FH-Freiburg: a novel missense mutation (C317Y) in growth factor repeat A of the low density lipoprotein receptor gene in a German patient with homozygous familial hypercholesterolemia

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Received 3 June 1999; received in revised form 13 September 1999; accepted 29 September 1999

Abstract

We describe the characterization of a novel mutation in the low density lipoprotein receptor (LDL-R) gene in a patient with true homozygous familial hypercholesterolemia (FH). The combined use of denaturing gradient gel electrophoresis (DGGE) and sequencing of genomic DNA revealed a guanine to adenine base substitution at nucleotide position 1013 of the LDL-R cDNA. This point mutation results in a change from cysteine to tyrosine at amino acid residue 317 of repeat A of the epidermal growth factor (EGF) precursor homology domain. Binding, uptake and degradation of iodinated LDL in skin fibroblasts from the homozygous patient were less than 10% of normal. In contrast, binding, uptake and degradation of iodinated VLDL was reduced by only 60, 30, and 38%, respectively. Incubation of the patient’s fibroblasts in the presence of cholesterol diminished the residual binding of VLDL by 50%, suggesting that the loss of the highly conserved cysteine at position 317 results in a LDL-R that fails to bind LDL, but retains some ability to bind VLDL by interacting with the apolipoprotein E. Both parents were heterozygous for the C317Y mutation. Interestingly, however, the father presented with markedly elevated levels of triglycerides and VLDL cholesterol, whereas his LDL cholesterol was unexpectedly low. The mother of the index patient had only slightly elevated LDL cholesterol. These observations testify to the biological complexity of genotype-environment interactions in individuals carrying mutations at the LDL-R locus and indicate that genetic analysis importantly complements the clinical and biochemical diagnosis of patients with hyperlipidemia. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Familial hypercholesterolemia; Low density lipoprotein receptor; Genetic disease; Polymerase chain reaction; Denaturing gradient gel electrophoresis; Low density lipoprotein binding

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant inherited disease caused by mutations in the gene coding for the low density lipoprotein receptor (LDL-R). The LDL-R mediates the specific uptake and catabolism of LDL into the liver and many other tissues of the body [1]. Heterozygous carriers of one defective LDL-R gene express only half the number of functional LDL receptors and have a markedly raised plasma cholesterol that is frequently associated with the occurrence of tendon xanthomata, accelerated atherosclerosis, and premature coronary artery disease. Homozygous individuals are more severely affected and may die from CHD before reaching the age of maturity.

The prevalence of heterozygous FH appears to be approximately 0.2% in European and North American populations [2]. In some culturally and geographically
isolated population groups, however, the frequency of the disease and of specific mutations is much higher, presumably as a result of a founder effect; examples include the French Canadians [3], Sephardic Jews [4], Lebanese Christian Arabs [5], South African Afrikaners [6] and the Finns [7].

So far, more than 500 different mutant alleles of the LDL-R gene have been reported [2,8–10] (website: www.ucl.ac.uk/fh), and except for the few populations dominated by founder mutations, each family is, a priori, expected to have a unique LDL-R mutation. For a number of mutations identified so far, the mutant LDL-R protein has been characterized, either in cultured cells derived from the patient or by mutagenesis and expression of the mutant allele in vitro.

Because the LDL-R consists of distinct structural domains, different mutations result in mutant proteins whose structure and function are impaired in different ways and to a different extent. Defining mutations at the protein level allows the distinction of five classes of functional defects. These classes include defects in synthesis, intracellular transport, ligand binding, internalization, and recycling of the receptor [2,8]. Consequently, the characterization of the specific mutation in the LDL-R gene of an FH patient not only provides further insights in the way the LDL-R functions in vivo, but also allows an accurate diagnosis to be made on which treatment and counselling can be based. It has long been recognized that there is considerable variation in the severity of the disease in FH patients, in both the degree of their hypercholesterolemia and the age of onset of clinical symptoms of coronary heart disease. Comparing groups of patients with either the same or different mutations in the LDL-R gene may allow a better assessment of the underlying genetic or environmental causes of this variation. In addition, there is an ongoing debate whether or not the knowledge of the molecular defect may allow to better predict the response to treatment [11–14].

