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Tangier disease caused by compound heterozygosity for ABCA1 mutations R282X and Y1532C

Jamie Cameron[a], Trine Ranheim[a], Bente Halvorsen[b,c], Mari Ann Kulseth[a], Trond P. Leren[a], Knut Erik Berge[a,*]

[a] Medical Genetics Laboratory, Department of Medical Genetics, Rikshospitalet, Oslo University Hospital, NO-0027 Oslo, Norway
[b] Research Institute of Internal Medicine, Rikshospitalet, Oslo University Hospital, Norway
[c] University of Oslo, Norway

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ABSTRACT

Background: Inherited low levels of high density lipoprotein (HDL) cholesterol may be due to mutations in the genes encoding the ATP-binding cassette transporter A1 (ABCA1), apolipoprotein (apo) A-I or lecithin:cholesterol acyltransferase (LCAT).
Methods: The ABCA1, apoA-I and LCAT genes of a 40-year-old male subject with serum HDL cholesterol of 0.06 mmol/l were subjected to DNA sequencing. The proband's family was examined for co-segregation between mutations and levels of HDL cholesterol. Cholesterol efflux in fibroblasts from the proband and a normocholesterolemic subject was compared. The effects of an ABCA1 mutation on cholesterol efflux and membrane localization of ABCA1 were studied in transfected HEK293 and HeLa cells, respectively.
Results: The proband was a compound heterozygote for ABCA1 mutations R282X (c.844 C>T) and Y1532C (c.4595 A>G). Relatives who were heterozygous for one of these mutations, had about half-normal HDL cholesterol levels. Cholesterol efflux was reduced in fibroblasts from the proband, as was cholesterol efflux from HEK293 cells transfected with an human (h) ABCA1 expression plasmid harboring the Y1532C mutation. Confocal microscopy of HeLa cells transfected with the Y1532C–hABCA1 plasmid revealed that the Y1532C mutation inhibits ABCA1 from reaching the cellular membrane.
Conclusion: Compound heterozygosity for the nonsense mutation R282X and the missense mutation Y1532C in the ABCA1 gene causes Tangier disease. R282X has a detrimental effect on the function of ABCA1 since a premature stop codon is introduced. Mutation Y1532C disrupts the normal function of ABCA1 as determined by in vitro analyses.

1. Introduction

Deficiency of high density lipoprotein (HDL) cholesterol might be caused by mutations in the genes encoding apolipoprotein (apo) A-I [1], lecithin:cholesterol acyltransferase (LCAT) [2] or ATP-binding cassette transporter A1 (ABCA1) [3]. The proteins encoded by these genes are important players in the HDL-pathway. ABCA1 is involved in the initial step of reverse cholesterol transport by mediating the efflux of cellular cholesterol and phospholipids to nascent apoA-I containing HDL particles [4]. This cholesterol is then esterified by the action of LCAT [5], which is activated by apoA-I. In the liver ABCA1 initiates the HDL formation [4]. In the absence of functional ABCA1, the efflux of free cholesterol onto apoA-I is prevented and the lipid-poor apoA-I particles are rapidly metabolized, resulting in low levels of HDL cholesterol [5].

Mutations in the apoA-I gene have been found in patients with low HDL cholesterol levels [1,6] and individuals homozygous for apoA-I mutations may have reduced HDL cholesterol levels to 10–15% of what is found in controls [7]. The mechanisms by which mutations in the apoA-I gene give rise to low HDL cholesterol levels might be through effects on cholesterol efflux and/or activation of LCAT [8]. Epidemiological studies have shown that low plasma HDL cholesterol levels are associated with an increased risk for ischemic heart disease (IHD) [9]. However, low levels of HDL cholesterol caused by mutations in the apoA-I gene, do not necessarily result in increased risk for IHD. In fact, one mutation, apoA-I Milano, which causes low levels of HDL cholesterol, seems to protect against IHD [10].

Homozygozity or compound heterozygosity for mutations in the LCAT gene is found in patients with familial LCAT deficiency (OMIM# 245900) or Fish eye disease (OMIM# 131620). Both conditions are characterized by corneal opacities and low levels of HDL cholesterol, but a more severe deficiency of LCAT is found in patients with familial LCAT deficiency [2]. Other signs and symp-

* Corresponding author. Tel.: +47 23075563; fax: +47 23075561.
E-mail address: knut.erik.berge@rikshospitalet.no (K.E. Berge).

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toms, such as renal failure due to accumulation of cholesterol and phospholipids, may develop in familial LCAT deficiency. Whether mutations in the LCAT gene increase the risk of IHD is debated, and studies with conflicting results have been published. Calabresi et al. [11] could not reveal any increased number of individuals with IHD among LCAT gene mutation carriers compared to the controls, even though the carriers had lower HDL cholesterol levels. On the other hand, Hovingh et al. [12] found lower HDL cholesterol levels and increased intima media thickness among heterozygotes for LCAT mutations compared to non-carriers. Using increased intima media thickness as a surrogate marker for atherosclerosis, their study [12] indicated that LCAT gene mutation carriers had increased atherosclerosis.

