Atypical Presentation and Treatment Response in a Child with Familial Hypercholesterolemia Having a Novel LDLR Mutation

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Abstract Familial hypercholesterolemia (FH) is an autosomal codominantly inherited disease. The severity of clinical presentation depends on the zygosity of the mutations in the *LDLR*, *APOB*, or *PCSK9* genes. The homozygous form (HoFH) is associated with high mortality rate by third decade of life, while individuals with HeFH begin to suffer from premature cardiovascular disease in fourth or fifth decade of life. Statin drugs have helped to improve the biochemical profile and life expectancy in HeFH, while they are only minimally effective in HoFH. LDL apheresis remains an effective treatment option in HoFH, though limited by its availability and affordability issues. We present the case that highlights a few novel aspects of clinical and genetic heterogeneity in FH, wherein a child presented with features of both HeFH and HoFH. His clinical picture was that of HoFH; however he responded well clinically and biochemically to pharmacologic treatment only. DNA sequencing showed a novel heterozygous rare splicing variant in the *LDLR* gene in addition to a relatively high polygenic trait score comprised of LDL-C raising alleles from common polymorphic sites. Interestingly his normolipemic mother showed the same heterozy-

gous mutation. Thus this novel splicing variant in *LDLR* showed nonclassical co-segregation with the disease phenotype and was associated with a high polygenic trait score comprised of common LDL-C raising polymorphic alleles in the affected proband. Thus it indicates the phenotypic heterogeneity of FH and suggests that secondary causes, such as polygenic factors and possibly as yet undetermined genetic or environmental factors, can exacerbate the metabolic phenotype in an individual who is genotypically heterozygous for FH.

Abbreviations

ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
FH	Familial hypercholesterolemia
HDL	High-density lipoproteins
HeFH	Heterozygous familial hypercholesterolemia
HoFH	Homozygous familial hypercholesterolemia
LDL-C	Low-density lipoprotein cholesterol
TG	Triglycerides

Introduction

Familial hypercholesterolemia (FH) is a rare and life-threatening disease. FH is classically an autosomal codominant disorder: heterozygous FH (HeFH), due to a single mutant allele, has a population prevalence of ~1:250, while the clinically more severe homozygous FH (HoFH) due to bi-allelic mutations has a population prevalence estimated between 1 in 160,000 and 300,000 (Cuchel et al. 2014). Though the categorization as HoFH or HeFH is based on clinical and genetic parameters there is not a linear genotype-phenotype correlation in FH. Both HeFH and

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HoFH are characterized by xanthomas, elevated cholesterol levels, and premature atherosclerotic cardiovascular disease (CVD), although levels of low-density lipoprotein (LDL) cholesterol are elevated to a much greater degree in HoFH (up to tenfold normal) compared to HeFH (up to fourfold normal). Both HeFH and HoFH can result from one or two rare mutations, respectively, in *LDLR*, *APOB*, and *PCSK9* genes. In contrast, carriers of one mutant allele of the *LDLRAP1* gene have no phenotype: HoFH is expressed phenotypically only in individuals with two rare mutations in the *LDLRAP1* gene (Cuchel et al. 2014). Interestingly, with increased genetic analysis, there is clearly some phenotypic overlap between certain individuals who genetically would be considered to have HeFH or HoFH based on molecular genetic criteria alone.

If left untreated HoFH has been reported to be almost universally fatal by the second or third decade of life, while individuals with HeFH begin to suffer from premature CVD in the fourth or fifth decade of life. Statin drugs have helped to normalize life expectancy in HeFH, while they are only minimally effective in HoFH (Cuchel et al. 2014; Marais et al. 2002; Gagne et al. 2002). The mainstay of treatment in HoFH has been weekly or biweekly plasmapheresis or selective LDL apheresis. Newer treatments recently approved or under development, including gene therapy, are providing new hope for children and young adults afflicted with HoFH (Cuchel et al. 2014). Given the proven ability of various LDL-lowering treatments to alter disease course and extend life, it is imperative to diagnose FH early and to begin appropriate management (Wiegman et al. 2015). Also the clinical presentation of HeFH and HoFH can represent a continuum; while genetic confirmation though beneficial has its own drawbacks in lacking a robust genotype-phenotype correlate (Cuchel et al. 2014; Soutar and Naoumova 2007; Talmud et al. 2013).

Case Report

We present an 18-month-old boy, born to non-consanguineous parents who presented with diffuse cutaneous xanthomas especially over the hands, legs, and face while the rest of clinical examination was unremarkable (Fig. 1). His blood examination (Table 1) revealed elevated total cholesterol level. Further lipid analysis showed elevations in both LDL cholesterol (LDL-C) and triglycerides (TG), with decreased high-density lipoprotein cholesterol (HDL-C). Apolipoprotein A-1 (apo A-1) level was decreased while apolipoprotein B (Apo B) level was increased. Based on the impressive xanthomatosis and very high LDL-C for age, a provisional diagnosis of HoFH was made, as he satisfied essentially all the diagnostic criteria (Cuchel et al. 2014; Wiegman et al. 2015). Causes for secondary

Fig. 1 Cutaneous xanthomas seen the child (index case)

hypercholesterolemia including nephrotic syndrome, hypothyroidism, primary biliary disease, and liver dysfunction were ruled out. Other diagnostic considerations included sitosterolemia, lipoprotein lipase deficiency, lysosomal acid lipase deficiency (cholesterol ester storage disease – CESD), apo A-1 or apo C-2 deficiency, apo E variant or E2/E2 homozygosity (type III hyperlipoproteinemia), and LCAT deficiency. Echocardiography and carotid ultrasound to assess intimal thickness measurement were both normal. He was started on a low plant sterol diet and statin treatment. Subsequently ezetimibe and cholestyramine were added. With this treatment his xanthomas and lipid profile showed substantial improvement (Tables 1 and 2) over the next one and a half years until his last consultation.

