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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Hyperlipoproteinemia, TYPE II

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Hyperlipoproteinaemia, Type II
Hypercholesterolaemia, Familial
Hyperlipoproteinemia, Type IIA
Hyper-Low-Density-Lipoproteinaemia

1.2 OMIM# of the disease

143890.

1.3 Name of the analysed genes or DNA/chromosome segments:

LDLR (LRG_274), *APOB* (NM_000384.2) and *PCSK9* (LRG_275).

1.4 OMIM# of the gene(s):

LDLR (606945), *APOB* (107730), *PCSK9* (607786).

1.5 Mutational spectrum

About 1.300 variants in the most commonly affected gene in the autosomal dominant form of Familial Hypercholesterolemia (FH), *LDLR*, are listed in the UCL *LDLR* variant database (http://www.ucl.ac.uk/ldlr/Current/index.php?select_db=LDLR) and the LOVDv2.0 platform (https://grenada.lumc.nl/LOVD2/UCL-Heart/home.php?select_db=LDLR). These variants are equally distributed over the gene and include exonic substitutions, small exonic rearrangements, large rearrangements, promoter variants, intronic variants and a variant in the 3' untranslated sequence, point mutations, splice site mutations, large deletions, with approximately 80% being likely to be disease causing.[1] Another reference database with known LDLR variants is maintained by Inserm (http://www.umd.be/LDLR/).

One major disease causing mutation in the *APOB* gene, c.10580 G>A (p.Arg3527Gln).[2–4]

Only a few *PCSK9* mutations, all of the missense type and resulting in a 'gain of function', have been found to be associated with the Hypercholesterolaemia phenotype, with some of them being restricted to certain ethnic groups.[5]

1.6 Analytical methods

Genetic testing: DNA extraction from peripheral blood (leucocytes), amplification of all 18 exons (*LDLR*) or 12 exons (*PCSK9*) and the promoter sequences or the part of *APOB* containing the major mutation, c.10580 G>A (p.Arg3527Gln), by PCR using flanking oligonucleotides, followed by direct DNA sequencing. Other methods such as Multiplex Ligation-dependent Probe Amplification can be used to detect deletions and duplications of one or more exons in the *LDLR* gene. A stepwise procedure according to the proportion of mutations found in the three genes involved in the disease has been recommended (*LDLR*>*APOB*>*PCSK9*), however, with the development of next generation sequencing methods in diagnostic laboratories, the approach of library capture of all exons promoters etc. of the three genes and simultaneous sequencing is becoming feasible and should be considered.[6] Where homozygous FH or compound heterozygous FH is suspected and neither of the three genes have been found mutated, consider sequence analysis of the *LDLRAP1* gene, responsible for the autosomal-recessive form of FH (ARH).

1.7 Analytical validation

The analysis of an independent biological sample is recommended in order to confirm an identified mutation. Analysis of samples from family members for segregation of the identified variant with the disease might be helpful, especially when the identified variation was not described before.

1.8 Estimated frequency of the disease

(Incidence at birth ('birth prevalence') or population prevalence. If known to be variable between ethnic groups, please report):

The general prevalence is reported to be about 1:500, with a higher frequency because of founder effects in the following populations: Lebanese, Afrikaners in South Africa and French Canadians (reviewed in Liyanage KE *et al.*[7]), Finnish/North Karelia[8] and Danish.[9] Based on this frequency FH is dramatically underdiagnosed in most countries.[3]

1.9 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☐	☒

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Comment:
Prenatal testing is possible, but not carried out frequently because testing can be done soon after birth. Prenatal testing for homozygosity might be considered in families presenting a relevant constellation, however, there are differences in laws on prenatal testing between countries.

2. TEST CHARACTERISTICS

	genotype or disease		A: True positives B: False positives	C: False negatives D: True negatives
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A + C)
			Specificity:	D/(D + B)
Negative	C	D	Pos. predict. value:	A/(A + B)
			Neg. predict. value:	D/(C + D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)
Close to 100%.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
Close to 100%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)
The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Based on clinical criteria patients can be classified as definite FH or possible FH. In a UK study the mutation detection rate was roughly 60% for patients with definite FH and 30% for patients with possible FH.[10] When patients are classified on the basis of the Dutch Lipid Clinic Network Criteria (DLCNC) score as 'possible'(> 3 and < 5), 'probable' (> 5 and < 8), or 'definite' FH (> 8), 70% of 'definite' FH patients were found to carry a mutation, only 29% of 'probable' and 11% of 'possible' FH patients were mutation-positive.[11] Considering a similar mutation screening strategy these rates can be expected to be similar for most European populations.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)
The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Approximately 100%. A negative FH diagnosis based on the LDL-C level made before the age of 2 (reviewed in Haney EM *et al.*[12]) and the rare paradox phenotype of relatively low LDL-C levels in people with a positive genetic test are reasons for not reaching the 100%.

