MOLECULAR SCREENING FOR FAMILIAL HYPERCHOLESTEROLEMIA IN THE NORTH EAST OF ENGLAND USING A TWO TIER APPROACH

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The Northern Genetics Service, in partnership with NewGene, have developed a two tier approach to the molecular investigation of Familial Hypercholesterolemia (FH) patients.

Patient samples are initially tested for 52 pathogenic mutations (50 *LDLR*, 1 in *APOB* and 1 in *PCSK9*) using a bespoke Agena (MALDI-TOF) assay. These pathogenic mutations represent the most common mutations previously identified in the North East of England region in an earlier nationwide pilot project, accounting for 70% of pathogenic alleles. Those samples that test negative following Agena analysis proceed directly to Massively Parallel Sequencing if the proband was referred with a Dutch Lipid Clinic Network Score of greater than nine or if still required following consultation with the referring clinician for lower scoring probands. Sequencing libraries are prepared using the commercially available Multiplicom kit (ADH Mastr assay) and sequenced using the Illumina MiSeq platform. The assay captures a region of *ApoB* exon 26 in addition to the entire coding sequence and 5′UTR for the *LDLR*, *PCSK9* and *ApoE* genes.
The sequence data is initially analysed using NextGENe software (SoftGenetics) and the variants are then filtered using a locally curated variant database. All variants are subject to confirmation by Sanger sequencing before reporting. Dosage analysis for *LDLR* is performed separately by MLPA on request.
Variants of uncertain clinical significance are investigated according to ACGS best practice guidelines, utilizing publically available databases and the Alamut variant interpretation support software (Interactive Biosoftware). Where appropriate, the NGS will undertake mRNA studies to investigate the impact of variants on gene splicing.
Of the 229 Agena tests undertaken to date, pathogenic mutations have been identified in 41 cases. Within our cohort of 103 sequencing screens we have identified 28 positive cases. We report here the effectiveness of a two tier approach to the molecular investigation of FH patients.

AN APPROACH TO GENETIC DIAGNOSIS OF SEVERE HYPERTRIGLYCERIDAEMIA

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Severe hypertriglyceridaemia defined as higher than 20 mmol/L carries a high risk for acute pancreatitis. Although secondary factors contribute to many cases of hypertriglyceridaemia, presence of a primary disorder of triglyceride metabolism contribute to severity of hypertriglyceridaemia.

We carried out genetic testing of subjects presented to our lipid clinic with severe hypertriglyceridaemia. Patients were selected to have genetic testing if they satisfied one or more of the following criteria. a) history of pancreatitis b) presenting with triglycerides >15 mmol/L without any secondary factors c) severe hypertriglyceridaemia in during pregnancy. Mutations analysis was carried out by sequence analysis of the LPL-, APOC2-, APOA5- and GPI-HBP-genes on 15 subjects who satisfied one or more of above criteria.
There was no difference in age, BMI or pancreatitis between the mutation negative and mutation positive subjects Those with mutation 37% were diabetic or had impaired fasting glycaemia and 75% had previous history of pancreatitis, 2 out of 8 subjects consumed >21u of alcohol per week
Among those who had no mutation detected 57% were diabetic and 71 % had pancreatitis, no one consumed >10units of alcohol per week.

Age (yrs)	Gender	Ethnicity	mutation	BMI (kg/m^2)	Highest triglycerides recorded (mmol/l)	diabetes	Lowest triglycerides achieved (mmol/l)	Frequency of pancreatitis
34	F	Portuguese	Heterozygous, R197L; CGT > CTT in exon5 of *LPL*	28.9	43.2	GSD	3.7	None
35	M	Indian	Heterozygous, c.249+1G>A in intron2 of *LPL*	32.6	10*	None	5.9	1
55	M	Caucasian	c.447delGinsCTC in exon 4 of *APOA5*	28.9	40	Impaired fasting Glycemia	31.6	1
55	M	Indian	Heterozygous, p.V227A in exon 5 of *LPL*	22.7	18	None	8.2	None
43	M	South Asian	Homozygous for p.G215E-mutation of *LPL*	22	35	Present	10.0	4+
42	M	South Asian	Heterozygous, p.N3185 in exon 6 of *LPL*	27	17**	None	1.5	1
55	F	Caucasian	Heterozygous,p.I252T in exon 5 of *LPL*	23	35	None	2.3	1
43	F	Bangladeshi	Homozygous c. 15+2 delT, *APOC2*; heterozygous p.Thr108Met *GPIHBP1*	35	15.8*	None	6.26	5

LPL = lipoprotein lipase, *on treatment, ** immediate post pancreatitis.

The criteria applied gave a pick up rate of >50% for diagnosis of familial hypertriglyceridaemia.
Early diagnosis and genetic confirmation may enhance compliance with lifestyle management and therapy and may prevent and reduce complications such as diabetes and pancreatitis.