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Variable phenotypic expression of nonsense mutation p.Thr5* in the APOE gene

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ABSTRACT

Subjects with hypercholesterolemia who do not carry a mutation in the low density lipoprotein receptor gene, in the apolipoprotein B gene or in the proprotein convertase subtilisin/kexin type 9 gene, could possible carry a mutation in the apolipoprotein E (APOE) gene. DNA from 844 unrelated hypercholesterolemic subjects who did not carry a mutation in any of the three above mentioned genes, was subjected to DNA sequencing of the APOE gene. Two subjects were found to be heterozygous for mutation p.Thr5*. This mutation which generates a stop codon in the signal peptide, is assumed to prevent the synthesis of APOE. Family studies revealed that the mutation was carried on an APOE4 allele in both families. In one of the families only those who had an APOE2 allele as the second allele, had hypercholesterolemia. These were functionally hemizygous for APOE2 and presented with a Type III hyperlipoproteinemia phenotype. However, in the second family, hypercholesterolemia was observed in the index patient who had APOE3 as the second allele, but not in four heterozygous family members who also had APOE3 as the second allele. These findings underscore that the phenotypic expression of mutations in the APOE gene is variable and that the trait exhibits reduced penetrance.

1. Introduction

The low density lipoprotein receptor (LDLR) plays a key role in cholesterol metabolism by clearing atherogenic low density lipoprotein (LDL) from plasma by receptor-mediated endocytosis [1]. Disrupted LDLR-mediated endocytosis of LDL causes autosomal dominant hypercholesterolemia [1]. The underlying genetic defect may be a mutation in the LDLR gene, in the apolipoprotein B (APOB) gene or in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene [2]. Due to the increased level of plasma LDL cholesterol, subjects with autosomal dominant hypercholesterolemia have a markedly increased risk of coronary heart disease [1]. However, efficient lipid-lowering therapy is available which may normalize the levels of plasma LDL cholesterol and reduce the risk of coronary heart disease [1,3]. Thus, it is important to diagnose patients with autosomal dominant hypercholesterolemia and this can be done by molecular genetic testing.

However, a portion of subjects with hypercholesterolemia who are referred for molecular genetic testing, does not carry a mutation in the LDLR, APOB or PCSK9 genes [4]. These subjects may therefore actually not have a monogenic hypercholesterolemia or they may carry a mutation in another gene. One such gene could be the apolipoprotein E (APOE) gene where mutations p.Leu167del (c.500_502delTCC) [5–7] and p.Arg163Cys (c.487C > T) [8] recently have been found to cause autosomal dominant hypercholesterolemia.

APOE is a constituent of chylomicrons, very low density lipoprotein, intermediate density lipoprotein and high density lipoprotein and plays a role in clearing triglyceride-rich lipoproteins from plasma [9]. It is primarily synthesized by the liver. The APOE gene contains four exons and is located on the short arm of chromosome 19. It encodes a 317 residue protein which is secreted as a mature 299 residue protein after the 18 residue signal peptide has been cleaved off during translocation to the endoplasmic reticulum [10,11]. There are three major isoforms of APOE which differ at residues 112 and 158. APOE3 (Cys_{112}, Arg_{158}) is the most common isoform with a frequency of approximately 0.7, while the frequencies of APOE4 (Arg_{112}, Arg_{158}) and APOE2 (Cys_{112}, Cys_{158}) are approximately 0.2 and 0.1, respectively [9]. Thus, approximately 1% of subjects are homozygous for APOE2 and of these, roughly 1% develop Type III hyperlipoproteinemia which is characterized by hypercholesterolemia and hypertriglyceridemia [9]. The underlying mechanism for the role of APOE2 in Type III hyperlipoproteinemia is that APOE2 in contrast to APOE3 and APOE4, exhibits defective binding to the LDLR [12].

To further study the role of mutations in the APOE gene as a cause of autosomal dominant hypercholesterolemia, we have screened 844 unrelated hypercholesterolemic subjects for mutations in the APOE gene.