Tangier disease (OMIM# 205400) is the most severe form of HDL deficiency, and it is inherited as an autosomal recessive trait [3]. Tangier disease was first described by Fredrickson et al. in 1961 [13]. Almost 40 years later, the disease was shown to be caused by mutations in the ABCA1 gene [14–16]. Tangier disease is characterized by a near absence of HDL cholesterol and very low levels of apoA-I in serum. Another common feature of the disease is an abnormal deposition of cholesteryl esters in different tissues leading to clinical signs such as hyperplastic orange tonsils, hepatosplenomegaly and neuropathy [3]. Individuals heterozygous for ABCA1 mutations have a less severe HDL cholesterol reduction compared to Tangier disease patients, and do not develop accumulation of cholesteryl esters [3]. Uncertainties exist whether mutations in ABCA1 that cause low levels of HDL cholesterol, increase the risk for IHD. Clee et al. [17] reported that obligate carriers of ABCA1 mutations had lower HDL cholesterol levels and increased risk for IHD [17]. On the other hand, Frikke-Schmidt et al. [18] did not reveal any increased risk for IHD among heterozygotes for ABCA1 mutations, even though these mutation were associated with an reduction in HDL cholesterol. One explanation for the conflicting results between these two studies might be that in the latter study [18] only modest reduction in HDL cholesterol levels were observed among mutation carriers.

In this study we have set out to identify the underlying cause of a HDL cholesterol level <0.1 mmol/l in a 40-year-old male subject. The ABCA1, apoA-I and LCAT genes were subjected to DNA sequencing, and the proband was found to be a compound heterozygote for two mutations in the ABCA1 gene.

2. Materials and methods

2.1. Clinical characteristics of the proband

The proband was a 40-year-old male subject who participated in a screening program for cardiovascular risk factors among 40-year-old subjects in Oslo. The following non-fasting lipid profile was obtained at this screening and at the age of 65 years, respectively: total serum cholesterol 1.73 and 3.1 mmol/l, HDL cholesterol 0.06 and 0.2 mmol/l and triglycerides 1.27 and 3.3 mmol/l. A medical history obtained at age 49, revealed that he had developed bilateral sensory hearing deficit at the age of 18–19 and since then had to use hearing aids in both ears. He had also been diagnosed with transient palsy of nervus radialis at the age of 26 and right-sided palsy of nervus peroneus at age 32. A neurological examination at the age of 36 revealed slight bilateral nystagmus. He underwent tonsillectomy at the age of 30 because of chronic tonsillitis. A full clinical chemistry profile at the age of 50 revealed thrombocytopenia (95×10^9/l, reference value 150×10^9/l to 450×10^9/l). Hemoglobin concentration was normal (15.0 g/100 ml, reference value 12.5–16.5 g/100 ml), but the mean corpuscular volume of erythrocytes was increased (109 fL, reference value 82–96 fL) as was the mean corpuscular hemoglobin of erythrocytes (37 pg, reference value 27–32 pg). The level of ferritin was increased (470 μg/l, reference value 25–200 μg/l), and there was a somewhat low level of iron (15.5 μmol/l, reference value 15.0–30.0 μmol/l) and of total iron-binding capacity (52 μmol/l, reference value 55–90 μmol/l). Otherwise, the clinical chemistry profile was normal. By the age of 65 he had no symptoms of cardiovascular disease.

Methods are described in online supplementary material.

3. Results and discussion

3.1. Identification of the cause of low HDL cholesterol levels

To identify the underlying mutation causing the low levels of HDL cholesterol in the proband, DNA sequencing of individual exons with flanking intron sequences of the genes apoA-I, LCAT and ABCA1 was performed.

No mutations in the genes encoding LCAT or apoA-I gene were identified as possible causes of low levels of HDL cholesterol. However, DNA sequencing of the ABCA1 gene suggested that the proband was a compound heterozygote for the nonsense mutation R282X (c.844 C>T) in exon 9 and the missense mutation Y1532C (c.4595 A>G) in exon 34. R282X has previously been reported by Altilia et al. [19] as a cause of defective function of ABCA1, while Y1532C is a novel mutation. As depicted in Fig. 1, all first degree relatives of the proband have about half-normal HDL cholesterol levels. With respect to the genotypes, the proband's mother and brother were both heterozygous for mutation R282X, while his father and daughter were heterozygous for mutation Y1532C. The genotypes of the proband's parents confirm that he was a compound heterozygote. Additionally, the low levels of HDL cholesterol were compatible with a co-dominant inheritance of mutations in the ABCA1 gene.

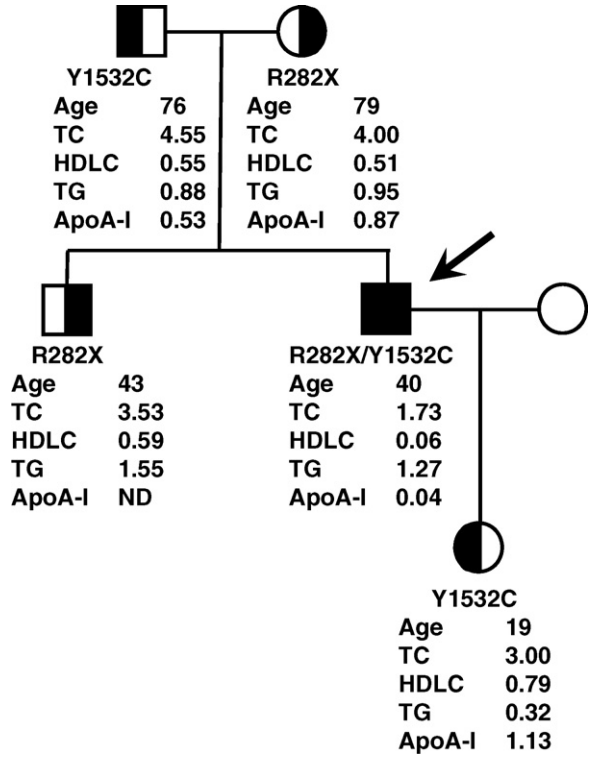

Fig. 1. Pedigree of the proband's family. The mutations found in the ABCA1 gene, the age of the individuals and the measured values for serum total cholesterol (TC), HDL cholesterol (HDLC), triglycerides (TG) (all in mmol/l), and apoA-I (g/l) are shown. The proband is indicated by an arrow. ND: not determined.