In this report we describe the identification of a novel mutation in the LDL-R gene in a FH family of German origin. The molecular defect consists of a G to A transition at nucleotide position 1013 in exon 7, which results in an amino acid substitution of cysteine by tyrosine at position 317. The index patient is a true homozygote for this mutation, thus affording the rare opportunity to unequivocally study the phenotypic effects of that particular mutation. The consanguineous parents as well as two siblings are heterozygous carriers of the mutant allele and show considerable variations in the biochemical expression of the genetic defect.

2. Methods

2.1. Subjects

The index patient (Fig. 1, PID V-2) described in this study is a now 11-year-old German boy who was referred to the lipid outpatient clinic because of xan-
thomas on the proximal interphalangeal joints of the fingers and bilateral lipid arcs. No tendon xanthomas were observed. The boy had no angina, but the electrocardiogram showed discrete repolarisation abnormalities in the anterior leads. Initial lipoprotein analysis showed total cholesterol of 27.5, HDL-cholesterol of 1.0, LDL-cholesterol of 25.9, VLDL-cholesterol of 0.6 and triglycerides of 1.9 mmol/l, respectively. The index patient has one sister and one brother, born in 1985 and 1982, respectively, who are both in good health. The parents of the index patient (Fig. 1, PID IV-1 and -2) are first degree cousins, as their mothers were sisters. In addition, as the parents of the index patient’s mother were first cousins as well, the extent of consanguinity in this family is extremely high. Unfortunately, only the parents and siblings of the index patient were available for DNA studies and analysis of serum lipid levels.

2.2. Biochemical analysis

Plasma lipoproteins were separated by preparative ultracentrifugation and precipitation and cholesterol and triglycerides were assayed by enzymatic methods (Roche Diagnostics, Mannheim, Germany) [15]. Plasma A-I and B apolipoproteins were measured by immunonephelometry (Behringwerke AG, Marburg, Germany). Lp (a) was determined with polyclonal antibodies (Inkstar, Stillwater, MN) on a Behring nephelometer as described in detail by Nauck et al. [16]. Apo E phenotyping was performed by agarose gel by isoelectric focusing and immunofixation as described previously [17].

2.3. DNA isolation and Southern blot analysis

High molecular weight genomic DNA was extracted from peripheral leukocytes according to standard procedures [18]. Southern blot analysis of the LDL-R gene was performed essentially as described by Rüdiger et al. [19]. DNA (10 μg) was digested using 10 U/μg of BglII or BamHI under conditions recommended by the manufacturer (New England Biolabs, Beverly, MA), separated by agarose gel electrophoresis and transferred to nylon membranes (Hybond-N, Amersham International, Buckinghamshire, UK). Two cDNAs were used for hybridization: a 1.7 kb HindIII/BglII fragment containing sequences from exons 1 through 11 and a 1.9 kb BamHI fragment encompassing sequences from exons 11 through 18 of the cDNA clone pLDLR3 (American Type Culture Collection, Manassas, VA).

2.4. Haplotype analysis of the LDL receptor locus

The haplotypes of the FH-family members were determined by seven known polymorphisms in the LDL-R gene. The variable restriction sites, SfαNI in exon 2 [20], Stul in exon 8 [21], AciI in exon 11 [22], HincII in exon 12 [23], AvaII in exon 13 [24], MspI in exon 15 [25] and NeoI in exon 18 [26] were detected by PCR amplification and restriction enzyme digestion. Alleles were designated as ‘+’ or ‘−’ indicating the presence or absence, respectively, of the cutting sites.

