EAS19-0321

Confirmation of Pathogenicity of a Rare Low Density Lipoprotein Receptor Mutation Causing Homozygous and Heterozygous Familial Hypercholesterolemia (FH)

T. Turner[1], E.M. Bahassi[1], E.Stein[2]

[1]Medpace and Medpace Reference Laboratories (MRL), Cincinnati, OH, USA;
[2]Universisty of Chicago Pritzker School of Medicine, Department of Medicine, Cardiology. Chicago, IL, USA

BACKGROUND

Familial hypercholesterolemia (FH) is most commonly due to a pathogenic mutation in the *LDL receptor* (*LDLR*). The *LDLR* was the first gene identified where mutations cause FH. *LDLR* is located on the short arm of chromosome 19 and consists of 18 exons that translate into five distinct domains which form the cell surface of *LDLR*. More than 2900 different variants in the *LDLR* have been identified[1] with over 1600 variants considered pathogenic[2] resulting in the FH phenotype. Here, we describe a family with a rare mutation in *LDLR* now confirmed as pathogenic for FH.

METHODS

The proband presented with a history of acute Achilles tendinitis at age 13, and a biopsy of extensor tendon nodules at age 18 was reported as cholesterol xanthoma. Subsequent lipid testing showed a total cholesterol of greater than 400 mg/dL, but no treatment was instituted. At age 35, she was seen by a lipid expert complaining of tachycardia at rest, and told she had FH. A stress echo was normal, and her LDL-C was greater than 300 mg/dL. She was prescribed statins, but took them intermittently and did not follow up regularly for evaluations. She was next seen in late 2017 at age 40 with large tendinous xanthomas on the extensor tendons of the hands and Achilles tendons bilaterally (Figure 1).

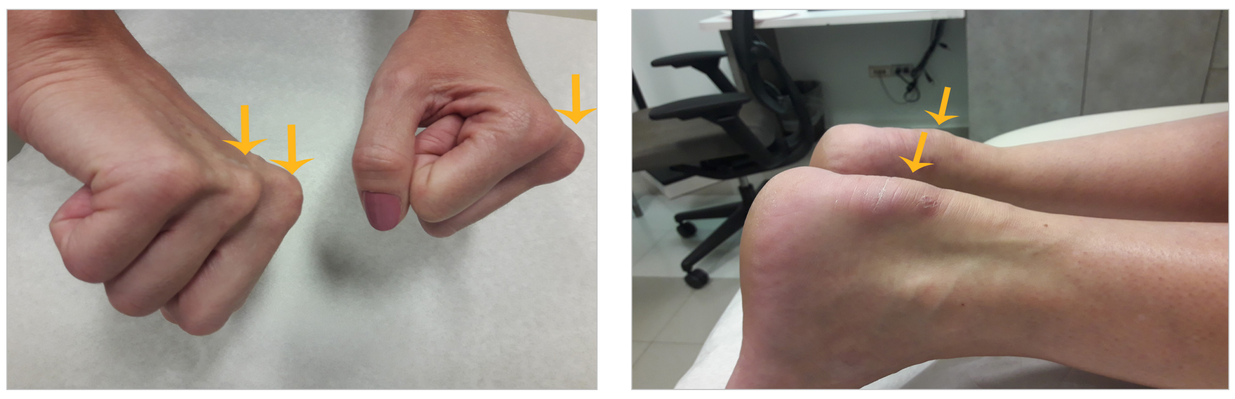


Figure 1: Proband's hands and Achilles tendons affected by xanthomas

She had not taken any lipid-lowering medication for the past 3 years and her LDL-C was 359 mg/dL. Despite being asymptomatic, CTA showed significant CAD, confirmed on angiography, and she had decreased flow reserve which resulted in LAD stenting. Her family history was not significant for CVD, especially no early CVD, and she indicated no known hyperlipidemia in either parent or in her 3 siblings. After intense counseling regarding her FH and CAD, she began treatment with rosuvastatin 40 mg daily and ezetimibe 10 mg daily; after 4 weeks of treatment, her LDL-C decreased to ~122 mg/dL. Evolocumab 140 mg every 2 weeks was added, and her LDL-C was further reduced to 52 mg/dL (Table 1).

