Familial Dysbetalipoproteinemia: A Genetically Heterogenous Disease Caused by Mutations of the Ligand Apolipoprotein E.

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Apolipoprotein E is present on the surface of very-low-density lipoprotein (VLDL) and chylomicron-remnants and is essential for the receptor mediated endocytosis of these particles via hepatic receptors. Several types of mutations of the apoE can cause a deficiency in the clearance of these remnant particles. An accumulation of lipoprotein-remnant particles may occur and familial dysbetalipoproteinemia (FD) develops. Genotyping of the various apoE variants and relation of these mutations with their effect on the lipoprotein-remnant removal have provided more insight in the structure-relationship of apoE ligand-receptor interactions. It is postulated that the apoE2 (Arg158 → Cys) mutation is just outside the binding domain and that its deficient binding can be stimulated by exogenous factors. This hypothesis can explain why apoE2/E2 homozygosity can only induce FD under certain circumstances.

ApoE mutations that occur in the binding domain, e.g., apoE2 (Lys146 → Gln) have a direct effect on the ligand-receptor binding and, in these individuals, FD is inherited in an autosomal dominant way.

Finally, apoE3-Leiden has an arginine residue at 112 and has a repeat of seven extra amino acid residues just outside the binding domain. Because of this repeat, conformational changes of the binding domain can ensue. Due to the fact that in apoE3-Leiden the arginine residue is present at 112, apoE3 Leiden is predominantly present on chylomicron and VLDL remnants. In these persons FD is also inherited in an autosomal dominant way. J Invest Dermatol 98:578–60S, 1992

Familial dysbetalipoproteinemia (FD) is a metabolic disorder characterized by elevated serum cholesterol and serum triglyceride levels. The clinical picture of FD is characterized by premature coronary and/or peripheral vascular disease. In 50% of patients with FD typical xanthomatosus lesions can be observed. The xanthomatosus lesions in the palms of the hands (xanthochromia striata palmatis) are nearly pathognomonic (Figs 1a, b). Tuberous xanthomas, mainly present on the elbows, can also be found in other lipoprotein disorders [1,2].

The metabolic disorder that is responsible for the development of FD is characterized by the accumulation of lipoprotein-remnant particles. These remnants are called chylomicron remnants when derived from the lipoproteins synthesized in the gut and VLDL remnants when derived from the liver. The remnant particles are relatively enriched in cholesterol and apolipoprotein E (apoE). The apoE present on the surface of the remnant particles binds as a high-affinity ligand to specific hepatic lipoprotein receptors [3]. The primary metabolic defect in FD is due to mutant forms of the ligand apoE that leads to impaired clearance of these lipoprotein-remnant particles [4]. Apolipoprotein E has a molecular weight of 34 kD and contains 299 amino acids. In the general population, three common genetic variants of apoE can be discriminated: E2, E3, and E4. These apoE isoforms differ in isoelectric point by one charge unit; apoE4 is the most basic and apoE2 the most acidic form. The isoforms are encoded for by three codominant alleles; E2, E3, and E4 at a single apoE gene locus. The gene consists of four exons separated by three introns at a locus on the long arm of chromosome 19 [5]. The common apoE2 is derived from the wild type apoE3 by a cysteine for arginine substitution at amino acid residue 158 [E2 (Arg158 → Cys)], whereas apoE4 is derived from apoE3 by an arginine for cysteine substitution at residue 112 (Cys112 → Arg).

It has been shown that homozygosity for the E2 (Arg158 → Cys) variant is most common in FD patients. FD is inherited in an autosomal recessive way in these patients [1]. Contrary to these findings, there are also FD patients described who are heterozygous for rare apoE variants. In these families FD is inherited in an autosomal dominant way with a high degree of penetrance. The above-mentioned inheritance pattern will be further discussed.

At first some techniques that enable us to discriminate between the various apoE mutations will be described.

APOLIPOPROTEIN E QUANTIFICATION, PHENOTYPING, AND GENOTYPING

Apolipoprotein E concentrations in plasma were measured by enzyme-linked immunos sorbent assay (ELISA) as described by Bury et al [6].
The apoE phenotype was determined by isoelectric focusing of delipidated plasma samples before and after cysteamine treatment followed by immunoblotting as described in [7].

