

# What matters most in pediatric familial hypercholesterolemia, genotype or phenotype?<sup>1</sup>

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Familial hypercholesterolemia (FH) is an inherited clinical disorder of lipoprotein metabolism characterized by life-long elevated levels of LDL-cholesterol (LDL-C) and increased risk of premature cardiovascular disease (pCVD) (1). The condition affects between 1:250 to 1:500 or 12 to 25 million people worldwide but in the vast majority remains undiagnosed and untreated (2).

FH is most frequently due to *LDLR* mutations and, to date, over 1,200 different mutations have been reported. Less commonly, the condition is due to mutations in *APOB* or *PCSK9* (3). However, even with the latest next-generation sequencing techniques, a mutation in these genes is not detected in up to 30% of subjects diagnosed by clinical criteria with definite FH and over 50% of subjects with clinical diagnosis of probable or possible FH (4).

The study by Medeiros et al. (5) in this issue of the *Journal of Lipid Research* evaluated lipid biomarkers in 237 unrelated children between the ages of 2 and 17 years diagnosed with FH based on clinical criteria slightly modified from the Simon Broome registry (6), but which required a total cholesterol (TC) >260 mg/dl or LDL-C >155 mg/dl plus either a family history of hypercholesterolemia (defined as TC >290 mg/dl in at least one parent) or pCVD. All but a few of the younger children met the TC or LDL-C criteria, about 25% met the pCVD criteria, and 75% had a documented TC >290 mg/dl in a parent. Consistent with similar studies in subjects selected based on clinical criteria as having FH, they reported 38% of their cohort had a mutation in either *LDLR* or *APOB*. These genetically confirmed FH children tended to have higher apoB and lower apoA1 levels and had a higher apoB/A1 ratio compared with those without a molecular diagnosis of FH, or 'non-genetically confirmed' FH (nonGC-FH). As would be expected, those children carrying null *LDLR* mutations had a more severe phenotype with significantly higher LDL-C levels compared with those carrying defective mutations. The authors then proceeded to subdivide the original clinical FH cohort into 'FH' and 'nonFH', despite the use of rather strict initial clinical criteria where virtually all children had TC or LDL-C close to or above

the age-specific 99th percentile and at least in 75% had a parent with a TC >290 mg/dl, clearly indicating a "familial" disorder and genetic predisposition to their elevated LDL-C. Thus, it appears inappropriate to relabel such children as 'nonFH' as it is likely that as technology advances, more will have a genetic cause identified. This may not even be a single major gene change but, as been proposed by Talmud et al. (4), a constellation of minor genes, or "polygenic" FH. In the interim, perhaps a better classification would be "genetically confirmed" FH (GC-FH) and "nonGC-FH".

As the authors point out, the reason for identifying children as having clinical FH in the first place is to initiate therapy to reduce the pCVD risk associated with FH. However, all pediatric studies, including those that have used ultrasound to monitor carotid intima-medial thickness changes during statin therapy, have enrolled and treated FH children based on clinical, not genetic, criteria (7, 8). None of these trials in children have shown or even suggested that treatment responses differ in GC-FH versus nonGC-FH or that LDL-C treatment goals based on genetic mutations are or should be different. This raises the obvious but important question, especially for pediatricians: is it important to make a genetic diagnosis of FH?

In general, the risk and age of onset of atherosclerosis and CVD is related to the extent and duration of raised LDL-C calculated as the cholesterol-year-score (9). Subjects with hypercholesterolemia in whom a mutation cannot be identified but with similar LDL-C levels to subjects with GC-FH likely have a similar risk for CVD. The benefit of identifying a causative mutation in a subject with clinical FH is that it allows for screening and identification of affected family relatives who are often asymptomatic but, if found to be mutation positive with high LDL-C levels, would benefit from lipid-lowering therapy. Mutation negative, nonGC-FH, patients may, however, have an as yet unidentified mutation and cascade testing of family members based on LDL-C, and clinical criteria is still warranted in view of the increased cardiovascular morbidity and mortality

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associated with their early and life-long elevated LDL-C. In fact, in this study more than half the children fell into this latter category.