2.5 Positive clinical predictive value
(life time risk to develop the disease if the test is positive)

Close to 100%. The paradox phenotype of relatively low LDL-C levels in genetically diagnosed FH patients was observed in different studies with a very low frequency, e.g. with a proportion of 1.6% in a Dutch cohort.[13]

2.6 Negative clinical predictive value
(Probability not to develop the disease if the test is negative)
Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:
Close to 100%.
Index case in that family had not been tested:
Close to 100%.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.9 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☒
	Electrophysiology	☐
	Other (please describe)	familial segregation

3.1.2 Describe the burden of alternative diagnostic methods to the patient
The clinical diagnosis of definite FH or possible FH can be made based on the National Institute for Clinical Health and Excellence (NICE)-endorsed Simon Broome Criteria or the Dutch Lipid Clinic Network Criteria. The criteria consider LDL-Cholesterol levels, clinical findings (Xanthoma, premature atherosclerosis) and family history of hypercholesterolaemia or premature coronary heart disease.[14,15]

There is an overlap with the autosomal-recessive form of hypercholesterolemia (OMIM #603813) and polygenic hypercholesterolaemia which can only be ruled out by a genetic diagnosis.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Not applicable.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	FH patients are at a significantly increased lifetime risk for cardiovascular disease (CVD).[14] In FH patients all modifiable cardiovascular risk factors should be aggressively treated. According to the ESC/EAS guidelines for management of dyslipidaemias in FH patients a treatment goal is to achieve an LDL-C value below 100 mg/dl or 2.6 mmol/l (in the presence of CVD < 70 mg/dl or 1.8 mmol/l) and according to the NICE guidance an LDL-C reduction of at least 50% from the level before treatment.[16] Modification of the baseline lifestyle including smoking cessation, physical activity and diet to reduce LDL-C, containing less than 7% saturated fat, less than 1% trans fats, and less than 200 mg of cholesterol per day but rich in Omega-3 fatty-acids should be encouraged, though the

	LDL-C lowering effect might be modest (10% reduction).[17] Statin therapy is the drug treatment with primary priority. Statins have been shown to reduce LDL-C, decrease inflammation and oxidative stress, to stabilize atherosclerotic plaques, inhibit the thrombogenic response and improve endothelial function.[18] Statins have proven to reduce cardiovascular mortality and morbidity.[19] First-line treatment of FH patients should be started with the more potent statins (Simvastatin, Atorvastatin and Rosuvastatin). If LDL-C target values can not be achieved by high dose statin treatment alone, which is common in FH patients, adding ezetimib or a bile acid binding resin should be considered. In severe cases of heterozygous FH, in particular with concomitant CVD or homozygous FH LDL-apheresis might be the only effective means to achieve target LDL-C levels (reviewed in Thompsen J *et al*.[20]). The main clinical utility of a DNA test result is to enable unambiguous cascade testing in relatives, since LDL-C levels in FH and non-FH relatives overlap considerably, especially in adults.[21] While in most cases the genetic test results will not influence the therapeutic strategy, there are several scenarios where the therapeutic route is based on the genetic test result: According to the EAS consensus statement initiation of statin treatment in children at the age of 8–10 is recommended on the basis of a positive genetic test result or strong clinical arguments including LDL-C >135 mg/dl (>3.5 mmol/l). The EAS expert panel advises to offer lipoprotein apheresis in children with homozygous FH. Lipid lowering therapy as described above should be offered to people with a genetically diagnosed FH even if the clinical diagnoses can not made based on the LDL-C level in order to reduce the lifetime LDL-C exposure.[3] The latter patients are typically diagnosed as a result of mutation analysis in relatives of an index patient, emphasising the usefulness of the cascade screening strategy. Considering that several new strategies to reduce LDL-C levels are under development[22] and a successful treatment with some of them might depend on the knowledge of the gene mutated in a specific patient, it can be expected, that the impact of the genetic test result on the disease management will rather increase in the near future. In the case of PCSK9 inhibitors for example, a treatment will most likely not be successful in patients with two *LDLR* null mutations.
Prognosis (please describe)	Depending on the age of initiating the statin therapy the cumulative LDL-C burden can be lowered to an extent that, with a low dose statin therapy starting between the age of 8 and 10 followed by a high dose statin therapy with the 3rd life decade, the LDL-C burden in the patient is comparable to a non affected individual.[21]
Management (please describe)	Once a person is identified as FH positive, a family cascade screening should be initiated in order to be able to treat affected family members as early as possible to prevent premature atherosclerosis. Since FH patients are at a high risk for cardiovascular disease they should be evaluated for the presence of cardiovascular disease and monitored closely for therapy adherence.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe):
Yes, the patient would be aware of an increased risk for atherosclerotic events which might be prevented by changes in lifestyle, including fat modified diet with a high content of polyunsaturated fatty acids, Mediterranean diet, physical activity and lipid-lowering medication treatment.[23]
If the test result is negative (please describe):
Persons with affected family members who suffered from early myocardial infarction or other consequences of FH will be relieved by the knowledge of being unaffected.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
There is no difference in the options with respect to lifestyle and prevention between genetically tested and non-tested individuals.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.9 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
A genetic test would show which family members are affected and need treatment.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
A positive genetic test will lead to additional genetic testing when cascade screening is initiated. However, knowledge of the mutation segregating in the family of the index patient will simplify genetic testing in further family members.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes.

3.4 Prenatal diagnosis
(To be answered if in 1.9 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Not applicable.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Even if LDL-C is currently low such that statin treatment is not recommended, the person is aware that he is a carrier and therefore at risk for developing elevated LDL-C in later life, and this may encourage maintaining a healthy life style, and lead to regular monitoring of lipid levels by their doctor. Their first degree relatives (ie their children and brothers and sisters) who are at 50% risk of also having inherited the FH-causing mutation, may not also have inherited the genetic factors protecting the index case from having elevated LDL-C and should certainly be advised to have their genotype and lipid levels tested.

CONFLICT OF INTEREST

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