European Journal of Human Genetics (2015) 23, doi:10.1038/ejhg.2014.225

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CLINICAL UTILITY GENE CARD UPDATE

Clinical utility gene card for: Familial hypobetalipoproteinaemia (*APOB*) – Update 2014

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European Journal of Human Genetics (2015) **23,** doi:10.1038/ejhg.2014.225; published online 22 October 2014

Update to: *European Journal of Human Genetics* (2012) **20**, doi:10.1038/ejhg.2012.85; published online 16 May 2012

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)
Familial hypobetalipoproteinaemia (Familial hypobetalipoproteinaemia type 1).

1.2 OMIM# of the disease
615558.

1.3 Name of the analysed genes or DNA/chromosome segments
APOB.

1.4 OMIM# of the gene(s)
107730.

1.5 Mutational spectrum
Over 90 variants have been reported, mostly nonsense and frameshift, occurring throughout the 29 exons of *APOB*.[1–4] Missense variants affecting the amino terminal region of the apolipoprotein B (apoB) protein have also been described in familial hypobetalipoproteinaemia.[5]

1.6 Analytical methods
DNA sequencing of genomic-exonic DNA with at least 20 bp of flanking intronic sequence. Western blotting can be used to detect truncated apoB species that are >30% of full-length protein size, to estimate where the variant occurs within *APOB*. If western blotting is negative, sequencing of the 5' 30% of the gene (exons 1 through 25) is recommended. Where homozygous familial hypobetalipoproteinaemia is suspected and a variant(s) in *APOB* cannot be found, consider sequencing *MTTP*.

1.7 Analytical validation
Where a variant is identified using bi-directional DNA sequencing, the test should be repeated from a fresh dilution of DNA for confirmation. For homozygous familial hypobetalipoproteinaemia, testing of the patient's parents is recommended, to confirm that the two variants are present in trans (ie, on opposite chromosomes).

1.8 Estimated frequency of the disease
(Incidence at birth ('birth prevalence') or population prevalence)
Approximately 1 in 1000–3000 are estimated to carry apoB truncations (heterozygous familial hypobetalipoproteinaemia).[6]

1.9 If applicable, prevalence in the ethnic group of investigated person
Not applicable.

1.10 Diagnostic setting

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

Comment:
Familial hypobetalipoproteinaemia is primarily a biochemical diagnosis; heterozygotes have plasma apoB levels one-quarter to one-third of normal, while in homozygotes apoB is very low or undetectable. Prenatal diagnosis may be required by parents who are both known to carry short truncations leading to severe apoB deficiency.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

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

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

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Received 17 June 2014; revised 8 September 2014; accepted 19 September 2014

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.

Many factors, such as a strict vegan diet, illness and high-dose statin therapy, can cause hypobetalipoproteinaemia. The more common secondary causes in the hospital setting include cachexia, malabsorption, malnutrition, severe liver disease and hyperthyroidism. Familial hypobetalipoproteinaemia is characterised by low levels (<5th percentile for age and sex) of plasma apoB-containing lipoproteins.[1,2] It has been suggested that familial hypobetalipoproteinaemia might represent a longevity syndrome and be associated with cardiovascular protection due to resistance to atherosclerosis. Heterozygotes are usually asymptomatic with low-density lipoprotein (LDL) cholesterol and apoB concentrations approximately one-third of those in normal plasma. Most of them have increased serum aminotransaminases due to hepatic steatosis and sometimes mild fat malabsorption.

Homozygous familial hypobetalipoproteinaemia is biochemically characterised by the absence of apoB-containing lipoproteins from plasma. The clinical features may resemble those of abetalipoproteinaemia: fat malabsorption, fatty liver, acanthocytosis, retinitis pigmentosa, spinocerebellar ataxia and myopathy, particularly in those with truncating variants shorter in length than apoB-48. Should this constellation of findings be present there are two possibilities, homozygous familial hypobetalipoproteinaemia or abetalipoproteinaemia caused by variants in *MTTP*. Family screening is useful in delineating between these conditions, as while obligate heterozygote parents of abetalipoproteinaemia patients have normal plasma lipid profiles, obligate heterozygous parents of homozygous familial hypobetalipoproteinaemia patients have plasma LDL cholesterol and apoB concentrations approximately one-third of normal.