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2. Material and methods

2.1. Subjects

The subjects included in this study had been referred for genetic testing with respect to autosomal dominant hypercholesterolemia as part of ordinary health care. However, no mutations had been identified in the LDLR gene or in the PCSK9 gene, and no-one carried mutation p.Arg3527Glu (c.10579C > T, ref. seq.: NM_000384.2) in the APOB gene. Subjects who also presented with hypertriglyceridemia and who had been found to be homozygous for APOE2, were excluded from the study. A total of 844 unrelated subjects were included of which 540 were females and 304 were males. The mean age was 49.6 (±12.1) years. Their mean values for total serum cholesterol, high density lipoprotein cholesterol, triglycerides before lipid-lowering therapy was started, were 10.2 (±12.6) mmol/l, 1.6 (±0.7) mmol/l and 1.8 (±1.0) mmol/l, respectively. Their mean value for LDL cholesterol calculated according to the formula of Friedewald et al. [13], was 7.4 (±1.1) mmol/l. However, because some uncertainty may exist regarding the fasting state at the time of measurement of lipid levels, the values for triglycerides and LDL cholesterol must be interpreted with caution.

2.2. Molecular genetic screening of the APOE gene and measurement of serum APOE levels

Screening for mutations in the APOE gene was performed by using the Sanger dideoxy sequencing method of the translated exons (exons 2–4) with flanking intron sequences. A 3730xl DNA analyzer (Applied Biosystems, Foster City, CA) was used for DNA sequencing. Regarding codon and nucleotide numbering of the APOE gene, the ATG initiation codon was codon #1 and adenosine of the initiation codon was nucleotide #1. The reference sequence used for nucleotide numbering of the APOE gene was NM_000041.3. The conditions and primer sequences for polymerase chain reaction and DNA sequencing are available upon request. Serum levels of APOE were determined by ELISA using Human Apolipoprotein E ELISA Kit (Abcam, Cambridge, UK).

3. Results and discussion

3.1. Identification of mutation p.Thr5*

DNA sequencing of the APOE gene in 844 unrelated hypercholesterolemia subjects identified several normal genetic variants of which the majority has been previously reported (data not shown). None of the subjects were heterozygous for mutations p.Leu167del or p.Arg163Cys and none of the subjects were homozygous for APOE2. However, two subjects were heterozygous for mutation p.Thr5* (c.15G > A) in exon 2. To our knowledge this nonsense mutation has not previously been reported. Codon 5 is within the signal peptide and mutation p.Thr5* is expected to prevent the synthesis of APOE. Both subjects heterozygous for mutation p.Thr5* presented with hypercholesterolemia (total serum cholesterol levels of 16.0 mmol/l and 11.7 mmol/l, respectively) and hypertriglyceridemia (triglyceride levels of 6.8 mmol/l and 10.0 mmol/l, respectively) (Figs. 1 and 2). The index patient (subject II.3) in Family T0657 (Fig. 1) had xanthomas and xanthelasms, whereas the index patient (subject III.3) in Family T5044 (Fig. 2) neither had xanthomas nor xanthelasms. To study whether mutation p.Thr5* segregated with hypercholesterolemia, available family members were tested for this mutation and had their lipid levels measured (Figs. 1 and 2).

3.2. Family T0657

A total of 12 family members were studied in Family T0657 (Fig. 1). Of these, four subjects were heterozygous for mutation p.Thr5* of which only subject II.1 had hypercholesterolemia. Her values for total serum cholesterol and triglycerides at an age of 58 were 18.4 mmol/l and 5.9 mmol/l, respectively. Thus, she had a lipid profile similar to that of the index patient. However, she did not have xanthomas or xanthelasms. The levels of total serum cholesterol and triglycerides in her two sisters and one niece who were also heterozygous for mutation p.Thr5*, ranged from 6.5 mmol/l to 7.7 mmol/l and from 1.6 mmol/l to 3.2 mmol/l, respectively (Fig. 1). Thus, mutation p.Thr5* did apparently not segregate with hypercholesterolemia in this family. Mutation p.Thr5* heterozygotes who were APOE4 homozygotes had slightly