Table 1: Proband lipid data

Date	8-Dec-17	19-Jan-18	2-Feb-18	2-Mar-18	4-Apr-18	6-Jul-18	25-Feb-19
Treatment	none	Rosuvastatin 40 mg QD					
		Ezetimibe 10 mg QD					
			Evolocumab 140 mg Q2W				
Cholesterol (mg/dL)	468	198	161	149	142	132	113
HDL-C (mg/dL)	98	67	78	86	79	65	68
Triglycerides (mg/dL)	56	47	56	54	50	39	54
LDL-C, Calc (mg/dL)	359	122	72	52	53	59	34

Based on her clinical history and the very early development of xanthomas, a genotypic evaluation for homozygous familial hypercholesterolemia (HoFH) was performed using next generation sequencing (NGS) on the MiSeq platform (Illumina) for *LDLR* (18 exons), *ApoB* (regions of exons 26, 29), *PCSK9* (12 exons) and *LDLRAP1* (9 exons). *In silico* analysis of missense mutations was performed using VarSeq software to determine pathogenicity status (Pathogenic, Likely Pathogenic, or Uncertain Significance).

RESULTS

NGS of the proband revealed identical mutations in *LDLR* [c.788A>G (p.Asp263Gly)]. The c.788A>G mutation in Exon 5 of *LDLR* affects the ligand binding domain of the LDLR protein (Figure 2), confirming the diagnosis of HoFH; clinical findings corroborated pathogenicity.