For genotyping, genomic DNA was isolated from leukocytes by standard methods [8]. The 5'-part of exon 4 of the apoE gene was amplified by the polymerase chain reaction (PCR) using the primers 402 (nucleotides 3,555–3,574, coding strand) and 401 (nucleotides 3,932–3,913, non-coding strand) giving a fragment of 378 bp (or 399 bp in the case of the apoE*3-Leiden allele [9]). This fragment encodes the amino acid residues 61–174 of the mature protein [10].

After amplification, the DNA fragment is cut with the restriction enzyme Hha I. With this enzyme the alleles for E*3, E*4 and E*2 and E*3-Leiden (vide infra) can be specifically recognized, as each allele will show a specific binding pattern after cleavage with this enzyme (Table I).

For the presence of the E*2 (Lys 146 -> Gln) allele, the amplified 5’ part of exon 4 is spotted on nitrocellulose and hybridized with synthetic oligonucleotides specifically recognizing either the E*2 (Lys 146 -> Gln) allele or the wild type E* as 3 allele [17].

**Table I.** Characteristics of ApoE

<table>
<thead>
<tr>
<th>ApoE Variant</th>
<th>Relative Electric Charge</th>
<th>Mutation</th>
<th>Genotyping Using Hha I Diagnostic Fragments in Basepairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo E3</td>
<td>0</td>
<td>Wild type</td>
<td>91/48</td>
</tr>
<tr>
<td>Apo E4</td>
<td>+1</td>
<td>Cys 112 -&gt; Arg</td>
<td>72/48</td>
</tr>
<tr>
<td>Apo E2</td>
<td>-1</td>
<td>Arg 158 -&gt; Cys</td>
<td>91/83</td>
</tr>
<tr>
<td>Apo E3</td>
<td>0</td>
<td>Duplication, 7 amino acids and Cys 112 -&gt; Arg</td>
<td>93/48</td>
</tr>
<tr>
<td>Leiden</td>
<td>-1</td>
<td>Lys 146 -&gt; Gln</td>
<td>Discrimination only via allele specific oligonucleotide hybridization</td>
</tr>
</tbody>
</table>

**DISTRIBUTION OF apoE ISOFORMS**

The distribution of apoE isoforms in 2018 randomly selected 35-year old men in the Netherlands are shown in Table II. The respective gene frequencies are (E*4, 0.167; E*3, 0.750; E*2, 0.082) [12]. These findings are similar with the gene frequencies observed in random population samples in Scotland and Germany [13,14]. From these population-based data it can be calculated that about 1% of the general population is homozygous for apoE2. Furthermore when the apoE isoforms were investigated in 72 FD patients it was found that 60 patients were homozygous E2/E2, six patients had E2/E3, and six patients had E3/E3 phenotypes. It was suspected that in these latter two groups of patients at least one “abnormal” apoE variant could be present. Protein analysis combined with DNA technique enabled us to characterize these apoE variants (vide infra).

**ApoE2/E2 Homozygosity** In vitro experiments showed that this variant exhibits only 1% of the receptor-binding capacity of normal apoE3 [15]. Despite this phenomenon only 4% of the persons homozygous for apoE2 will eventually develop FD. Therefore, other genetic and/or environmental factors will determine whether these homozygous persons develop FD [4].

Clinical observations have provided evidence that age, hormones (male sex, menopausal women), body mass index, and nutritional status belong to these environmental factors [1,4].

Also when apoE2/E2 homozygous persons have inherited another lipoprotein disorder (e.g. familial hypercholesterolemia, combined hyperlipidemia) they may be prone to develop FD [1,16].

In conclusion, aggravating factors may cause FD in persons homozygous apoE2/E2 via two mechanisms: 1) Additional effect on the removal of lipoprotein-remnant particles; and 2) Increased synthesis of lipoprotein-remnant particles (Fig 2).

**Rare apoE Variants in FD** As already mentioned, the presence of rare apoE variants has also been detected in FD. The great majority of these apoE variants cause FD in an autosomal dominant way with high penetrance. The clinical picture of these patients is different from FD patients homozygous for apoE2 [17].

