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RESEARCH LETTER

The Lebanese allele at the *LDLR* in normocholesterolemic people merits reconsideration of genotype phenotype correlations in familial hypercholesterolemia

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Familial hypercholesterolemia (FH) was first described in Lebanon in 1964 by Khachadurian [13]; however, it took 20 years for the gene responsible for the disease to be cloned [19, 23]. In the mid 1980s, mutations in the gene encoding the low density lipoprotein receptor (LDLR) were being reported in FH patients [4, 10, 14, 16, 22]. One of the earliest mutations to be described was the 2463C>A in exon 14, a nonsense mutation that results in substitution of cysteine for a stop codon and production of a truncated receptor [15]. The mutation was termed the "Lebanese allele," and resulted in a severe phenotype due to a functionally defective receptor. The mutation is responsible for the very high prevalence of FH in Lebanon due to a presumed founder effect, as shown by studies on Lebanese immigrants [5, 9, 18] and more recently on FH cohorts in Lebanon [1, 8]. There has been considerable evidence for the high penetrance of this particular mutation. The Lebanese allele segregated with the phenotype in all the reported families, and was the only *LDLR* mutation in most FH patients [1, 2]. Classically, patients with one allele were observed to have LDL-C levels that are twice the normal and were termed heterozygous, while those with two alleles had LDL-C levels that are four times the normal and were termed homozygous. For many years, FH was thought of as a monogenetic disease and the terms homozygous and heterozygous are still used for clinical diagnosis without mutational screening. Nevertheless, over the last decade, three new genes other than the *LDLR* were linked to FH, and a mutation-negative gap still remains in most FH cohorts where there is no mutation after screening for all the four known genes. More importantly, there have been reports of modifier genes and non-genetic factors that can decrease LDL-C levels in carriers of *LDLR* mutations [7, 12].

We have been studying Lebanese families with severe (clinically homozygous) FH, which to our knowledge is the largest reported cohort. We had confirmed in an earlier study that the majority of FH in Lebanon is caused by the *LDLR* Lebanese allele [8]. Through screening large families, we were able to detect, for the first time, normocholesterolemic probands heterozygous for the Lebanese allele. This finding was surprising since we have experienced several Lebanese allele heterozygous patients from our cohort with severe elevations of LDL-C levels to the extent that they were clinically diagnosed as homozygous and placed on LDL apheresis. This was the first time that we see the Lebanese allele in normocholesterolemic patients.

We genotyped 23 families with index members clinically diagnosed with homozygous FH as reported in our previously published article [8]. Fourteen of the 23 families

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had the *LDLR* mutation at the Lebanese allele. The number of patients homozygous for this mutation was 18 and their average lipid profile while on LDL apheresis was as follows: TC 600 mg/dL; LDL 493 mg/dL; HDL 36 mg/dL; triglycerides 144 mg/dL (All values were taken before an LDL apheresis session) [8]. Twenty-one genotyped family members were heterozygous for the *LDLR* Lebanese allele. Four members belong to two kindreds (C and K) and have normal LDL-C levels (Fig. 1). One subject is a young child that had a normal lipid profile, and eleven members did not have fasting lipid profiles. The remaining five members were all aged $\geq$50 belonging to three families, and three of the five subjects were on LDL apheresis for many years due to a severe elevation of LDL-C levels at diagnosis. The current average lipid levels of these five subjects are as follows: TC 294 mg/dL; LDL 194 mg/dL; 61 mg/dL; and triglycerides 200 mg/dL.

Families C and K illustrate the absent clinical expression of FH in heterozygotes for the *LDLR* Lebanese allele (Fig. 1). In Family C, two siblings (HC6 and HC7) are homozygous for the Lebanese allele and have a severe phenotype (LDL-C of 544 and 451 mg/dL, respectively while on LDL-apheresis therapy for many years). The father and older sibling, both heterozygous for the *LDLR* Lebanese allele, have LDL-C levels of 74 and 70 mg/dL. respectively. The mother in Family C is an obligate heterozygote and expressed the FH phenotype by hearsay, but she was not genotyped and phenotype. In Family K, both parents (HC70 and HC71) have LDL-C levels at the upper level of normal on no treatment, yet they are heterozygous for the *LDLR* Lebanese allele. The parents in both families are non-consanguineous; however they are from the same ethnicities within Lebanon.

