Red, enabling measurement of lipid accumulation and Neutral Red, enabling measurement of cell viability. One-way analysis of variance with Tukey’s Multiple Comparison test was used to compare the effects of the phytochemicals to the control. P-values <0.05 were considered to be statistically significant.

Results: For the experimental model, lipid accumulation was successfully produced (p < 0.001) but reduced cell viability was not. After phytochemical treatment, only indole-3-carbinol was found to alter lipid accumulation, which produced a decrease (p<0.001). No effect on cell viability was observed with any phytochemical apart from indole-3-carbinol, which produced a decrease (p<0.001).

Conclusion: A robust experimental model of steatosis was produced but failed to display lipotoxicity. This was due to no significant decrease in cell viability following co-supplementation of HepG2 cells with oleate and palmitate. Overall, the study found that the phytochemicals did not exhibit protective actions against oleate-induced lipid accumulation. Their actions on lipotoxicity were inconclusive due to the inability of replicating lipotoxicity in vitro. Therefore, it was concluded that phytochemicals do not possess potent anti-steatoctatic actions and an alternative experimental design is warranted for determining the actions of phytochemicals on lipotoxicity.

(26) VEGF IS INDIRECTLY ASSOCIATED WITH NO PRODUCTION AND ACUTELY INCREASES IN RESPONSE TO HYPERGlyCAEMIA

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Background: Increased levels of vascular endothelial growth factor (VEGF) have been observed in patients with metabolic syndrome (MetS). Nitric oxide (NO) formation is reduced in MetS, but its relationship to VEGF production remains poorly defined. We evaluated the association between VEGF/NO synthesis and insulin sensitivity in obese subjects and investigated the secretory response of VEGF to an acute elevation of glucose.

Materials and methods: Seven healthy normal weight subjects, seven obese subjects without MetS and seven obese subjects with MetS were recruited. Anthropometry, body composition and cardio-metabolic functions (blood pressure, glucose, insulin, triglycerides, total cholesterol, HDL-C and VEGF) were measured, and a novel stable-isotope method was used to assess in vivo rates of NO production. A frequent sampling intravenous glucose tolerance test was performed to study the dynamics of VEGF release.

Results: Fasting VEGF levels were significantly higher in the two obese groups compared to the control group (p for trend = 0.02), but the difference was not significant after adjustment for age. VEGF levels were associated with systolic blood pressure (rho = 0.54; p = 0.01) and NO production (rho = 0.44; p = 0.04). VEGF levels increased in response to acute hyperglycaemia in normal weight and obese subjects (p<0.001).

Conclusions: VEGF levels rapidly increase during hyperglycaemia and are inversely related to NO production at steady-state. The potential link between the acute secretion of VEGF and atherosclerotic risk in subjects with poorly controlled glycaemia as well as the potential of lowering
elevated VEGF levels by increasing NO production and/or availability warrants further investigation.

(27) DNA SEQUENCE VARIANT C.932A > C, P.LYS311THR IN LDLR GENE IN FAMILIAL HYPERCHOLESTEROLAEMIA (FH): OBSERVATIONS FROM FAMILY STUDIES

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Detection of a pathogenic variant in LDLR gives an unequivocal diagnosis of FH and allows cascade testing. Bioinformatic analysis and family segregation studies enable classification of most variants as pathogenic or benign, however, for some variants pathogenicity remains uncertain. Laboratories in Cardiff and Bristol have identified the variant LDLR c.932A > C, p.Lys311Thr in trans with a known pathogenic LDLR variant in 2 two patients. The variant was identified by the Cardiff laboratory in trans with LDLR c.1285 G > A, p.Val429Met in a patient with extensive Xanthomata and a cholesterol 13.4 mmol/L at age 10 (LDL cholesterol reduced by 51% with rosuvastatin, ezetimibe and colestipol). In two relatives heterozygous for c.932A > C cholesterol was 7.0 and 4.9 mmol/L. The variant was identified by the Bristol laboratory in trans with LDLR c.1766delA, p.Asparate589ValfsX76 in a second patient (diagnosed age 8), with cholesterol of 20 mmol/L. A relative heterozygous for the c.932A > C variant had equivocal cholesterol (5.1 mmol/L). In silico predictions for this variant are different depending on the algorithm used. In vitro techniques which examine the characteristics of the region where the amino acid is indicate that the substitution of the highly conserved Lys311 by a Threonine could affect the ability of the LDLR to release the captured LDL in the endosome, leading to a decrease of LDLR activity. The c.932A > C variant on its own appears to have a mild effect, which is additive when in combination with a pathogenic variant, leading to a clinical presentation consistent with a compound heterozygote.