2.5. Polymerase chain reaction and DGGE

To screen for small deletions, insertions or point mutations in the LDL-R gene and in the sequence of the apolipoprotein B-100 gene encompassing the domain relevant for receptor binding, denaturing gradient gel electrophoresis (DGGE) was applied according to Nissen et al. [27]. Briefly, genomic DNA encoding the LDL-R was amplified using intron-specific primers for each of the 18 exons encompassing the coding and the splice site consensus sequences at the intron/exon junctions. The gene regions screened by the exon 1 and LDL-R promoter primers are overlapping and in combination screen all of exon 1 and the first 233 bp of the LDL-R promoter sequences, thus including all the known regulatory elements of the LDL-R gene [28]. With the primers specific for the apo B gene only codons 3456–3553 are screened, but these include all known mutations resulting in defective binding of apo B to the LDL-R [29].

2.6. Sequencing of exon 7 of the LDL receptor gene

PCR fragments of exon 7 that displayed an abnormal pattern in DGGE were purified by glass milk elution of the appropriate band from 2% agarose (Jet Quick, Genomed GmbH, Bad Oeynhausen, Germany) and ethanol precipitation. The purified fragments were sequenced directly in both directions using the fluorescence-labeled primers SP 66 and 67 [8] and the thermo sequenase cycle sequencing kit (US Biochemical, Cleveland, OH) according to the manufacturer’s instructions. Aliquots of the sequencing reactions were then analyzed using an automated sequencer (ALF, Pharmacia, Uppsala, Sweden).

2.7. Restriction analysis of the C317Y mutation

Because the G→A transition at nucleotide position 1013 generates a new restriction site for Rsal, aliquots of the PCR products obtained by the amplification of exon 7 were digested for 4 h with 5 U of Rsal according to the manufacturer’s instructions (New England Biolabs). The digested products were electrophoresed on 3.5% GTG Agarose (FMC Bioproducts, Rockland, ME) stained with ethidium bromide and examined under UV illumination.
2.8. Binding, uptake and degradation of $^{125}$I-labeled lipoproteins

VLDL ($d < 1.0063$ kg/l) and LDL ($1.019 < d < 1.063$ kg/l) were isolated by preparative ultracentrifugation from a pool of normolipemic donors and iodinated using the iodine monochloride method [30]. Human skin fibroblasts were obtained from skin biopsies of the patient and a normolipemic individual, respectively. Cells were grown in 24-well polystyrene plates. Prior to the experiments, cells were pre-incubated for 40 h in medium containing 10% (v/v) human lipoprotein-deficient serum in order to up-regulate LDL receptors. Binding, uptake and degradation of $^{125}$I-labeled lipoproteins were measured according to the procedure described by Goldstein et al. [31] with slight modifications [32]. To measure cell surface binding, cells were incubated for 1 h at 4°C with $^{125}$I-labeled lipoproteins in DMEM medium containing 10 mM HEPES. To determine uptake (surface binding plus internalization) and degradation, cells were incubated for 4 h at 37°C with $^{125}$I-labeled lipoproteins in DMEM medium containing 24 mM bicarbonate (pH 7.4). The amount of $^{125}$I-labeled material associated with the cells (binding and internalization) was determined as $^{125}$I-labeled trichloracetic acid-soluble (non-iodine) material in the conditioned medium. Values were corrected for protein concentrations using the Lowry assay and BSA as standards (Bio-Rad, Hercules, CA).

3. Results

3.1. Family analysis

The pedigree of the G. family is shown in Fig. 1 and the plasma lipid values are given in Table 1. The parents of the patient were first degree cousins, as the parents’ mothers were sisters (PID III-2 and -3). Further, the maternal grandparents of the patient were also first degree cousins (PID III-1 and -2), so that an extraordinary extent of consanguinity is evident in this family. The patient showed lipid values that definitively met the biochemical criteria for homozygous FH. The patient presented with xanthomas on the proximal interphalangeal joints of the fingers and bilateral lipid arcs characteristic of FH. On ECG, discrete repolarisation abnormalities were detected, which we interpret as an early sign of ischemic cardiomyopathy. The patient is now treated with colesteryamine (16 g/d) and LDL-apheresis which is performed every 2 weeks.