In an attempt to explain the findings, we tried to look at known factors that modify the phenotype in FH. Table 1 shows the characteristics of the subjects with respect to some of these factors. Clinically, the four heterozygous patients with normal phenotype have no unique exposures or environmental risk factors. They are all previously healthy, have a normal BMI, and eat a regular diet. They have repeatedly measured their lipid profile, which is consistently normal and hence never were on any lipid-lowering medications. All the four subjects were carefully examined for secondary causes of decreased LDL-C such as liver disease or cancer, and none was found.

Previous studies since the late 1980s have observed normocholesterolemics carrying various heterozygous *LDLR* mutant alleles [11, 17]. In one of the studies, the inheritance of a dominant gene that prevents the expression of the FH phenotype was noted [11]. In another, the β^0-thalassemia trait was shown to decrease LDL-C levels in heterozygous FH patients in Sardinia [6]. A more recent study has looked at the effect of LDL-lowering genes *APOB*, *PCSK9*, and *ANGPTL3* on heterozygous FH patients with unexpectedly low LDL-C levels [12]. Out of a total of 75 subjects studied, only five had rare loss of function variants in *APOB* that corrected the phenotype, while no such variants were found in *PCSK9* and *ANGPTL3* [12]. Apart from that, there has been no success in identifying a single gene that would totally correct the LDL-C levels in FH patients, as would be required in our case. GWAS studies on the other hand have identified a

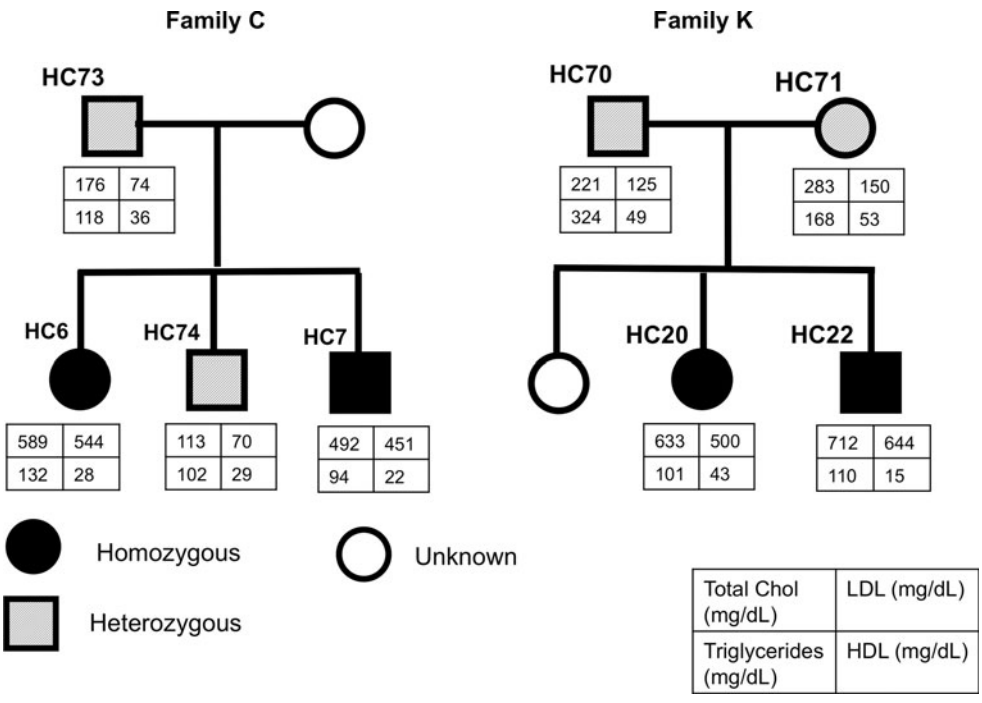

Fig. 1 Pedigrees of families C and K form the Lebanese FH cohort

Table 1 Characteristics of homozygous and heterozygous subjects from families C and K

