PATIENT INFORMATION		SAMPLE		REFERRING PHYSICIAN	
Patient Id	John Smith	Sample number	22B54321	Name	Jane Doe, MD
Sex	Male	Source	Saliva	Institution	Local Hospital
DOB	April 22, 1973	Date received	Jan 16, 2016		
Reference	A-0987654321	Date of report	Feb 8, 2016		

Test results of: John Smith

Reason for the study: Homozygotic familial hypercholesterolemia

Test(s) requested: Dyslipidemia and premature atherosclerosis panel

RESULT: POSITIVE

We have identified a mutation in homozygosis in the LDLR gene. This mutation has been previously described in association with familial hypercholesterolemia. This result is in agreement with the informed clinical picture of the submitted patient. We suggest performing the familial screening of the mutation. Heterozygous family carriers would also be affected.

Gene	Variant	Result	Pathogenicity	Population frequency	Number of references
LDLR	**NP_000518.1:p.Pro105_Gly314delinsArg NM_000527.4:c.314-1121_941-1446del NC_000019.9:g.11214785_11219892del**	Homozygosis	**Pathogenic or disease-causing**	**Mutation (not found in controls)**	11

Clinical interpretation

The identified mutation has been extensively described and is known as FH-Valencia 4 and FH Vancouver-6. It consists of the in frame deletion of 3 exons, producing the loss of a crucial region for the protein function: the LDL-binding site. All of the described carriers presented a definite diagnosis of familial hypercholesterolemia. No other homozygous carriers have been previously described.

Technical aspects of the study

This sample has been studied by massive parallel sequencing method using a library that included genes related to dyslipidemia and premature atherosclerosis.

Signatures

James Dermody, PhD
Laboratory Director
ABMG Certified, Clinical Molecular Genetics
Admera Health LLC

Doc. Lorenzo Monserrat Iglesias
Cardiologist and Scientist Director
Health in Code

DETAILED RESULTS

Gene: LDLR (Encoding the protein: Low density lipoprotein receptor) NP_000518.1:p.Pro105_Gly314delinsArg/NC_000019.9:g.11214785_11219892del

Homozygous carrier: mutation occurs in both copies of the gene.

Mutation nomenclature: Nucleotide code:NM_000527.4:c.314-1121_941-1446del, NC_000019.9:g.11214785_11219892del. Amino acid code: NP_000518.1:p.Pro105_Gly314delinsArg. Alternative names at the DNA level: NM_000527.4:IVS3-1121_IVS6-1446del; characterized LDLR Ex3_6del. Alternative names at the protein level: NP_000518.1:p.P105_G314delinsR. Located in: Initial intron: 3, Final intron: 6.

Pathogenicity: pathogenic or disease-causing.

Population frequency: mutation (not found in controls).

Clinical information

The identified mutation has been widely described in association with heterozygous familial hypercholesterolemia (FH). In previous years, mutations in LDLR were named according to the site where they were first identified. In this case, the mutation is called FH Valencia-4 and FH Vancouver-6. Both mutations are very similar and consist of an in-frame deletion of exons 4 to 6. This means that the mutation leads to the synthesis of a shortened protein lacking the sequence encoded by these exons. Deleted exons include the binding site of lipoprotein LDL receptor, thereby altering hepatic LDL uptake for metabolism and increasing the value of this lipoprotein in plasma.

As the name suggests, this mutation was firstly identified in Spanish and Canadian families. It has also been identified in Dutch individuals. In some Spanish HF cohorts, this mutation presented a relatively high frequency, representing up to 5% of cases. It cannot be determined whether it is due to a founder effect. It is also possible that mutation arose *de novo* in different populations as a result of the mechanism that causes the mutation (see details in bioinformatics section). As far as we know, no homozygous carriers have been described.

In a paper published by Chaves et al. (2001), carriers of this variant had a poorer response to simvastatin treatment compared to carriers of other defective missense mutations.

At least 6 similar mutations have been previously published, consisting of in-frame deletions of exons encoding the LDL ligand site. All reported cases had clinical criteria for heterozygous familial hypercholesterolemia, with no carriers presenting normal or borderline lipid profile.

Bioinformatics study

A deletion of exons 4 to 6 of the LDL gene has been identified in this sample. As the rest of protein synthesis is not affected, this is an in-frame mutation (p. Pro105_Gly314delinsArg). The deletion causes a loss of the protein segment located between regions LDL-receptor Class A3 and LDL-receptor Class A7. The molecular mechanism leading to the deletion of these exons is known as "microhomology-mediated break-induced replication". It is based on homologous recombination between two Alu sequences, in this case between AluSq in intron 3 and AluSc in intron 6 (see figures 1 and 2 at the end of the report).

Gene comment

The low-density lipoprotein receptor (LDLR) gene is located in chromosome 19 and encodes the LDLR protein. LDLR is a cell surface receptor predominantly present in the liver and also found in most other tissues. It binds to particles containing apoB-100 and ApoE (mostly LDL, chylomicron remnants, and IDL), removing them from the blood via endocytosis. Lipoprotein particles are degraded in lysosomes and cholesterol is released. An increased amount of cholesterol in cells inhibits HMGCoA reductase activity and inner cholesterol synthesis and also decreases the LDLR activity. LDLR may be targeted for degradation by PCSK9 in lysosome or recycled for the cell surface. LDLR in the liver plays a major role in determining plasma LDL levels: a low number of LDLR is associated with high plasma LDL levels, while a high number of hepatic LDLRs is associated with low plasma LDL levels.

Mutations in the LDLR gene lead to familial hypercholesterolemia (FH), a disease with an autosomal dominant pattern of inheritance. Over 1,700 mutations have been identified in the LDLR gene, of which 79% are probably expressed as a hypercholesterolaemic phenotype. Mutations in the LDLR gene comprise small deletions, insertions, duplications, and missense mutations, as well as large splicing defects. Pathogenic variants can occur in the promoter or in introns or exons. The majority of pathogenic variants fall within the ligand-binding (40%) or epidermal growth factor precursor-like (47%) domains, with the highest frequency of pathogenic variants reported in exon 4 (20%) [Leigh et al., 2008; Usifo et al., 2012]. In heterozygotes for the LDLR pathogenic variant, penetrance for FH approaches 90%.

Familial hypercholesterolemia (FH) is a result of the absence or dysfunction of LDLR on the surface of hepatocytes due to a mutation in the LDLR gene, leading to a dramatic increase in LDL cholesterol and total cholesterol blood levels and to an early onset of atherosclerosis and cardiovascular complications. Clinical features of the disease are arcus corneae, xanthelasmas, xanthomas, and premature atherosclerosis, which are more severe in homozygous cases compared to heterozygotes. Individuals with heterozygous FH may have no visible signs of the disease, especially in children and in individuals being in lipid lowering treatment.

Conclusions

This mutation has been clearly associated with familial hypercholesterolemia. Its presence in homozygosis is in agreement with the clinical picture reported in the case. Heterozygous carriers of the family would also be affected, therefore we suggest completing the familial genetic and clinical screening.