Both siblings of the patient also showed concentrations of LDL-chol that were above the 95th percentile for age and gender in the German population. Both siblings were doing well and had no clinical signs of FH. The mother showed moderate hypercholes-
terolemia only with LDL-chol and HDL-chol concentrations that were both above the 75th percentile for age and sex. Fasting triglycerides, however, were significantly elevated.

Of special interest was the biochemical analysis of the patient’s father, who showed an extreme fasting hypertriglyceridemia and an unusually high VLDL-chol concentration. The concentration of LDL-chol, however, was 70 mg/dl, thus being below the 5th percentile of age — and sex adjusted reference values. Consequently, at a glance, the presence of a mutated LDL-R allele appeared to be rather unlikely in this individual. Both parents showed no clinical signs of FH. The self-reported alcohol consumption of the parents was 150 and 600 g ethanol per week for mother and father, respectively. The γ-glutamyl-transferase activity of the father was elevated at 87 U/l (local reference range: 0–24 U/l).

Unfortunately, because the patient’s parents have completely lost contact with their relatives, further members of the G. family were not available for clinical examination, DNA testing, or biochemical analyses. Information on the causes of death in previous generations was also not obtainable.

3.2. Identification of a sequence alteration by DGGE

Southern blot analysis after BglII or XbaI digestion of genomic DNA isolated from the patient, his parents and his siblings did not reveal any major abnormality in the LDL-R gene (not shown), suggesting the presence of a point mutation or minor rearrangement. To determine the exact location of the mutation, the amplified products of all 18 exons, including the splice site sequences, and of the promoter of the LDL-R of the patient, his parents and his siblings were subjected to DGGE analysis.

As shown in Fig. 2, evidence for a single mutation was found in exon 7 or in its splice junctions.

The DGGE analysis for the patient revealed a single homoduplex band as it is seen in individuals with two identical alleles. However, the electrophoretic mobility of this homoduplex band was clearly distinct from that of two normolipemic controls. The mutant homoduplex band migrated a shorter distance in the denaturing gradient gel, indicating a lower melting temperature of the altered sequence. The appearance of this single homoduplex band with an aberrant melting profile clearly indicated a true homozygous state with the presence of an identical mutation on both alleles.

In contrast, in the DGGE analysis of the patient’s parents and his siblings an identical four band pattern was apparent, indicating the presence of two different alleles of the LDL-R gene. While the two lower bands were caused by the two different alleles, the remaining two bands were caused by formation of heteroduplexes between the two alleles. The lowest band migrated at
Table 1
Biochemical data on members of the G. family with the C317Y mutation of the LDL-R gene

<table>
<thead>
<tr>
<th>PID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Chol (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>LDL-chol (mmol/l)</th>
<th>HDL-chol (mmol/l)</th>
<th>VLDL-chol (mmol/l)</th>
<th>C317Y</th>
<th>Apo E</th>
<th>Apo B (mg/dl)</th>
<th>Apo AI (mg/dl)</th>
<th>Lp(a) (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 1</td>
<td>F</td>
<td>47</td>
<td>7.3</td>
<td>2.8</td>
<td>4.2</td>
<td>2.1</td>
<td>1.0</td>
<td>Heteroz.</td>
<td>2/4</td>
<td>139</td>
<td>227</td>
<td>110</td>
</tr>
<tr>
<td>IV 2</td>
<td>M</td>
<td>37</td>
<td>9.4</td>
<td>21.3</td>
<td>1.8</td>
<td>1.1</td>
<td>6.5</td>
<td>Heteroz.</td>
<td>2/3</td>
<td>119</td>
<td>179</td>
<td>3</td>
</tr>
<tr>
<td>V 1</td>
<td>F</td>
<td>14</td>
<td>8.1</td>
<td>1.8</td>
<td>6.2</td>
<td>1.1</td>
<td>0.8</td>
<td>Heteroz.</td>
<td>3/4</td>
<td>164</td>
<td>129</td>
<td>53</td>
</tr>
<tr>
<td>V 2</td>
<td>M</td>
<td>9</td>
<td>27.8</td>
<td>1.9</td>
<td>25.9</td>
<td>1.0</td>
<td>0.9</td>
<td>Homoz.</td>
<td>2/4</td>
<td>564</td>
<td>110</td>
<td>219</td>
</tr>
<tr>
<td>V 3</td>
<td>M</td>
<td>11</td>
<td>7.1</td>
<td>0.9</td>
<td>5.6</td>
<td>1.3</td>
<td>0.1</td>
<td>Heteroz.</td>
<td>2/3</td>
<td>126</td>
<td>128</td>
<td>65</td>
</tr>
</tbody>
</table>

