Proposal form for the evaluation of a genetic test for NHS Service Gene Dossier

Test – Disease – Population Triad

Disease – name and description (please provide any alternative names you wish listed) (A)-Testing Criteria	Familial hypercholesterolaemia (FH) is an autosomal dominant disorder caused by mutations that directly affect the rate at which low density lipoprotein cholesterol (LDL-C) is cleared from the blood. The subsequent elevated levels of LDL-C cause accelerated atherosclerosis resulting in increased risk of coronary heart disease (CHD).
OMIM number for disease	#143890**,** #144010, #603776
Gene – name and description (please provide any alternative names you wish listed)	LDLR, low density lipoprotein receptor 606945 APOB, apolipoprotein B100, 107730 PCSK9, proprotein convertase, subtilisin/kexin-type, 9, 607786
OMIM number for Gene	606945, 107730, 607786
Mutational spectrum for which you test	Point mutations, small and larger deletions and duplications, splice site mutations, Pm mutations for LDLR Missense mutation for APOB and for PCSK9
Technical Method (s)	Amplification refractory mutation analysis (ARMs) -pre-screen Direct sequence analysis Multiplex ligation-dependent probe amplification analysis (MLPA) - Dosage analysis
Validation Process Note please explain how this test has been validated for use in your laboratory	The ARMS analysis for ADH is a commercial kit developed and validated by our group in collaboration with Tepnel (Taylor A, Tabrah S, Wang D, Sozen M, Duxbury N, Whittall R, Humphries SE, Norbury G. Multiplex ARMS analysis to detect 13 common mutations in familial hypercholesterolaemia. Clin Genet. 2007 Jun; 71(6):561-8). Direct sequence analysis is a standard platform used in the CPA accredited laboratory for most mutation analysis, and multiplex ligation probe amplification is also widely used in the laboratory for a number of diagnostic services. The laboratory successfully participates in EQA for sequence analysis (& dHPLC)
Are you providing this test already? If yes, how many reports have you produced? Please give the number of mutation positive/negative samples you have reported	Over 1400 reports have been produced, April 2002 to Feb 2008, that includes around 600 probands received for the DH funded FH cascade (GKP) study. Around 84% of reports are mutation screens and 16% are mutation tests. From the above study with samples from 5 centres across the UK and using the Simon Broom criteria for definite and probable FH, we found a detection rate of 59% (13-75% range) in probands referred with a diagnosis of definite FH (DFH) and 31% (21-33%) in probands referred with a diagnosis of possible FH (PFH). Of the cascade mutation tests, around 67% were positive.
For how long have you been providing this service?	A service for familial hypercholesterolaemia has been available from this laboratory since 2001

Is there specialised local clinical/research expertise for this disease?	**Yes √**	**No**	**Please provide details** Prof Steve Humphries at UCL has a 25 year track record of research in FH. Dr Philip Lee is a national expert in FH particularly in children.
Are you testing for other genes/diseases closely allied to this one? Please give details	Yes. We have established services for Fabry disease (GLA), hypertrophic cardiomyopathy and dilated cardiomyopathy (MYBPC3, MYH7, TNNT2, TNNI3)		
Your Activity How many tests do you (intend to) provide annually in your laboratory?	With our high through-put system we currently screen over 500 FH samples per annum. We would be able to handle up to 1000 probands for ADH, aided by our move to purpose-built premises and developments in our IT interfacing systems (Oct 2008)		
Based on experience how many tests will be required nationally (UK)? Please identify the information on which this is based	Total National Provision The estimates are based on the incidence of FH, a 2007 HEART UK survey of the number of patients known and being treated by 130 UK lipid clinics and our DH funded study across 5 centres (with a diverse ethnic mix of patients) on the uptake of mutation screen tests and cascade tests. The activity can be broken down into a backlog and an incident cases workload. Backlog Cases is estimated at 8250 of the currently known FH patients that may request DNA testing in England. Incident cases is estimated at around 20 new FH patients/year/clinic or up to 2000 new FH patients /year/100 English clinics.. Relative (cascade) testing estimated at ~2400 family mutation tests per year.		

