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Case Report Open Access

First Detection of Hypercholesterolemia Causing ApoB-100 R3527Q Mutation in a Family in Greece

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Abstract

Familial defective apolipoprotein B (FDB) is an autosomal dominant genetic disorder causing hypercholesterolemia in affected patients. It is occurring due to mutation of apoB gene leading to a decreased low density lipoprotein (LDL) particles clearance. The R3527Q mutation is one of the disease's causative mutations. Data support mutation's origin 6000-7000 years ago in Central Europe and its prevalence is decreasing in relation to the distance from where initially occurred. Until now, the presence of R3527Q mutation had not been confirmed in Greece. This is the first report of FDB due to R3527Q mutation in a family in Northwest Greece.

Keywords: ApoB-100; Dyslipidemia; Familial defective apolipoprotein B; R3527Q

Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominant genetic disorder leading to plasma accumulation of low-density lipoprotein (LDL) cholesterol in affected patients. It is also characterized by tendon xanthomatosis and premature atherosclerosis associated with cardiovascular disease. Symptoms are mainly a result of abnormal cholesterol deposition due to ineffective LDL clearance. LDL particles are removed from circulation through binding to LDL receptors at liver cells. Apolipoprotein B-100 (ApoB-100) is the protein component of LDL particles which serves as a ligand to LDL receptors. Mutations of the LDL receptor or ApoB-100 (familial defective apolipoprotein B or FDB) may lead to defective LDL clearance and subsequent FH [1]. Recently, mutations of proprotein convertase subtilisin/kexin type 9 (PCSK9) genes have also been associated with FH [2].

Various mutations of ApoB-100 have been related with FDB: R3480W [3], R3527Q [4], R3500W [5], R3531C [6] and H3543Y [7]. The glutamine to arginine substitution at position 3527 (R3527Q, previously R3500Q) can be found among FDB patients of Caucasian origin with noteworthy geographical spread. The R3527Q mutation prevalence is higher in Central Europe (Rhein-Main area of Germany, Switzerland and Belgium) [8-11] and becomes less common as distance from Central Europe increases, suggesting a common ancestor in this area. Data regarding its prevalence in Eastern and Southeast Europe were scarce. Recently, the R3527Q mutation was found in Bulgaria [12] but it was absent from subjects screened in Turkey [13-15]. A study from Greece [16] also failed to detect R3527Q mutation.

In this report, we present the first family carrying the R3527Q mutation found in Greece, further expanding the R3527Q mutation spread in Europe, with the description of the phenotype.

Clinical Report

A 42-years-old patient, attending the Outpatient Lipid Clinic of University Hospital of Ioannina, Northwest Greece and receiving lipid lowering treatment, was diagnosed with FH, with his sons, 2 and 5 years old respectively, found with dyslipidemia as well. The patient was additionally attending the epilepsy outpatient Clinic because of epilepsy.

The patient was further tested for genetic characterization of his disease and was found to be heterozygous for the R3527Q mutation of ApoB-100 protein. Consequently, we further studied all available family members to determine mutation distribution in the family. Table 1 shows their lipid profile. All procedures were in accordance with Helsinki Declaration. Whole blood (EDTA-anticoagulated) was collected for genetic analysis from all subjects who gave their consent. Genomic DNA was extracted from whole blood. CVD StripAssay™ of ViennaLab Diagnostics GmbH was used for genotyping. PCR amplification was done by using biotinylated primers and hybridation of amplification products by a test strip containing allele specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrated. Analysis of clinical chemistry parameters of serum was carried out on an Olympus AU2700 analyzer (Olympus Diagnostica, Hamburg, Germany) by standard procedures. Total cholesterol and triglycerides were determined enzymatically and HDL-cholesterol by a direct assay (Olympus Diagnostica, Hamburg, Germany).

As seen in Figure 1, patient's mother was also detected as heterozygous for the R3527Q mutation. Mutation was also found in one patient's brother and one sister, who were screened, as well as in patient's both sons. Rest of the family members (marked with a "?" in Figure 1) were not screened as either we were not able to reach them or they denied to be screened. All known heterozygous family

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members are followed up because of dyslipidemia. Affected patients are effectively treated with statins. They were asked to inform other family members for regular lipid blood testing and/or genotyping in case of dyslipidemia.

Patient and his family live in a region in Northwest Greece, near Albania and FYROM (Figure 2), where the mutation has been detected.

Discussion

ApoB-100 protein is a very large molecule consisting of 4536 aminoacids with a total molecular mass of about 500 kDa. Gene

	Mother	Patient	Son 1	Son 2	Brother	Sister
Total Cholesterol	212 mg/dL	268 mg/dL	243 mg/dL	240 mg/dL	262 mg/dL	246 mg/dL
Triglycerides	206 mg/dL	171 mg/dL	140 mg/dL	42 mg/dL	193 mg/dL	69 mg/dL
HDL	38 mg/dL	47 mg/dL	42 mg/dL	42 mg/dL	44 mg/dL	55 mg/dL
LDL	133 mg/dL	186,8 mg/dL	173 mg/dL	190 mg/dL	179 mg/dL	177 mg/dL
Non-HDL	N/A	221 mg/dL	201 mg/dL	N/A	N/A	N/A
ApoA1	N/A	122 mg/dL	103 mg/dL	N/A	N/A	N/A
ApoB	N/A	165 mg/dL	151 mg/dL	N/A	N/A	N/A
Lp(a)	N/A	72 mg/dL	59 mg/dL	N/A	N/A	N/A

N/A: Not Available

Table 1: Lipid profile of R3527Q affected patients attending Outpatient Lipid Clinic.