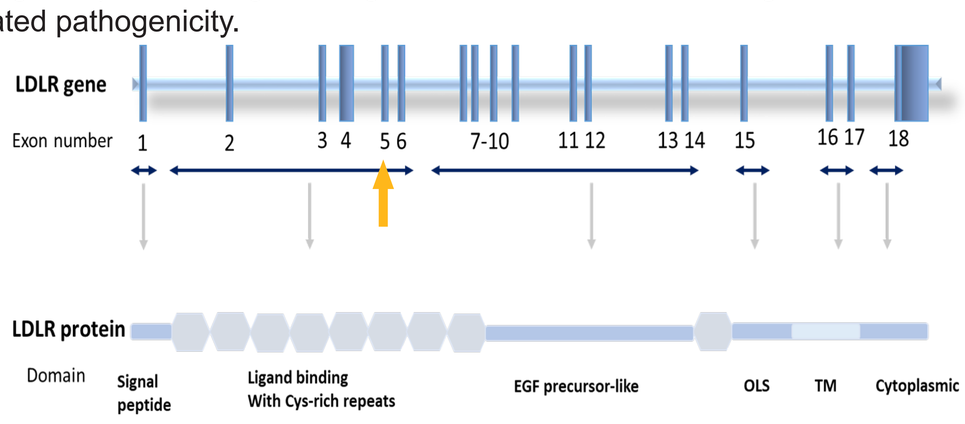


Figure 2: Proband variant (c.788A>G) in exon 5 of *LDLR*

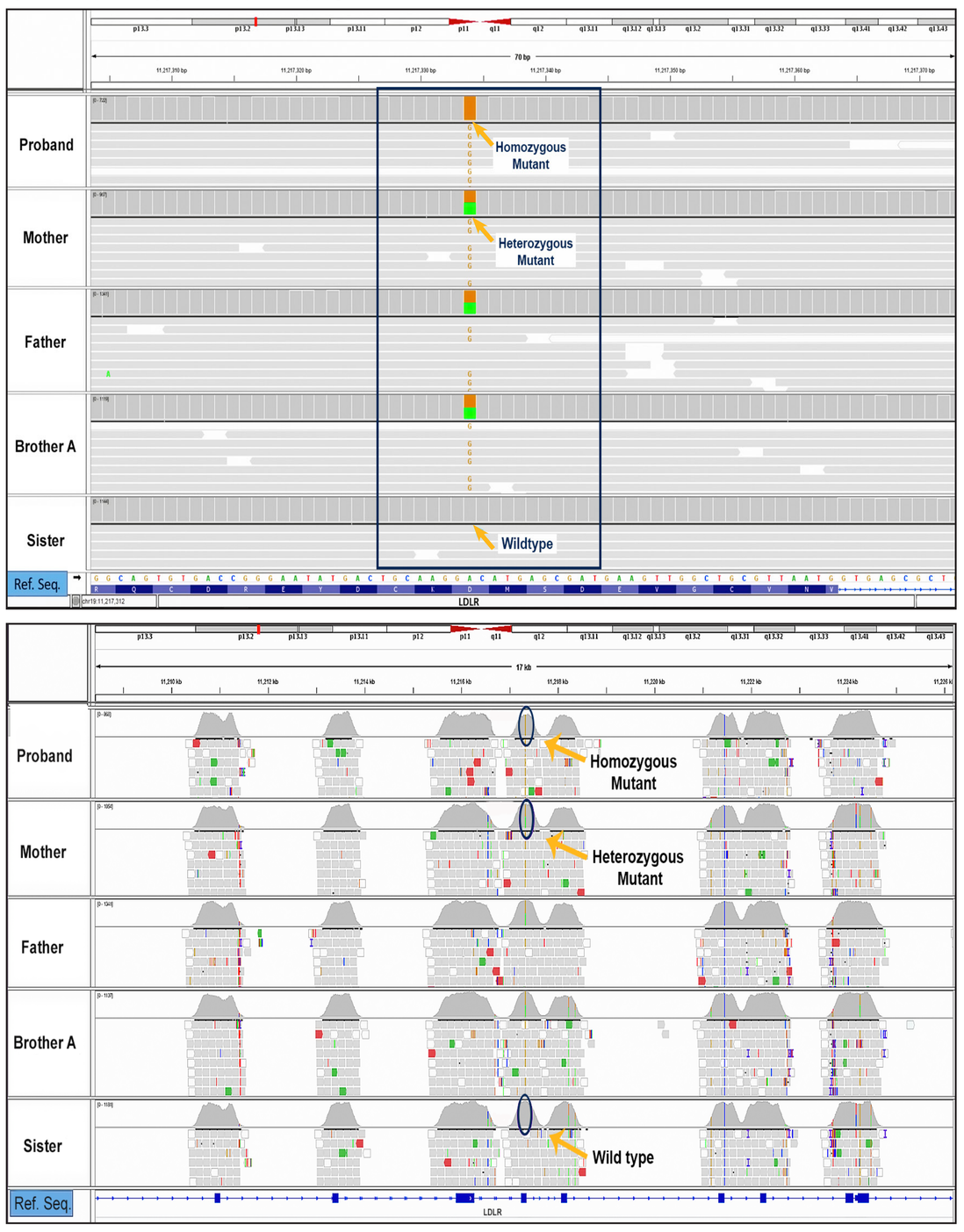
Further questioning revealed that her parents were first cousins, who originally lived in Calabria, Italy. Her parents, siblings and additional family members were subsequently screened for lipids and the *LDLR* mutation. Mutations were discovered in 6 of the 7 family members screened. The Integrative Genomics Viewer (IGV) confirmed the presence of the HoFH mutation in the proband, and the heterozygous family hypercholesterolemia (HeFH) mutation in the mother, father, both brothers and 2 of Brother B's 3 children (Table 2).

Table 2: *LDLR* mutants in proband and screened family members

Family Member	Age (years)	LDL-C (mg/dL)	ApoB (mg/dL)	Lp(a) (mg/dL)	FH status	*LDLR* Mutant	Xanthoma	CVD
Proband	40	359	224	9	HoFH	c.788A>G/c.788A>G	+++++	+
Father	69	125*	99	27	HeFH	c.788A>G	-	-
Mother	62	186	123	29	HeFH	c.788A>G	-	-
Brother A	44	243*	88	39	HeFH	c.788A>G	-	-
Sister	41	113						
Brother B	43	212	152	10	HeFH	c.788A>G	-	-
Daughter 1 of B	16	144	112	19	HeFH	c.788A>G	-	-
Son of B	14	79	56	22	HeFH	c.788A>G	-	-
Daughter 2 of B	6	79	65	17				

*indicates statin use

Uniform read coverage distribution was observed across the *LDLR* in the mutated area amongst the family members (Figures 3 and 4).



Figures 3 and 4: Next-Generation Sequencing (NGS) coverage in the *LDLR* of proband family members. In Figure 3, gray boxes indicate wild type bases, orange boxes indicate homozygous null mutants, and orange and green boxes indicate heterozygous mutants. In Figure 4, the mutant nucleotide c.788A>G is circled. The solid blue boxes at the bottom indicated the reference sequence.