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	FH has an estimated prevalence of roughly 1:500 i.e. 110,000 subjects in the UK Information review Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. Am J Epidemiol. 2004 Sep 1;160(5):421-9.
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	Since FH is an autosomal dominant disorder the estimated carrier frequency for mutations causing FH is 1:500. This has never been tested directly. Mutations in at least distinct three loci cause FH; LDLR mutations account for the majority of cases with currently over 1000 mutations identified world-wide, with more than 100 reported in UK patients to date The APOB p.R3527Q mutation occurs in about 5% of UK patients The *PCSK9* p.D374Y mutation occurs in ~2% of UK patients
Estimated penetrance Please identify the information on which this is based	Estimating the penetrance of FH-causing mutations is complicated by the fact that elevated levels of LDL-C occur commonly in the general population. Conclusions from available Danish and Dutch studies indicate that when a relative has inherited an FH-causing mutation the penetrance is 100%. However, other relatives who have not inherited the family mutation may also have elevated LDL-C for polygenic and environmental reasons (e.g. a poor diet)
Target Population The essential clinical or family history features defining the target population must be described. (C)-Testing Criteria	1- Children (below age 16 years)– in whom clinical or biochemical diagnosis is less sensitive than genetic diagnosis. Earlier diagnosis improves compliance with life style changes and assists in clinical management decisions. In general, children tested will be relatives from a family with an adult proband. However we will also accept proband referrals from paediatricians where the child meets the Simon Broome criteria (i.e. where the child has elevated LDL cholesterol levels and for example a family history of early CHD or the fatal MI of a parent). 2- People with at risk relatives who will benefit from early diagnosis The adult index cases will be Simon Broome Definite and Possible FH patients. The UK Simon Broome FH register criteria (Betteridge et al. 1991, 1999) are used to classify patients into definite or possible FH. The testing criteria are defined below with referral form (page 13)

Estimated prevalence of disease in the target population	By definition, all index cases with a clinical diagnosis of FH will have untreated LDL-C levels above the diagnostic criteria. Of their relatives, 50% will have the family mutation, and the majority of these will have elevated LDL levels and require treatment, Ethnicity Patients with a clinical diagnosis of FH of African, Oriental or South Indian origin are under-represented in UK lipid clinics compared to the general population prevalence (in the DH audit project database 3% of the FH patients were South-Indian and 1 % were Afro-Caribbean). While this may in part be due to under diagnosis in these subjects, it may also reflect a lower prevalence of FH-causing mutations in these different groups. FH in subjects from the Indian sub-continent is not uncommon, since a number of children with homozygous FH are known in the UK (most from consanguineous marriages).

Intended Use (Please use the questions in Annex A to inform your answers)

Please tick the relevant clinical purpose of testing	YES	NO
Diagnosis	√	
Treatment (and compliance)	√	
Prognosis & Management	√	
Presymptomatic testing	√	
Risk Assessment	√	

Test Characteristics

<table>
<tr>
<td>Analytical sensitivity and specificity

This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.

If a number of genes will be tested, please include your testing strategy and data on the expected proportions of positive results for each part of the process.

It may be helpful to include a diagram to illustrate the testing strategy.</td>
<td>Our study is based on over 600 samples from 5 centres, Newcastle, Harefield, Royal Free (London), Birmingham (Sandwell), Kent and Bournemouth using the Simon Broome referral criteria for definite (DFH), and possible FH (PFH), Testing was performed using a sequential strategy of ARMS, dHPLC/sequence and MLPA.

<table>
<tr><td>Analytical</td><td>v2.0 ARMS*</td><td>dHPLC /sequence**</td><td>MLPA**</td></tr>
<tr><td>Sensitivity</td><td>~43%</td><td>~95%</td><td>~5%</td></tr>
<tr><td>Specificity</td><td>~100%</td><td>~100%</td><td>~95%*</td></tr>
</table>

* Any mutations detected by ARMs are confirmed by sequence/restriction analysis to confirm the zygosity.
** Exon 12 was sequenced directly due to the presence of polymorphisms. Any shifts detected on dHPLC were sequenced.
*** As is standard for diagnostic use of MLPA, any results indicating deletion/duplication of a <u>single</u> probe require validation to exclude the rare possibility of a primer or probe polymorphism. We detected one such case in our cohort.</td>
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<td>Clinical sensitivity and specificity of test in target population

The clinical sensitivity of a test is the probability of a positive test result when disease is known to be present; the clinical specificity is the probability of a negative test result when disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without disease (for specificity)</td>
<td>In our study of over 600 samples from 5 centres, Newcastle, Harefield, Royal Free (London), Birmingham (Sandwell), Kent and Bournemouth using the Simon Broome referral criteria for DFH, and PFH,
Detection rate
DFH overall 59% (range 13-75%)
PFH overall 31% (range 0-42%)
The variation between the centres may reflect the way in which the Simon Broome criteria were interpreted.

Clinical
Sensitivity – the Simon Broome criteria that are recommended by the draft NICE guidelines and that are widely used in the UK gives a sensitivity of around 90%.

Unlike other disorders such as HCM, BRCA there seems little problem with unclassified variants. In our experience with the last 600 patients, the proportion of detected sequence changes where a definitive report cannot be written is less than 1%.