*a PID, pedigree identification number; F, female; M, male; ApoE, Apo E phenotype; age refers to years of age at blood sampling; lipid values are in mg/dl and represent maximal values obtained before any medical treatment.
Fig. 2. DGGE analysis of the patient and his family. Exon 7 was amplified from genomic DNA as described in Section 2. The PCR product, containing a 40-bp GC clamp was loaded on a 6% polyacrylamide gel containing a denaturing gradient of 40–80%. Pedigree identification number is with reference to Table 1. A single homoduplex band with an abnormal electrophoretic mobility, indicating the presence of an identical mutation on both alleles is seen in subject V2. All other family members examined display an identical four band pattern with a fast-migrating homoduplex band at the position of the normolipemic controls (lane 6 and 7) and a slow-migrating homoduplex band at the position corresponding to the mutant DNA.

the same position as the homoduplex band of the controls, representing the normal allele whereas the second lowest band displayed the same electrophoretic mobility as the homoduplex band seen in the patient’s sample.

3.3. Nucleotide sequence of the mutant allele and restriction genotyping of the C317Y mutation

To precisely identify the mutation in exon 7, the PCR product originating from the mutant allele of the patient was sequenced. Direct bidirectional sequencing revealed a single base substitution (G → A) at nucleotide 1013 of the LDL-R cDNA (not shown). This mutation results in the substitution of cysteine by tyrosine at residue 317 thereby eliminating the fourth of the six cysteine residues involved in the disulphide bonds that are located in repeat A of the EGF precursor homology domain of the LDL-R protein [2].

As the base substitution at position 1013 creates a recognition sequence of RsaI (GTAC), the presence of the mutation was confirmed by RsaI digestion of the PCR product of exon 7. As expected, the 253 bp PCR product originating from the patient was completely digested, generating two fragments with 146 and 107 bp, respectively. The PCR products of the controls remained completely uncut, whereas the other family members displayed restriction fragments consistent with a heterozygous state for this mutation (not shown).

3.4. Haplotype analysis

Genotyping at seven polymorphic sites showed that the index patient was homozygous for the SfaNI (+), StuI (+), AcI (+), HinclI (−), AvaII (+), MspI (+) and the NcoI (−) RFLPs. This genotype was identical for all family members except for the NcoI polymorphic site, at which the mother and the sister of the patient were heterozygous.

3.5. Interaction of lipoproteins with cultured cells

To examine the functional properties of the mutant LDL-R, skin biopsies of the patient and a normolipemic individual were obtained and primary fibroblast cell cultures were established. We studied binding (at 4°C), uptake and degradation (both at 37°C) of 125I-LDL and 125I-VLDL at protein concentrations of 5 through 40 mg/l. Binding, uptake and degradation of 125I-LDL in fibroblasts from the patient were less than 10% of those in normal cells (Fig. 3). The interaction of 125I-VLDL with cultured cells was also decreased, but to a lesser extent. On average, binding, uptake and degradation of VLDL in cells from the patient were reduced by 60, 30, and 38%, respectively, compared to normal cells (Fig. 4).
Binding of VLDL was also determined after down-regulating the expression of the LDL-R gene by incubating the cells for 24 h in the presence of sterols [33]. In the cells of the patient, sterol-dependent repression of the LDL-R gene diminished the residual binding activity for VLDL by 50% (Fig. 5).