The rationale expressed by the authors that GC-FH children may require more aggressive treatment is not substantiated from prior trials in children with FH where treatment is not gene-specific but LDL-C specific. All guidelines recommend a step-by-step approach beginning with diet and life-style modification and then, based on post diet LDL-C and age, commencing with statins, increasing doses as needed to achieve LDL-C targets and if necessary, combination therapy with bile binding sequestrants and/or ezetimibe (10). Thus while those with nonGC-FH in this study tended to be more overweight or obese and had marginally but not statistically significant higher triglyceride levels, in practice, treatment for most would be no different from those with GC-FH. In fact, in both FH groups, the prevalence of obesity or overweight children was concerning, with 44% of GC-FH children having a BMI >75th percentile, not much less than the 58% of nonGC-FH children. However, while the initial LDL-C levels in the GC-FH cohort were higher, there tended to be more clustering of multiple CVD risk factors in the nonGC-FH children, which would tend to neutralize any differences in the aggressiveness of treatment as LDL-C targets should take global risk into account.

Medeiros and colleagues also evaluated a number of other lipid and apolipoprotein parameters and assessed them alone, in combination, or as ratios to determine if they would add greater diagnostic specificity or sensitivity in diagnosing GC-FH. They concluded that apoB/AI ratio of >0.68 when added to Simon Broome criteria was the best marker for differentiating GC-FH from nonGC-FH. However, such a ratio is almost completely dependent on the validity of the measurements of these two apolipoproteins and, from the data presented, a serious question arises for at least apoB. From the data presented in Table 2 of the main article and Table II in the supplementary online material, there is inconsistency in the reported levels of apoB relative to the LDL-C levels, as well as relative to those in different cohorts. The relationship in well-standardized laboratories indicate that for a mean LDL-C of around 233 mg/dl, as seen in the GC-FH cohort, an apoB of at least 140 to 180 mg/dl would be expected, substantially higher than the reported mean of 118.6 mg/dl. For example, in the FH lovastatin adolescent male study where measurements were performed in a highly standardized and certified central lipid laboratory, mean baseline LDL-C was ~ 250 mg/dl and apoB 195 mg/dl, while in the rosuvastatin pediatric FH trial (PLUTO), where mean baseline LDL-C in the various treatment groups ranged from 229 to 238 mg/dl, the mean apoB ranged from 140 to 150 mg/dl (11, 12). This inconsistency was also seen in the nonGC-FH cohort, where a mean LDL-C of 179 mg/dl was associated with a mean apoB of only 92 mg/dl (Table 2), well below values seen in previously reported pediatric and adult populations with similar LDL-C levels (13, 14). In the children identified by cascade screening and whose lipids were compared with those of the GC-FH cohort, the mean

LDL-C was 204 mg/dl, nearly 30 mg/dl lower than the index cohort, yet their apoB was 122.8 mg/dl, 4 mg/dl higher. Differences in levels of other lipid fractions such as lipoprotein(a), non HDL-C, or small dense LDL (sLDL) cannot explain this discrepancy. Thus it is premature, based on this study, to include such a ratio as it is likely very different ratios would be obtained in different laboratories. Certainly the use of apoB has been suggested as a better marker for FH in children than LDL-C (14).

The results reported for sLDL, on the surface appear perhaps counterintuitive, as sLDLs tend to be disproportionately increased in subjects who are overweight or obese or who have features of the metabolic syndrome, features seen to a greater extent in the nonGC-FH cohort. On the contrary, subjects with FH tend to have large LDL particles. However, higher sLDLs were reported in the GC-FH children who had lower triglycerides and less obesity, but as these children had higher LDL-C and apoB, the entire spectrum of apoB particles would be expected to be increased, including sLDL, even though they may have been lower as a percentage of total particles than in the nonGC-FH group had all particles been measured. However as it turned out and as one would expect, measurement of sLDL provided no additional discriminating information to the quest of differentiating GC-FH and nonGC-FH.

While only lipid biomarkers were evaluated in this study, it would have been a good opportunity to determine whether other nonlipid inflammatory biomarkers such as hsCRP differed between the groups. Hypercholesterolemia due to elevated remnant cholesterol levels as occurs in obesity tends to be associated with inflammation and raised hsCRP levels, whereas elevated LDL-C alone as occurs in FH is not associated with raised hsCRP levels (15).

In summary, all children with a clinical diagnosis of FH based on criteria such as Simon Broome need to be identified, any underlying medical conditions contributing to secondary LDL-C elevation corrected, and then counseling regarding diet, lifestyle, and regular exercise encouraged. If the LDL-C does not respond, or responds inadequately, to such lifestyle measures, irrespective of genetic confirmation or not, they should, in our view, be considered for lipid-lowering therapy. At present, the use of molecular diagnosis in FH remains confined to improving cascade screening and there does not appear to be a role for other lipid biomarkers to either guide diagnosis or therapy. 

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