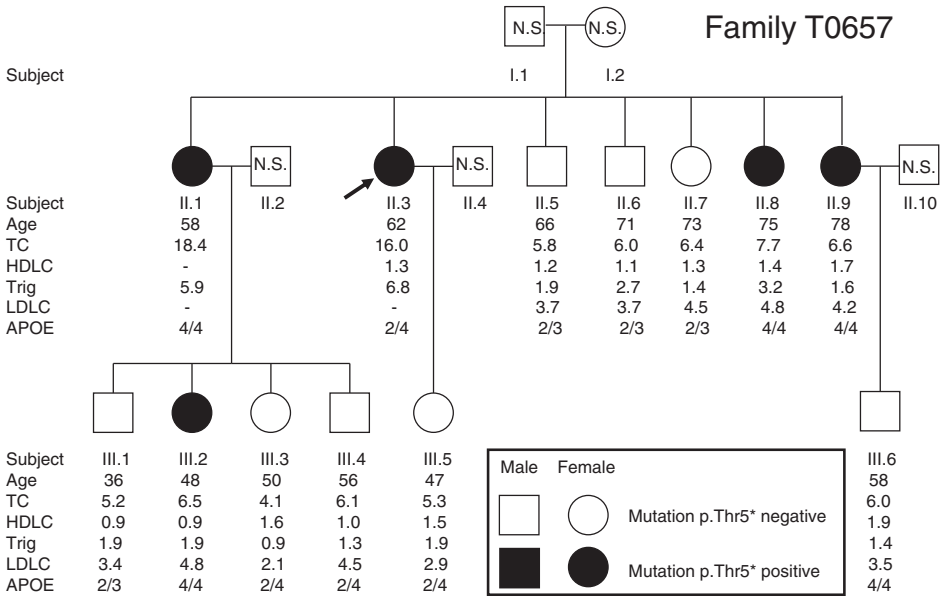

Fig. 1. Pedigree and clinical characteristics of subjects in Family T0657 Age and values for total serum cholesterol (TC), high density lipoprotein cholesterol (HDLC), triglycerides (Trig) and LDL cholesterol (LDLC) (all mmol/l) as well as APOE genotype are indicated in the family members. Levels of LDL cholesterol in the family members were measured directly and not calculated by the use of the formula of Friedewald et al. [13]. The index patient is indicated by an arrow. N.S.: not sampled. -: Missing value.