The mutation appears to affect both protein structure and stability determined using POLYVIEW, a protein structure visualization software and MUPro, a protein stability prediction tool. POLYVIEW results showed a dramatic change in the LDLR ligand binding domain containing the Asp263Gly mutation when compared to Wild Type (WT) (Figure 5).

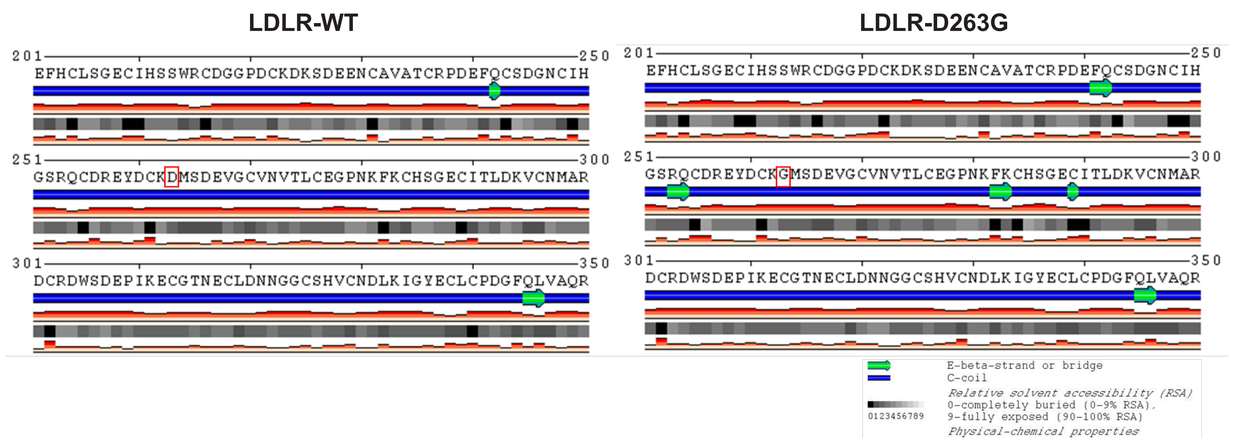


Figure 5: POLYVIEW results of the Asp263Gly mutation in LDLR

Family members identified with the HeFH mutation had clearly marked mutants, and two of the children had very low LDL-C levels (Table 2 and Figure 6).

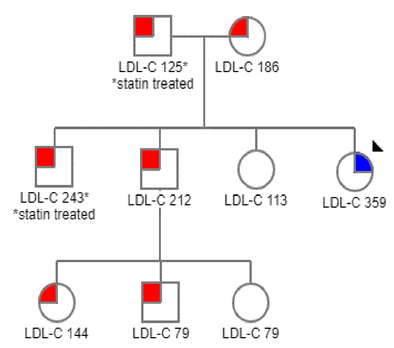


Figure 6: Pedigree for the *LDLR* c.788A>G (p.Asp263Gly) genotype

CONCLUSION

The very low frequency *LDLR* variant was only recently reported by Italian investigators as pathogenic. Interestingly, the reported variants came from a city in Sicily, a short ferry ride from Calabria[3,4].

REFERENCES

1. Sharifi, M., et al. Genetic Architecture of Familial Hypercholesterolaemia. *Curr Cardiol Rep* 2017;19(5): 44
2. Benito-Vincente, A., et al. Validation of LDLr Activity as a Tool to Improve Genetic Diagnosis of Familial Hypercholesterolemia: A Retrospective on Functional Characterization of LDLr Variants. *Int. J. Mol. Sci.* 2018; 19:1676-1693
3. Scicali, R., Di Pino, A., Platania, R., et al. Detecting familial hypercholesterolemia by serum lipid profile screening in a hospital setting: Clinical, genetic and atherosclerotic burden profile. *Nutrition, Metabolism & Cardiovascular Diseases* 2018;28:35-43
4. Pirillo, A., Garlaschelli, K., Arca, M., et al. Spectrum of mutations in Italian patients with familial hypercholesterolemia: New results from the LIPIGEN study. *Atherosclerosis Supplements* 2017;29:17-24

MEDPACE
REFERENCE LABORATORIES