Family	Subject	LDL (mg/dL)	*LDLR* mutation	*ANGPTL3* sequencing	*APOB* sequencing	*PCSK9* sequencing	PCSK9 Exon1 polymorphism	Age (years)	Age at start of apheresis
C	HC6	544	Hom 2043C>A	No mutation	No mutation	No mutation	L9/L9	22	6
	HC7	451	Hom 2043C>A	No mutation	No mutation	No mutation	L10/L10	17	5
	HC73	74	Het 2043C>A	No mutation	No mutation	No mutation	L9/L10	–	–
	HC74	70	Het 2043C>A	No mutation	No mutation	No mutation	L9/L9	–	–
K	HC20	500	Hom 2043C>A	No mutation	No mutation	No mutation	L9/L9	17	6
	HC22	644	Hom 2043C>A	No mutation	No mutation	No mutation	L9/L10	11	6
	HC70	125	Het 2043C>A	No mutation	No mutation	No mutation	L9/L9	–	–
	HC71	150	Het 2043C>A	No mutation	No mutation	No mutation	L9/L10	–	–

L number of leucine residues in exon 1 of *PCSK9* on each of the two alleles; Fasting LDL levels were taken for patients randomly before one LDL-apheresis session. Sequencing of LDLR, ANGPTL3, and PCSK9 included all exons and intronic–exonic boundaries. Only exon 26 of *APOB* was sequenced

large number of loci that can modify LDL-C levels, yet each with very low effect [21]. Reports have also noted the importance of common variants in modifying lipid levels, yet with a lower effect [12]. Nonetheless, the cumulative dose of large number of variants can explain a decreased clinical expression of the FH phenotype. A recent report showed that a polymorphism in exon 1 of the *PCSK9*, particularly an insertion of a leucine to a stretch of nine leucine residues, correlated with lower LDL-C levels in FH patients heterozygous for the Lebanese allele [1].

Given the above data from the literature, we sequenced our four probands for all exons and intron–exon boundaries of *PCSK9* and *ANGPTL3*. We also sequenced exon 26 of the *APOB* gene, where the most known variants associated with decreased LDL-C levels are located [3, 20]. As seen in Table 1, the leucine stretch insertions in exon 1 of *PCSK9* did not explain the normal phenotype in our subjects. No coding or splice-site variation could be identified in *ANGPTL3*, *PCSK9*, and exon 26 of *APOB*.

This novel observation indicates that the Lebanese allele might not have a high penetrance as currently believed, and it might also be prevalent in normocholesterolemic people in the Lebanese population. The only screening of normocholesterolemic Lebanese controls was on 50 patients [1]. It is possible that screening of more controls is needed to determine the prevalence of this allele in the general population in Lebanon. This observation suggests that normocholesterolemic *LDLR* Lebanese allele carriers have another unknown genetic variation that is decreasing their LDL-C levels and blunting the otherwise deleterious effect of the mutant allele. Previously reported known genes that decrease the clinical expression of heterozygous FH could not explain the phenotype in our two kindreds. Although it is possible that a large set of common variants in many loci/genes contributes a cumulative effect to decreasing the LDL-C levels, it is less likely to be the cause in our subjects, particularly kindred C, where the LDL-C levels are very low. It is more likely that a mutation in another unidentified gene that results in a high hypocholesterolemic effect segregates in these families.

It would be extremely interesting to identify additional genes involved in decreasing LDL-C levels because these could potentially serve as therapeutic targets analogous to the *PCSK9* gene. More importantly, this finding merits reconsideration of genotype phenotype correlations in FH, a genetic disease where functional studies on effects of mutations are uncommonly performed. The heterozygous–homozygous nomenclature ought to be carefully used by clinicians and scientists to refer only to the genotype, because the genetic background of individuals can blunt the phenotype. We thus suggest using the terms mild and severe when a clinical diagnosis is performed on FH patients and using the terms heterozygous and homozygous only when referring to a particular mutation.

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