4. Discussion

A missense mutation in the LDL-R gene has been detected in a homozygous FH patient with the combined use of DGGE and sequence analysis. The mutant alleles inherited by the patient from both parents change the codon 317 from TGC to TAC. This transition results in a replacement of cysteine by tyrosine at position 317 of the LDL-R protein, thereby eliminating the fourth of six cysteine residues involved in the formation of disulphide bonds located in repeat A of the EGF precursor homology domain of the LDL-R [28]. This domain is the most highly conserved in evolution, and the cysteine residues in repeat A are absolutely conserved [34]. The mutation reported here is the second mutation affecting codon 317. A previous report described a homozygous FH patient from Japan with a transition from TGC to TAC [35]. That mutation, named FH-Wakayama, causes an amino acid change from cysteine to serine. In pulse chase experiments performed with fibroblasts from the Japanese homozygous patient, Funahashi et al. found that the posttranslational processing of the receptor was markedly impaired, and the majority of the receptors remained in the precursor form even after the chase period [36]. Accordingly, the allele FH-Wakayama encoded a protein that is delayed in transport between the endoplasmic reticulum and the Golgi apparatus. Although the mutation described in this paper is not a serine for cysteine_{317}, but a tyrosine for cysteine_{317} substitution, it is reasonable to assume that the functional consequences are similar, because a disulphide bond is lost in both cases. Thus, also in view of the similarity between serine and tyrosine, it is likely that a defect in the intracellular transport of the receptor is present in our patient as well, so that we propose to classify the newly discovered allele C317Y as transport defective, the most common type of mutation at the LDL-R locus [8].

In a previous study using site-directed mutagenesis, Esser et al. showed that repeat A of the EGF precursor homology region is required not only for the regular processing and normal stability of the receptor, but also for binding of LDL [37]. In line with this previous
studies using apo E containing 125I-VLDL as ligand, the expression of Funahashi et al. we also performed binding studies using apo E containing 125I-VLDL as ligand, the binding of which to the LDL-R has less stringent structural requirements than the binding of LDL [38]. We found that binding of VLDL was reduced to only about half the normal value. In addition, when the expression of the LDL-R gene was repressed by incubating the cells in the presence of sterols, the mutant cells showed a residual binding activity for VLDL that was further diminished by 50%, compared to cells cultured under sterol-depleted conditions. These results suggest that half of the residual binding of VLDL seen in sterol-depleted cells was as a result of the LDL-R and that the proportion of the mutant receptor that reaches the cell surface selectively looses the ability to bind 125I-LDL, but retains an ability to bind 125I-VLDL. This assumption is supported by experiments from Esser et al. showing that the replacement of either 1 or 2 cysteine residues in repeat A (cysteine297 and cysteine308) selectively decreases binding of LDL, but not of β-VLDL [37]. Taken together, the mutation C317Y presumably encodes a receptor protein that exhibits more than one type of functional defect. It is converted to the mature form at a very slow rate, and the reduced amounts of LDL-R reaching the cell surface specifically fails to bind LDL, but not VLDL. Accordingly, the type of mutation encoded by this allele has to be classified as class 2b mutation [8].

Further evidence that the mutation C317Y is causing FH comes from the family analysis. The homozygous patient is more severely affected than his two older siblings, who are heterozygous for the C317Y allele, so that a gene-dosage effect typical of autosomal-codominant disorders can be established. While both siblings had an intermediate level of LDL-chol consistent with the presence of only a single functional LDL-R gene, the family analysis turned more complicated when taking the parents into account. The patient’s mother showed concentrations of total cholesterol and triglycerides above the 95th percentile for age and gender, while LDL-chol was only moderately elevated being above the 75th percentile. HDL-chol was elevated (2.1 mmol/l) above the 75th percentile as well. The father showed an extremely elevated triglyceride level (20.7 mmol/l), while his LDL-chol concentration was strikingly low.