Molecular biologic techniques like sequencing and hybridization with mutation-specific oligonucleotide probes in combination with amplified DNA have unraveled the type of mutations present in

**Table II.** ApoE Phenotypes and Gene Frequencies in 2018 Randomly Selected 35-Year-Old Men in The Netherlands [12]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Phenotypes</th>
<th>Relative Frequency (percent)</th>
<th>Phenotypes</th>
<th>Relative Frequency (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo E4</td>
<td>0.16</td>
<td>E4/E4</td>
<td>2.9</td>
<td>E3/E4</td>
<td>25.4</td>
</tr>
<tr>
<td>Apo E3</td>
<td>0.75</td>
<td>E3/E3</td>
<td>55.9</td>
<td>E3/E2</td>
<td>12.9</td>
</tr>
<tr>
<td>Apo E2</td>
<td>0.08</td>
<td>E2/E2</td>
<td>0.7</td>
<td>E2/E4</td>
<td>2.2</td>
</tr>
</tbody>
</table>
genotype E2 (158 Arg→Cys)

E2 / E2

production more remnant particles
Aggravating factors
FD
additional effect on removal remnant particles

Figure 2. Schematic drawing of factors that may lead to FD in homozygous E2/E2. Note only 1–4% of these persons acquire FD.

some of these apoE variants. In our patients with rare apoE variants the mutation of apoE2 takes place at the amino acid residue 146, whereby the amino acid lysine was replaced by glutamine E2 (Lys146→Gln) [11]. Within the literature other rare apoE mutations have been found like E3 (Cys112→Arg; Arg142→Cys); El Harrisburg (Lys146→Glu); E2 (Arg145→Cys); E2 Churchstean (Arg136→Ser) [18–20].

In addition, we found a rare apoE3 variant, designated as E3-Leiden, that was also responsible for FD in an autosomal dominant way [21]. The exact nature of this defect was not due to a simple nucleotide substitution.

apoE3-Leiden: The identification of the DNA variant apoE3-Leiden in family members was performed by amplification of genomic DNA followed by hybridization with a specific synthetic oligonucleotide probe against the duplication of the junction region or by digestion with an allele-specific restriction endonuclease followed by polycrylamide gel electrophoresis.

The molecular defect was defined by PCR and sequencing. The 5′-part of exon 4 proved to be slightly longer and definitely elucidated by sequencing. The nature of the mutation was found to be localized in the apoE4 allele and consisted of partial gene duplication of 21 nucleotides forming a tandem repeat of codons 120–126 or 121–127 [10,22].

Genetic studies of five probands showed that the E3-Leiden probands share common ancestry in the seventeenth century [23]. The large number of carriers versus non-carriers for apoE3-Leiden enabled us to study the effect of apoE3-Leiden on the lipoprotein levels in more detail. It could be shown that age and body mass index of the E3-Leiden carriers display a strong effect on the expression of FD in these E3-Leiden families [23].

DISCUSSION

The various studies of apoE mutations and their effect on the removal of lipoprotein-remnant particles have provided data to devise a structure-function relationship of the apoE-receptor binding. Mahley and coworkers [24–26] suggest that the conformation of the 130–150 α-helical region of apoE is the putative receptor-binding domain.

It could be demonstrated that this receptor-binding activity can easily be modulated by small genetic changes. These authors suggest that the conformation of apoE2 (Arg158→Cys) is sensitive to its (lipid) environment and that the cysteine at 158 has a secondary, rather than a direct effect, on the ligand-receptor binding. This property could explain the requirement for additional factors for the expression of FD in persons who are homozygous apoE2/E2 [27].

The apoE2 [e.g., ApoE*2 (Lys146→Glu)] allele is almost absolutely associated with FD and has a molecular defect in the α-helical receptor binding domain. Mahley [27] suggest that the basic amino acid residues are essential for the binding of the ligand [28] to the receptor.

The apoE3-Leiden mutation is located just outside the putative receptor binding domain. The additional seven amino acids constitute nearly two turns of the α-helix and would affect the confirmation of the receptor-binding domain irreversibly. As a consequence, an interference takes place with the binding activity. Nevertheless the binding activity per se might be sensitive to factors such as age and body mass index. Moreover, on apoE3-Leiden an arginine residue is present on position 112 instead of cysteine. It has been shown by Weisgraber [29] that the arginine at 112 plays a crucial role in the distribution of apoE over the different lipoprotein fractions. For this reason the ApoE3-Leiden variant may, similar to apoE4, preferentially be localized on VLDL particles thus rendering the VLDL particle apparent homozygous for the apoE3-Leiden variant. This predomination of apoE3 Leiden on VLDL could explain its behavior as a dominant trait in the expression of FD.

We conclude that different apoE variants can impair the chylomicron and VLDL remnant clearance via different mechanisms [fig 3; 1] redistribution of the different apoE variants over the different lipoprotein fractions; 2) affecting the ligand receptor interaction by conformational changes; 3) affecting the binding domain in a direct way; and 4) affecting the binding in a reversible way.

REFERENCES