Additional comments – there are a small but significant number of families (3-5 per year) in which there is a risk of FH on both sides of the family and consequently the children may be homozygotes or compound heterozygotes. These are referred to us because ICH is a tertiary referral centre. When such a sample is received, testing is continued till both mutations are identified, with the use of samples from both parents included if</td>
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	possible. Specificity - large cohorts of patients without the clinical symptoms of FH have not been tested, so no data on specificity are available
Clinical validity (positive and negative predictive value in the target population) The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical disease or predisposition. It is measured by its *positive predictive value* (the probability of getting the disease given a positive test) and *negative predictive value* (the probability of not getting the disease given a negative test). The denominator in this case is the number of people with a positive or a negative test respectively - not the number with or without the disease. The clinical validity may be calculated knowing the sensitivity and the specificity and the prevalence of the disease in the population being studied. Positive and negative predictive values depend critically on the prevalence of the disease in the test population	The issue of the clinical validity of the DNA tests for FH is also complicated. This is primarily because of the overlap in plasma LDL-C levels between FH-mutation-positive and normal individuals based on lipid levels alone, and also the high prevalence of hyperlipidaemia in the general population which increases with age. In Index cases the PPV is 100%, NPV has not been determined. The frequency of detected mutations in FH patients is always less than 100% In relatives the PPV is 100%, and all mutation positive subjects require either treatment or monitoring and their 1st degree relatives need to be tested. While essentially the NPV in relatives is also 100% there will be a proportion of relatives who have not inherited the family mutation but who have elevated LDL-C levels because of (poly) genetic and environmental (e.g. dietary) causes. In addition there are rare cases where a relative has inherited a different mutation from the other side of family (or non paternity). There are essentially no cases of *de novo* mutations causing FH (<5 in the world literature). In the UK GRAFT study 20% of carriers and 7.45% of non-carriers would have been incorrectly diagnosed on the basis of their (age and gender specific) 95th percentile total cholesterol levels. This strengthens the utility of DNA testing in families for carrier status and unequivocal FH diagnosis reported by others

Clinical utility of test in target population (Please refer to Appendix A) Please provide a full description of the clinical care pathway for those individuals undergoing testing. This should include details of which medical specialties will be able to refer for testing. (B)-Testing Criteria	Lipidologists will refer the majority of referrals for testing, i.e. Consultants and their accredited Supporting Health Care professionals (i.e. Genetic Associates) running recognised lipid clinics. Index Subjects will be required to have elevated Total or LDL-C above the Simon Broome cut-off (7.5mmol/l and 5.0mmol/l respectively if an adult and 6.7mmol/l and 4.0mmol/l in children). They will also have a family history of hypercholesterolemia or premature heart disease (possible FH) or will have a personal or family history of tendinous xanthomata (definite FH). Paediatrician with Metabolic Expertise will also send referrals for testing. Children will be required to have lipid levels above the 95th percentile for age and gender and will have a family history of hypercholesterolemia or premature heart disease (possible FH) or will have a r family history of tendinous xanthomata (definite FH). Subjects may also be referred from a Cardiology setting, when subjects will have evidence of premature CHD (e.g. MI >55 years in males <65 in females) plus evidence of family history of CHD or personal or family history of hyperlipidaemia.
How will the test add to the management of the patient or alter clinical outcome? What impact will this test have on the NHS i.e. by removing the need for alternative management and/or investigations for this clinical population? Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test) please state the added advantage of the molecular test Are there specific ethical, legal or social issues with this test?	Management: Identification of a mutation can give an unequivocal diagnosis, and several studies of a DNA versus cholesterol-based diagnosis have shown that 15-20% of family members would have been incorrectly classified based on cholesterol testing alone. This has clinical utility in several ways. i) cascade testing to allow secondary testing ONLY in those carrying the family mutation and not from those with elevated LDL-C levels for non-monogenic reasons. (as recommended by NICE) ii) early identification of children (before the age of 10 years) who can be given appropriate dietary and lifestyle advice (exercise and not smoking) to reduce their subsequent risk of CHD. (as recommended by NICE) iii) Monitoring of all identified mutation carriers for LDL-C levels and treating appropriately with lipid-lowering statin therapy. iv) Identifying the patients that are at higher CHD risk who require more aggressive-lowering treatment and tracing of relatives (These being the mutation positive DFH patients with the PCSK9 p.D347Y or an LDLR mutation) Patient acceptance. The DH Cascade testing project which has been using both LDL-C and DNA testing has found a high take up rate and also a high level of satisfaction. Benefit to the NHS. Health economic modelling from the NICE FH guideline draft strongly support the beneficial impact of DNA diagnosis for 1) diagnosis of affected probands even allowing for the use of the more potent statins or possible combination therapies needed in most cases. 2) cascade testing of relatives using DNA is more cost effective than using LDL-C levels

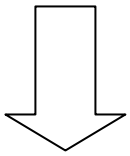
Please complete the referral pathway diagram on the following page and the testing criteria form.