The patient and his parents underwent targeted next-generation DNA sequencing (Hegele et al. 2015), which found that the patient and his mother were each heterozygous for a novel insertion/deletion mutation in the *LDLR* gene, namely in +8 delCGGGGCCAGGG in intron 4. DNA sequence analysis of other candidate genes in monogenic dyslipidemia, including *APOB*, *PCSK9*, *LDLRAP1* (*ARH*), *ABCG5*, *ABCG8*, *LIPA* (*CESD*), *APOA1*, *APOE*, *APOC2*, and *LCAT*, revealed no other rare and

Table 1 Lipid profile at baseline (at the time of diagnosis) and after 18 months of therapy with statin and ezetimibe

	Normal	Pretreatment	Posttreatment	Change with treatment
Total cholesterol (mg/dl)	<200	403	297	−26.3%
Triglycerides (mg/dl)	<150	211	200	−5.2%
HDL cholesterol (mg/dl)	<40	9	11	+22.2%
LDL cholesterol (mg/dl)	<130	352	249	−29.3%
VLDL cholesterol (mg/dl)	10–30	42	37	−11.9%
Apo A-I (mg/dl)	120–176	80	75	−6.2%
Apo B (mg/dl)	63–114	166	140	−15.6%

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very-low-density lipoprotein, *apo* apolipoprotein

Table 2 Lipid profile of parents as part of reverse cascade screening

	Normal	Mother	Father
Total cholesterol (mg/dl)	<200	162	203
Triglycerides (mg/dl)	<150	115	219
HDL cholesterol (mg/dl)	<40	41	41
LDL cholesterol (mg/dl)	<130	98	118.2
VLDL cholesterol (mg/dl)	10–30	23	43.8

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very-low-density lipoprotein, *apo* apolipoprotein

potentially disease-causing variants. The patient was also found to have a high polygenic trait-raising score for LDL-C comprised of multiple small-effect SNPs (15/20), meaning that only ~8% of people would have a higher burden of polygenic effects to raise LDL-C. The findings were interesting in that the patient's mother was not markedly dyslipidemic. Additional genetic studies, including multiplex ligation primer amplification to detect large-scale copy-number variation of the *LDLR* gene, were normal in the family. Using the Rogan method, bioinformatics based analysis of the impact of the detected mutation on the RNA splicing revealed that it was highly deleterious. The mutation abolished a natural RNA splicing site and it was predicted that a cryptic isoform would be created 60 base pairs downstream and this would result in an increase in the binding by 3321.8%. By contrast the RT-PCR analysis of the *LDLR* RNA from peripheral blood leucocytes showed no evidence of splicing defects in the proband or parents.

Discussion

This case description highlights a few novel aspects of clinical and genetic heterogeneity in FH. The child presented with a clinical picture suggesting HoFH; however, he responded well clinically and biochemically to

pharmacologic treatment only, indicating that he has a phenotype with features of both HeFH and HoFH. DNA sequencing of known candidate genes showed a novel heterozygous rare splicing variant in the *LDLR* gene in addition to a relatively high polygenic trait score comprised of LDL-C-raising alleles from common polymorphic sites. It is of interest that his essentially normolipemic mother is heterozygous for the same *LDLR* mutation. Thus this novel splicing variant in *LDLR* showed nonclassical co-segregation with the disease phenotype. The only probable explanatory factor being a high polygenic trait score comprised of common LDL-C-raising polymorphic alleles. Additionally there may be parental allele-of-origin epigenetic influences or perhaps an oligogenic interaction involving a novel mutation in new genes that has not yet been associated with lipid metabolism. While this might be resolved by whole-genome or whole-exome sequencing, it would be challenging to ascertain a single potentially causative interacting novel secondary factor from the whole genome of a single individual.

This case indicates the phenotypic heterogeneity of FH and suggests that secondary causes, such as polygenic factors and possibly as yet undetermined genetic or environmental factors, can exacerbate the metabolic phenotype in an individual who is genotypically heterozygous for FH.

Author Contributions

Varma S: (a) Physician involved in patient care, (b) planning, (c) drafting the chapter.

McIntyre AD and Hegele RA: (a) Design and conduct of genetic tests, (b) revising the chapter manuscript.

Conflict of Interest

Varma Sharat, McIntyre Adam, and Hegele Rob declare that they have no conflict of interest.

Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible committee (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from parents of the child for genetic testing and publication of the scientific results.

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