CAD, Coronary artery disease

Figure 1: Family tree of Q3527R affected patient.

Figure 2: Geographical location of family affected from Q3527R mutation in Greece.

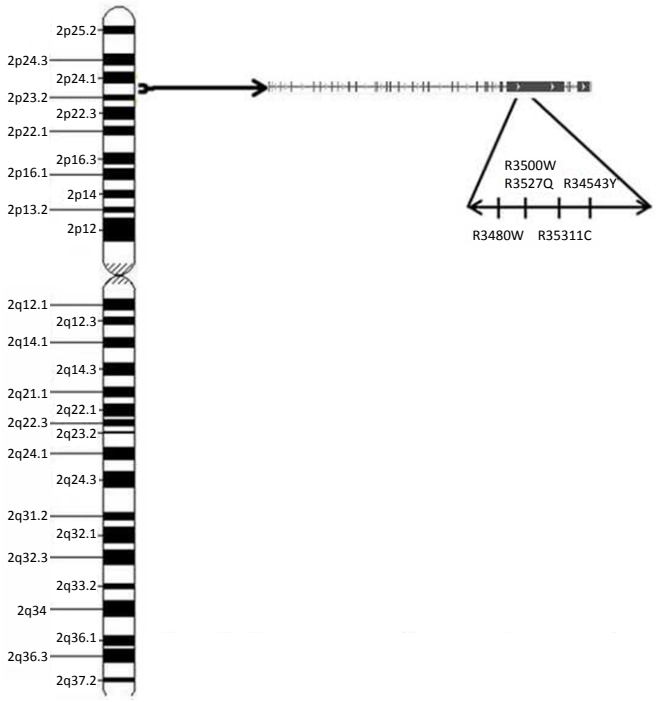

Figure 3: Chromosome position, gene structure and location of most common mutations.

encoding apoB-100 resides in chromosome 2 (2p24-p23) and consists of 28 introns and 29 exons. As shown in Figure 3, a large part of the protein is coded by exon 26, where most common mutations are to be found [17]. The R3527Q mutation leads to a glutamine-for-arginine substitution at position 3527 of ApoB-100 protein. Mutation is located at position 10708 in exon 26 of apoB gene [4].

Familial hypercholesterolemia is characterized by high plasma concentrations of LDL cholesterol. This lipoprotein consists of neutral lipids and a shell of phospholipids, cholesterol and apoB-100 protein. LDL clearance from circulation is achieved by binding to LDL receptors in liver via apoB-100. While FH has been found to be a result of defective variants or lack of LDL-receptors, FDB is a result of lower affinity of ApoB-100 for the LDL-receptor [18]. The glutamine-for-arginine substitution caused by R3527Q mutation is located in LDL receptor-binding domain of apoB-100 protein [4]. Findings support an altered secondary or tertiary structure of apoB-100 receptor-binding domain resulting to lower affinity and decreased LDL clearance [19].

The R3527Q mutation is almost exclusively found in Caucasian population. Its prevalence varies among different populations ranging from 1:71 [10] to 1:1250 [1]. The fact that almost all affected families have the mutation on the same haplotype, characterized as 194, lead Myant et al. [8] to suggest that this mutation originate even before 6000-7000 years. Other authors [20,21], using mutation's prevalence, expanded this theory and supported that initial mutation arose in Celtic populations of Central Europe and followed their expansion and migration across Europe. This is supported by mutation's decreasing prevalence with increasing distance from Central Europe. Especially in South Europe, this mutation has a very low prevalence in Italy [22] and Spain [23]. It is of interest that mutation prevalence in Spain seems to be greater in Galicia, a region in northwest Spain, known to have been inhabited by Celts during the 11th century BC [23]. Reports from Eastern Europe support the mutation's spread toward this area [24-28]. Recently, a report from Bulgaria supported mutation's existence in Southeast Europe. Authors reported an estimated prevalence of 1:451 in adult Bulgarians [12].

The mutation is almost universally absent from non-European populations around Europe. Researchers failed to detect the mutation in populations from Turkey [13-15], Lebanon [29], Morocco [30] and Iran [31]. Overseas, mutation can be found mainly in subjects of Caucasian origin. Of interest is a report regarding a very high prevalence of R3527Q mutation in the Older Order Amish of Lancaster County, Pennsylvania? As they are descendants of approximately 300 founders from Switzerland, data support a strong founder effect [32].

Regarding Greece, data are limited in only one report with molecular characterization of FH in 100 patients. Authors failed to detect R3527Q mutation [16] in the Greek population. To our knowledge, this is the first report for known R3527Q carriers in Greece; further supporting the theory of mutation's spread in European populations after its initial appearance in Central Europe. Our report fills the gap between mutation's presence in Bulgarian and absence from Turkish population and is a good example of how genetics can help understanding population movements around Europe.

Conclusion

The R3527Q mutation is a known mutation responsible for FDB. Its prevalence supports that mutation first arose in Central Europe and was further spread among European populations. Until now, data regarding its presence in Greek population were missing. To our knowledge, this is the first report confirming its presence in subjects of Greek origin. Further, large scale studies are needed to confirm its prevalence in various parts of Greece.

Conflict of Interes

Every author declares no conflict of interest.

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