Referral Pathway Template –

NOTE: Please use this page as a template. Please expand the test boxes manually as needed.

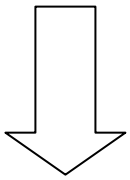
TARGET POPULATION
(Description)

Index Cases will be Simon Broome Definite and Possible FH patients. Index cases may also have a lower index of suspicion if referred from a cardiology setting (e.g. MI <55 years in males <65 years in females plus evidence of family history of CHD or personal or family history of hyperlipidaemia).



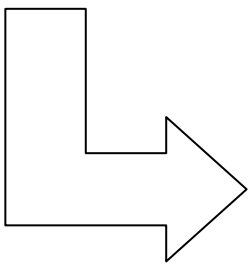
WHAT TYPE AND LEVEL OF PROFESSIONAL OR REFERRER DO YOU ACCEPT SAMPLES FROM?

Consultants and their accredited Supporting Health Care professionals (i.e. Genetic Nurses) running recognised lipid clinics, Pediatricians with Metabolic Expertise
Clinical Geneticists, Cardiologists with appropriate expertise



PLEASE PROVIDE DETAILS OF HOW REFERRALS WILL BE ASSESSED FOR APPROPRIATENESS?

Index Subjects will be required to have elevated Total or LDL-C above the Simon Broome cut-off (7.5mmol/l and 5.0mmol/l respectively if an adult and >95th percentile for age and gender in children). They will also have a family history of hypercholesterolemia or premature heart disease (possible FH) or will have a personal or family history of tendinous xanthomata (definite FH). Where referred from a cardiology setting subjects will have evidence of premature CHD (e.g. MI <55 years in males <65 years in females) plus evidence of family history of CHD or personal or family history of elevated lipid levels



HOW MANY TESTS DO YOU EXPECT TO PERFORM ANNUALLY?

500 →1000 probands
plus 1000-2000 relative samples
This number may increase as the clinical provision expands but is felt to be realistic in the first year(s) and based on experience with HCM. GOSH has capacity to handle this number in its new accommodation (Oct 2008).

Approval Date: Oct 2008 (revised Feb 2011)

Submitting laboratory: GOSH Molecular Genetics

UKGTN Testing criteria:

Name of Disease(s):
HYPERCHOLESTEROLEMIA, AUTOSOMAL DOMINANT (143890)
HYPERCHOLESTEROLEMIA, AUSTOSOMAL DOMINANT, TYPE B (144010)
HYPERCHOLESTEROLEMIA, AUTOSOMAL DOMINANT, TYPE 3 (603776)

Name of gene(s):
low density lipoprotein receptor (familial hypercholesterolemia); LDLR (606945)
apolipoprotein B (including Ag(x) antigen); APOB (107730)
proprotein convertase subtilisin/kexin type 9; PCSK9 (607786)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Clinical Geneticists	
Consultant Lipidologist	
Consultant in Metabolic Medicine	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria One of the following:	Tick if this patient meets criteria
Simon Broome Criteria for **definite FH** in adults*	
Simon Broome Criteria for **possible FH** in adults*	
Total or LDL-C above the 95th percentile for age and gender in children	
Family history of confirmed familial hypercholesterolaemia (provide details of mutation, family relationship and testing laboratory)	

*For mutation screen Simon Broome diagnostic criteria for probands
Definite familial hypercholesterolaemia is defined as:

1. Total cholesterol above 6.7mmol/l or LDL cholesterol above 4.0mmol/l in a child aged under 16 years or total cholesterol above 7.5mmol/l or LDL cholesterol above 4.9mmol/l in an adult (levels either pre-treatment or highest on treatment) **and**

2. Tendon xanthomas in patient, or in 1st degree relative (parent, sibling, child), or in 2nd degree relative (grandparent, uncle, aunt) **OR**

3. DNA-based evidence of an LDL receptor mutation, familial defective apo B-100, or a PCSK9 mutation.

Possible familial hypercholesterolaemia is defined as no.1 above and to include one of the criteria below:

1. Family history of myocardial infarction: below age of 50 years in 2nd degree relative or below age 60 years in 1st degree relative

2. Family history of raised total cholesterol: above 7.5mmol/l in adult 1st or 2nd degree relative or above 6.7mmol/l in child or sibling aged under 16 years.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Testing pathway