It is known that many genetic and environmental factors influence the LDL-chol so that a reasonable number of individuals is misclassified as having or not having FH when traditional clinical criteria are used to make the diagnosis. According to the probability assessments of Williams et al. for diagnosing FH in members of FH families, the mother and the father of the patient had probabilities of 11.2% and below 1%, respectively, for having FH if LDL-chol levels were used and it is assumed that a first degree relative has definite FH [39]. Although Williams et al. suggested using lower diagnostic lipid values in FH families than in the general population, based on the higher a priori possibility of having FH when belonging to an FH family than to the general population, these criteria would fail to diagnose the correct FH status of the parents in this case. In case of the father one would even tend to exclude the presence of a defective LDL-R allele because of the extremely low LDL-chol level. In addition, clinical symptoms of FH, such as tendon xanthomas or evidence for the presence of CAD were absent in both parents. These low probabilities on the basis of biochemical and clinical criteria, despite the presence of a disease-causing LDL-R allele, document the improved diagnostic precision obtained by introducing genetic diagnosis in FH families [40–42].

What are the factors that mitigate the hypercholesterolemic phenotype of the parents despite the presence of a disease-causing allele that clearly results in a characteristic FH-phenotype in the affected children? In our opinion, the uncharacteristic phenotype of the parents is most likely attributable to their nutritional behaviour, which is characterized by both strikingly hypercaloric diet and high alcohol consumption, both of which being present to a greater extent in the father. Ethanol ingestion is known to affect the lipid metabolism by stimulating the synthesis of VLDL triglycerides in the liver. In addition it is discussed that alcohol delays the metabolism of VLDL resulting in a decreased production rate of LDL [43,44]. Moreover, extreme alcohol consumption may also contribute to a reduction in LDL-chol, because of the low content of unsaturated fatty acids typically present in the diet of these individuals [45]. Here, it seems conceivable that the decreased metabolism of LDL as a result of the presence of a functionally defective LDL-R is compensated by an impaired lipolysis of VLDL resulting in a reduced production rate of LDL. As a consequence, the LDL steady state concentrations in plasma are not profoundly elevated, as found in the mother, or even unexpectedly low, as seen in the father.

In line with the well established positive correlation between alcohol consumption and HDL, the mother showed a notably elevated concentration of HDL-chol [44,46].

The mutation identified in this family does not appear to be a frequent cause of FH in South-West Germany. In a screening of 120 unrelated hypercholesterolemic patients attending the lipid outpatient clinic
at the University Hospital Freiburg, we did not detect any further carrier of the mutation C317Y. In view of the patient’s family exhibiting first degree consanguinity in two generations, one can speculate that both alleles affected by the same mutation present in the homozygous patient are derived from the same ancestor. This view is supported by the haplotype analysis showing homozygosity for all polymorphic sites tested. The family of the patient is of South-West German origin, and we propose that this mutation should be named FH-Freiburg according to the nomenclature of Hobbs et al. [2].

In conclusion, the mutant allele identified in the homozygous patient encodes a LDL-R that is defective for both transport and LDL binding. The allele is associated with a severe clinical phenotype in the homozygous patient and his heterozygous siblings. The presentation of the heterozygous parents, however, underlines the profound impact of life-style habits, which might blur the diagnosis of FH based on clinical criteria. These uncertainties seen in the parents call for simple and efficient methods for genetic diagnosis in order to improve diagnostic precision [40,47].

Acknowledgements

The excellent technical assistance of Ulrike Stein, Sabine von Karger, Sybille Rall and Brigitte Kreisel is gratefully acknowledged.

References