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%.

2.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive)

100%.

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:

100%.

Index case in that family had not been tested:

Should the constellation of clinical findings be present in an index case, it is possible that they might have abetalipoproteinaemia, rather than homozygous familial hypolipoproteinaemia. Abetalipoproteinaemia patients receive similar treatment advice as homozygous familial hypobetalipoproteinaemia patients. Also, there are even rarer conditions called homozygous proprotein convertase subtilisin/kexin type 9 (PCSK9) deficiency and familial combined hypolipidaemia (due to homozygous variants in angiopoietin-like protein 3, *ANGPTL3*) that present with extremely low (but not absent) levels of apoB-containing lipoproteins, but no systemic manifestations.[2] In familial combined hypolipidaemia, high-density lipoprotein (HDL) cholesterol levels are also very low. To date, there are only a handful of families in the world reported with these latter two genetic conditions.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.10 '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):	

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Heterozygotes for familial hypobetalipoproteinaemia are often asymptomatic, but have plasma LDL cholesterol and apoB concentrations that are approximately one-third of normal. Most of them have increased serum aminotransferases due to hepatic steatosis and sometimes mild fat malabsorption.[7,8] Homozygous familial hypobetalipoproteinaemia is characterised by the absence of plasma apoB-containing lipoproteins with marked hypocholesterolaemia, absence of LDL cholesterol and apoB and low triglyceride concentrations. In addition, increased serum aminotransferases due to hepatic steatosis, acanthocytosis and fat-soluble vitamin deficiency are found. Homozygous familial hypobetalipoproteinaemia, particularly those with truncating variants shorter in length than apoB-48, cannot be distinguished from abetalipoproteinaemia clinically.

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)	The cornerstone of treatment for homozygous familial hypobetalipoproteinaemia is dietary fat restriction with replacement of fat-soluble vitamins (A, D, E and K).[1,2,9] A low-fat diet (<30% of total calories) will eliminate steatorrhoea and allows absorption of other nutrients essential for growth and development. High-dose oral fat-soluble vitamins are thought to bypass the intestinal chylomicron assembly pathway via the portal circulation and are associated with improved clinical outcomes. High-dose oral vitamin E supplementation (100–300 mg/kg/day) is recommended to halt the progression of neurological disease; however, serum levels do not normalise. Supplementation with a combination of high-dose vitamins E and A is effective in reducing retinal degeneration. Although vitamin D and K deficiencies are inconsistent findings in homozygous familial

	hypobetalipoproteinaemia, oral replacement should be considered, along with other supplementary nutrients such as iron and folate if required. Vitamin E supplementation in heterozygous familial hypobetalipoproteinaemia has been recommended in those with low vitamin E concentrations to prevent neurological deficits;[1] however, more recently this advice has been called into question.[10]
Prognosis (please describe)	The impact of age at diagnosis, commencement of a low-fat diet and fat-soluble vitamin supplementation, and the findings from *APOB* genomic DNA sequencing in homozygous familial hypobetalipoproteinaemia are variable. Early treatment with high-dose oral vitamin E and A can reduce the potential severity of neuropathy and retinopathy.[1,11] A relative paucity of data makes it difficut to predict outcomes based on *APOB* genotype. The long-term outcome of hepatic steatosis in familial hypobetalipoproteinaemia is unknown, but associations with hepatic steatohepatitis, cirrhosis and hepatocarcinoma have been reported.[3,7,12,13]
Management (please describe)	The clinical follow-up and management of homozygous familial hypobetalipoproteinaemia focuses on evaluating symptoms and monitoring growth in children, detecting and preventing complications, and monitoring compliance with therapy by providing specialised dietary advice and fat-soluble vitamin therapeutic regimens.[9] Increased serum aminotransferases and hepatic steatosis are a common occurrence in heterozygous familial hypobetalipoproteinaemia; however, the long-term consequences are unknown. Therefore, it would seem prudent to monitor biochemically and by imaging techniques the livers of these individuals gven a potential increased risk of progression to cirrhosis, particularly in the presence of known risk factors, such as alcohol, caloric excess and liver injury.

