Frequent Detection of Familial Hypercholesterolemia Mutations in Familial Combined Hyperlipidemia*

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Familial combined hyperlipidemia (FCH) is the most common genetic cause of hyperlipidemia, affecting approximately 1% of the population. It was first described in the Seattle Myocardial Infarction Study in 1973 (1). This condition is characterized by variable lipid phenotypes (increased levels of triglycerides or cholesterol or both lipids) in the proband and in relatives that may vary within an individual person from time to time. Familial combined hyperlipidemia contributes to ≥20% of coronary artery disease in males under the age of 60 years (2,3).

The diagnosis of FCH is difficult, requiring data from family members who may not be available. Percentile cutoffs, often >90th or >95th percentile for cholesterol, low-density lipoprotein (LDL)-cholesterol, or triglyceride elevations, are utilized to establish the diagnosis. As lipid levels vary over time, both a negative and a positive diagnosis can be unreliable. An increase in apolipoprotein B (apoB) (4,5), together with elevated numbers of small, dense LDL particles, appears to be a more consistent phenotype for FCH than are total cholesterol and triglycerides, whose levels vary over time (6).

Although originally described as autosomal dominant, more recent data indicate a more complex inheritance. Several loci with major and minor impacts on risk of FCH, as well as environmental factors, have been demonstrated.

Variants in upstream transcription factor (USF)-1 were linked in a Finnish FCH cohort (7) and associated in Dutch patients (8) and Mexican patients (9). Paradoxically, a dyslipidemia haplotype was associated with lower cardiovascular disease risk (10). The USF-1 dyslipidemia haplotype has been associated with lower interleukin-6 and C-reactive protein levels increasing with age, suggesting that the reduced risk may not involve regulation of lipid genes (11).

However, this locus only accounts for some FCH families, suggesting other factors remain undetected. Other loci implicated include lipoprotein lipase (12) and the apolipoprotein A1/C3/A4/A5 gene cluster (13,14). Additionally, a large number of chromosomal regions have been implicated by linkage in FCH families. Clearly, FCH is genetically heterogeneous.

Heterozygous familial hypercholesterolemia (FH) is diagnosed based on high LDL-cholesterol levels (usually >250 mg/dl) in untreated adults and frequent tendinous xanthomas. Familial hypercholesterolemia, which is autosomal dominant and associated with early vascular disease, can be confirmed by molecular demonstration of a mutant LDL receptor (LDLR) gene. However, molecular studies are rarely done clinically, as the results do not influence treatment. A defect in the ligand for the LDLR, apoB, is a less common cause of FH, as are missense mutations of PCSK9 (15). FH occurs in about 1 in 500 persons in the population, and accounts for approximately 4% of premature myocardial infarctions (1). Hypertriglyceridemia can independently occur in FH, causing mixed hyperlipidemia and leading to confusion with FCH.

Against this background and potential uncertainties about the diagnosis of FCH, Civiera et al. (16) searched for classical FH mutations in FCH using probes for 203 LDLR mutations and 4 apoB mutations. Their novel microarray techniques (Lipochip) (17) recognized 88% of 230 different LDLR mutations from Spain. Methodology to find rearrangements in the LDLR gene was also utilized (18). In all, 143 unrelated middle-aged men and women with FCH were selected from the clinical records of 2 lipid clinics (16). Inclusion required LDL-cholesterol levels of >170 mg/dl or non–high-density lipoprotein-cholesterol of >220 mg/dl when triglyceride levels were over 400 mg/dl. At least 1 first-degree relative had to have hyperlipidemia, which was defined as total cholesterol and/or triglyceride levels >90th percentile. Functional LDLR mutations were found in 28 patients (20%). No apoB mutations were detected. Patients with LDLR mutations had higher levels of cholesterol LDL-cholesterol and apoB levels as compared with patients without these mutations, and their triglyceride levels were somewhat higher than in patients without LDLR mutations. Diabetes mellitus was found in 26 patients (23%) without LDLR mutations and was absent in all LDLR mutation carriers. Considering only nondiabetic patients with cholesterol levels of >355 mg/dl and apoB levels of >185 mg/dl, 48 patients (or 42% of study subjects) had LDLR mutations. The detected LDLR mutations were likely to be pathogenic, as most of the LDLR abnormalities...
in this study had been observed in other Spanish FH patients (17).

Although it is not surprising that “weaker” mutations in LDLR might result in diagnostic uncertainty in the absence of molecular diagnosis, the high frequency of FH mutations in nondiabetic patients with a presumptive clinical diagnosis of FCH is unexpected. Whereas part of this excess can be ascribed to selection or referral bias of more complicated patients to a referral center, and to the atypically high LDL levels in this cohort, these data indicate potential difficulties with the clinical diagnoses of FH and FCH. Subjects with LDLR mutations lacked tendon xanthomas, which would have suggested FH but frequently are absent. Furthermore, patients with LDLR mutations in this study had lower lipid levels than those typical for FH. The lack of persons with isolated elevated triglyceride levels in a family with presumptive FH should suggest the possibility of FH rather than FCH.

Similar studies on FCH using molecular search for LDLR mutations are strongly suggested. What should be the role of molecular testing for LDLR mutations in FCH? Currently, LDLR molecular studies are rarely done clinically, owing to the absence of both the impact on patient management and the ability to test for affected relatives using lipid determination rather than molecular studies, which are not generally available. Although the presence of a LDLR mutation allows definite diagnosis of FH, studies of lipid levels alone will give informative diagnostic results in affected and nonaffected family members in both FH and FCH.

Should molecular testing be more widely utilized, some consideration must be given to the optimal test. Available options include coverage of the whole gene with current sequencing methodology (19) or a microarray including individual known mutations such as used by the current study (16) and the study by Tejedor et al. (17). The content of a microarray mutation panel for FH detection must, of course, reflect the various mutations in a given population, which varies. Note that at least 800 different FH mutations exist. With the falling cost of comprehensive deoxyribonucleic acid sequencing, an approach covering the entire LDLR gene, including promoters, is likely to detect all LDLR mutations and ultimately will be possible.

A separate issue is the utility of APOE testing in subjects with a presumptive diagnosis of FCH. Again, it is useful to consider whether knowledge of the results of the diagnostic test will change management of the patient. For APOE, there are special considerations because the APOE e4 allele is a risk factor for late-onset Alzheimer’s disease and is found in approximately 25% of heterozygotes in the population (e2e4 and e3e4) and in <1% of homozygotes (e4e4) (20), depending on race. Testing for APOE, therefore, requires a tailored approach. In the study under discussion (16), 4 of 115 FCH patients carried the APOE e2e2 genotype. These patients have type III hyperlipidemia (dysbetalipoproteinemia or remnant removal disease), which can be diagnosed by examining very low-density lipoprotein composition (21) or with APOE phenotyping by gradient gel electrophoresis (21). Less than 1% of most populations carry such APOE e2e2. In view of the higher risk of Alzheimer’s disease—2 to 3 times higher for e4 heterozygotes and 15 times higher for e4e4 homozygotes (22) compared with non-e4 genotypes—and the fact that e2 and e4 are separately testable polymorphisms, many physicians request only APOE e2 testing or do not inform patients of their APOE e4 status and its predictive significance for Alzheimer’s disease. Ideally, before any testing that may disclose e4 status, all patients should be advised about potential risks for Alzheimer’s disease and offered clinical genetics consultation.

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