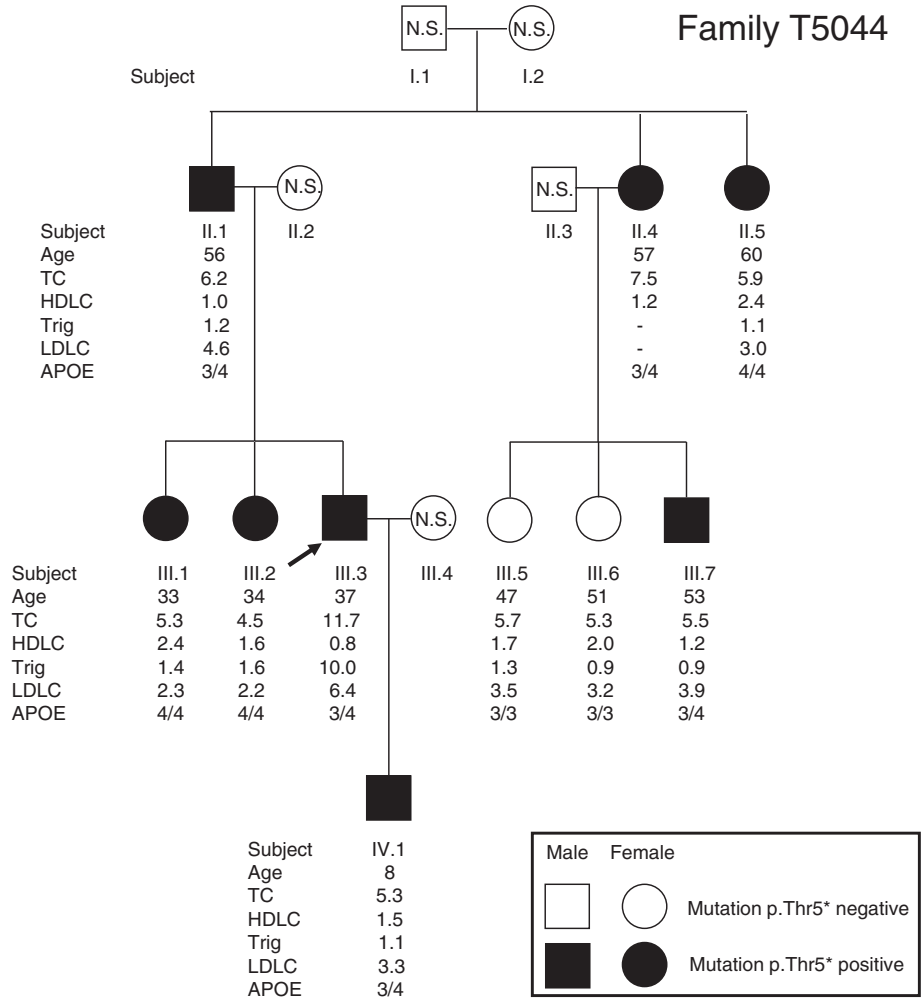

Fig. 2. Pedigree and clinical characteristics of subjects in Family T5044. Age and values for total serum cholesterol (TC), high density lipoprotein cholesterol (HDLC), triglycerides (Trig) and LDL cholesterol (LDLC) (all mmol/l) as well as APOE genotype are indicated in the family members. Levels of LDL cholesterol in the family members were measured directly and not calculated by the use of the formula of Friedewald et al. [13]. The index patient is indicated by an arrow. N.S.: not sampled.

higher total serum cholesterol levels than those who did not carry mutation p.Thr5*. This could suggest that heterozygosity for mutation p.Thr5* per se may have a mild effect on plasma cholesterol levels.

To further study the mechanism for hypercholesterolemia in the two mutation p.Thr5* heterozygotes, APOE genotyping was performed based upon the DNA sequencing data. This genotyping revealed that the two mutation p.Thr5* heterozygotes who had hypercholesterolemia (subjects II.1 and II.3), were APOE2/4 heterozygotes, whereas the three mutation p.Thr5* heterozygotes who did not have hypercholesterolemia (subjects II.8, II.9 and III.2), were APOE4 homozygotes (Fig. 1). From segregation analysis in this family it is evident that mutation p.Thr5* resided on an APOE4 allele. Thus, the two hypercholesterolemic subjects carrying mutation p.Thr5* and who were APOE2/4 heterozygotes, have no functional APOE with respect to binding to the LDLR. Being functionally hemizygous for APOE2, they are expected to present with a phenotype of Type III hyperlipoproteinemia, similar to those who are APOE2 homozygotes. This is also what was observed.

A similar finding to ours is that of Feussner et al. [14]. They identified mutation p.Thr38* (c.114G > A) which resided on an APOE3 allele. This mutation caused Type III hyperlipoproteinemia in subjects who had APOE2 as the second allele, but not in subjects who had APOE3 as the second allele. Moreover, Dijck-Brouwer et al. [15] have reported a frame-shift mutation (c.339dupG) leading to a premature stop codon (p.Glu114Glyfs*50) which resided on an APOE3 allele. This mutation caused Type III hyperlipoproteinemia only in subjects who had APOE2 as the second allele. Furthermore, also missense mutation p.Arg154Ser (c.460C > A) has been reported to cause hyperlipidemia if the second allele was an APOE2 allele [16].