3.2 Predictive setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.10 '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).
If the test result is **negative** (please describe).

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)?
Not applicable.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.10 'C' was marked).

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Not applicable.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Not applicable.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Not applicable.

3.4 Prenatal diagnosis
(To be answered if in 1.10 '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)

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by EuroGentest2 (Unit 2: 'Genetic testing as part of health care'), a Coordination Action under FP7 (Grant Agreement Number 261469) and the European Society of Human Genetics. RAH is supported by the Jacob J. Wolfe Distinguished Medical Research Chair at the University of Western Ontario, the Edith Schulich Vinet Canada Research Chair in Human Genetics (Tier I), the Martha G. Blackburn Chair in Cardiovascular Research, and operating grants from the CIHR (MOP-13430, MOP-79533), the Heart and Stroke Foundation of Ontario (T6066, 000353), and Genome Canada through Genome Quebec award 4530. JRB is supported by a Practitioner Fellowship from the Royal Perth Hospital Medical Research Foundation.

1 Kane JP, Havel RJ: Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins; in: Scriver CR, Beaudet AL, Sly WS *et al*: (eds): *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill, 20018th ednpp 2717–2752.
2 Hooper AJ, Burnett JR: Update on primary hypobetalipoproteinemia. *Curr Atheroscler Rep* 2014; **16**: 423.
3 Welty FK: Hypobetalipoproteinemia and abetalipoproteinemia. *Curr Opin Lipidol* 2014; **25**: 161–168.
4 APOB Leiden Open Variation Database. https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=APOB.
5 Burnett JR, Zhong S, Jiang ZG *et al*: Missense mutations in APOB within the betaalpha1 domain of human APOB-100 result in impaired secretion of ApoB and ApoB-containing lipoproteins in familial hypobetalipoproteinemia. *J Biol Chem* 2007; **282**: 24270–24283.
6 Welty FK, Lahoz C, Tucker KL, Ordovas JM, Wilson PW, Schaefer EJ: Frequency of apoB and apoE gene mutations as causes of hypobetalipoproteinemia in the Framingham offspring population. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1745–1751.
7 Hooper AJ, Adams LA, Burnett JR: Genetic determinants of hepatic steatosis in man. *J Lipid Res* 2011; **52**: 593–617.
8 Schonfeld G, Patterson BW, Yablonskiy DA *et al*: Fatty liver in familial hypobetalipoproteinemia: triglyceride assembly into VLDL particles is affected by the extent of hepatic steatosis. *J Lipid Res* 2003; **44**: 470–478.
9 Lee J, Hegele RA: Abetalipoproteinemia and homozygous hypobetalipoproteinemia: a framework for diagnosis and management. *J Inherit Metab Dis* 2014; **37**: 333–339.
10 Clarke MW, Hooper AJ, Headlam HA *et al*: Assessment of tocopherol metabolism and oxidative stress in familial hypobetalipoproteinemia. *Clin Chem* 2006; **52**: 1339–1345.
11 Chowers I, Banin E, Merin S *et al*: Long-term assessment of combined vitamin A and E treatment for the prevention of retinal degeneration in abetalipoproteinaemia and hypobetalipoproteinaemia patients. *Eye* 2001; **15**: 525–530.
12 Tarugi P, Lonardo A, Ballarini G *et al*: A study of fatty liver disease and plasma lipoproteins in a kindred with familial hypobetalipoproteinemia due to a novel truncated form of apolipoprotein B (APO B-54.5). *J Hepatol* 2000; **33**: 361–370.
13 Di Filippo M, Moulin P, Roy P *et al*: Hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia due to homozygous MTTP and APOB mutations. *J Hepatol* 2014; **61**: 191–902.