3.3. Family T0544

A total of nine family members were studied in Family T0544. Of these, seven were heterozygous for mutation p.Thr5* (Fig. 2). However, none of these had hypercholesterolemia or hypertriglyceridemia. Segregation analysis revealed that mutation p.Thr5* resided on an APOE4 allele also in this family. Because families T0657 and T5044 are from a restricted area in the most Southern part of Norway, it is assumed that there is a common gene source for mutation p.Thr5*.

In contrast to the index patient in Family T0657 who was an APOE2/4 heterozygote, the index patient in Family T5044 was an APOE3/4 heterozygote. The latter patient therefore has one functioning APOE allele with respect to LDLR binding. It was therefore surprising that this subject presented with hyperlipidemia. In this situation one could speculate that his APOE3 allele somehow could be defective due to inactivating mutations. However, no such mutations had been detected by DNA sequencing of the translated exons with flanking intron sequences of the APOE gene. Moreover, the finding that heterozygosity was observed in exons 2 and 4, indicate that these exons have not been deleted.

From the literature there are other examples of subjects with one mutant APOE allele and one normal APOE3 allele who have autosomal

dominant hyperlipidemia [17]. However, in Family T5044 there were four other mutation p.Thr5* carriers who were also APOE3/4 heterozygotes, and who did not have hypercholesterolemia. Similar findings of reduced penetrance of mutations in the APOE gene have previously been reported [18,19]. Thus, other factors affecting lipid metabolism must have contributed to the hypercholesterolemia in the index patient in this family.

3.4. Measurement of APOE

Based upon the observation that subjects lacking APOE have severe hypercholesterolemia and hypertriglyceridemia [20], it is assumed that a certain serum concentration of APOE is required for normal APOE-dependent lipid metabolism. Moreover, because a wide variation is observed in the plasma levels of APOE [21], one could speculate that the mechanism for the index patient in Family T5044 being hyperlipidemic, was that he had very low levels of APOE. Therefore, the serum levels of APOE were measured in the index patient in Family T5044 and in eight subjects from the two families who were also heterozygous for mutation p.Thr5*. Nine subjects who had Type III hyperlipoproteinemia due to homozygosity for APOE2 and 21 healthy individuals were included as controls. The mean serum level of APOE in the mutation p.Thr5* carriers was 42 (±17) μg/ml. The corresponding serum levels in the Type III hyperlipoproteinemia patients and in the healthy controls were 164 (±57) μg/ml and 63 (±20) μg/ml, respectively. Thus, the mean APOE level in mutation p.Thr5* carriers was 67% of that of the normal controls. The serum level of APOE in the index patient in Family T5044 was 47 μg/ml. This value was similar to that of subjects heterozygous for mutation p.Thr5* who did not present with hyperlipidemia.

Thus, there is no obvious explanation for the hyperlipidemia observed in the index patient in Family T5044. One could speculate that having approximately half normal levels of APOE due to the p.Thr5* mutation, could be insufficient to clear triglyceride-rich lipoproteins from plasma in situations where the metabolism of these lipoproteins is stressed due to factors such as high intake of fat or alcohol. However, we do not have data regarding life-style or dietary habits of this subject. Neither do we have follow-up data regarding the lipid profile. In this situation it is assumed that polygenic factors which may be difficult to identify, cause hyperlipidemia in the index patient in Family T5044. These factors could be similar to those that contribute to hyperlipidemia in the 1% of APOE2 homozygotes who develop Type III hyperlipoproteinemia.

The findings in these two families underscore that our understanding of the factors that precipitate hyperlipidemia in individuals carrying a mutation in the APOE gene, is limited.

Our data also indicate that mutations in the APOE gene are not a common cause of hypercholesterolemia in the Norwegian population. Thus, there is no reason to include DNA sequencing of the APOE gene in routine genetic testing for autosomal dominant hypercholesterolemia.

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