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Clinical characterization and mutation spectrum of German patients with familial hypercholesterolemia

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ABSTRACT

Background and aims: Autosomal-dominant familial hypercholesterolemia (FH) is characterized by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) and a dramatically increased risk to develop cardiovascular disease (CVD). Mutations in three major genes have been associated with FH: the LDL receptor gene (*LDLR*), the apolipoprotein B gene (*APOB*), and the proprotein convertase subtilisin/kexin 9 gene (*PCSK9*). Here we investigated the frequency and the spectrum of FH causing mutations in Germany.

Methods: We screened 206 hypercholesterolemic patients, of whom 192 were apparently unrelated, for mutations in the coding region of the genes *LDLR*, *PCSK9* and the *APOB* [c.10580G > A (p.Arg3527Gln)]. We also categorized the patients according to the Dutch Lipid Clinic Network Criteria (DLCNC) in order to allow a comparison between the mutations identified and the clinical phenotypes observed. Including data from previous studies on German FH patients enabled us to analyse data from 479 individuals.

Results: Ninety-eight FH causing variants were found in 92 patients (nine in related patients and 6 patients with two variants and likely two affected alleles), of which 90 were located in the *LDLR* gene and eight mutations were identified in the *APOB* gene (c.10580G > A). No mutation was found in the *PCSK9* gene. While 48 of the *LDLR* mutations were previously described as disease causing, we found 9 new *LDLR* variants which were rated as “pathogenic” or “likely pathogenic” based on the predicted effect on the corresponding protein. The proportions of different types of *LDLR* mutations and their localization within the gene was similar in the group of patients screened for mutations here and in the combined analysis of 479 patients (current study/cases from the literature) and also to other studies on the LDLR mutation spectrum, with about half of the variants being of the missense type and clustering of mutations in exons 4, 5 and 9.

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The mutation detection rate in the 35 *definite* and 45 *probable* FH patients (according to DLCNC) was 77.1% and 68.9%, respectively. The data show a similar discriminatory power between the DLCNC score (AUC = 0.789 (95% CI 0.721–0,857)) and baseline LDL-C levels (AUC = 0.799 (95% CI = 0.732–0.866)). *Conclusions:* This study further substantiates the mutation spectrum for FH in German patients and confirms the clinical and genetic heterogeneity of the disease.

1. Introduction

Familial hypercholesterolemia (FH, OMIM 143890) is an inherited autosomal-dominant disorder, which is characterized by a reduced hepatic capacity to clear atherogenic cholesterol-rich low-density lipoproteins (LDL) from the circulation, leading to a marked elevation of serum LDL-cholesterol (LDL-C). Consequently, FH causes early morbidity and mortality from cardiovascular disease (CVD) (reviewed in Ref. [1]). Common tools for the clinical FH diagnosis are the Dutch Lipid Clinic network (DLCN) or the Simon Broome criteria, both representing a weighted score of the clinical FH characteristics including plasma LDL-C concentration, presence of tendon xanthoma, premature corneal arcus lipoides, cardiovascular disease and also the clinical family history because of the high likelihood of close relatives to be also affected from the disease. The prevalence of FH is estimated to be as high as one in 200–500 and might be even higher in populations with founder effects [2–4].

FH is caused by defects in one of at least three different genes, *LDLR*, *APOB* or *PCSK9*, coding either for the low density lipoprotein (LDL) receptor, its ligand apolipoprotein B, or for proprotein convertase subtilisin/kexin type 9, a protein involved in LDL receptor turnover [2,3,5,6]. Autosomal-recessive hypercholesterolemia (ARH) is a rare disorder, which is clinically indistinguishable from FH and caused by mutations in the *LDLRAP1* gene, leading to a complete loss of function of an adaptor protein required for receptor-mediated hepatic uptake of LDL-C.

About 60% of patients with the clinical diagnosis of FH may be mutation negative, i.e. no mutation is identified in the three established FH genes. This might partly be explained by an accumulation of common small-effect LDL-C raising alleles [7]. Another possibility is that variants in other genes, which have not yet been associated with FH, may cause the disease in patients negative for mutations in the known FH genes. Indeed, Fouchier and colleagues recently identified *STAP1* as a possible fourth gene causing FH [8].

FH therapy includes lifestyle modification and treatment with a statin, first line therapy choice. International guidelines for prevention of CVD recommend rigorous lipid lowering treatment especially in FH patients. Early effective treatment is associated with a 75% reduction in lifetime risk of death from CVD [9]. Some countries, such as Netherlands and UK, already implement FH cascade family screening as a cost-effective tool to deal with FH and initiate lipid lowering treatment to prevent early CVD [10]. In most parts of Europe, however, FH is still underdiagnosed, with a detection rate below 15% [11]. In Germany, the diagnosis rate is not known.

Early diagnosis is essential for prevention of cardiovascular events in FH-patients. In the case of a clinically definite or probable FH (DLCN or Simon Broome criteria), genetic testing remains the gold standard in diagnostics and enables cost-effective cascade screening [12]. In the current study we report on the clinical characteristics of over 200 patients with hypercholesterolemia from Germany and the genetic variations identified in these patients. Additional consideration of some 200 published cases on German FH patients [13–16] allowed us to describe the FH mutation spectrum and phenotype-genotype correlations based on data from >400 patients.

2. Materials and methods

2.1. Patients and recruitment

The current study includes 164 patients diagnosed between 2012 and 2015 in the specialized Lipid Clinic at the Interdisciplinary Metabolism Center, Charité – Universitätsmedizin Berlin, Germany. In order to increase the total number of analyzed patients other specialized lipid clinics from different parts of Germany were asked to join the study. Four other centers (Homburg, Giessen, Kempten and Magdeburg) provided additional data on 42 patients. For the analysis of the mutation spectrum we used additional data from the literature on German patients studied earlier [13–16].

2.2. Clinical diagnostics

FH patients were diagnosed clinically based on patient and familial anamnesis, physical examination and laboratory parameters including total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG) and lipoprotein (a) [Lp(a)]. Where possible, we calculated scores based on the Dutch Lipid Clinic Network criteria (DLCNC) [17]. This score allows to group patients according to the likelihood of having FH based on clinical parameters and anamnesis. In patients receiving lipid lowering medication, we calculated medication-naïve LDL-C using conversion factors reported in the literature [18–26]. A DLCNC score of >8 suggests a “definite”, 6–8 a “probable”, 3–5 a “possible” and <3 an “unlikely” FH. The capacity of the DLCNC to distinguish between carriers and non-carriers of disease causing mutations was estimated with the area under the receiver operating characteristic (ROC) curve.

LDL-C serum levels were given in mg/dL (1 mmol/L = 38.66 mg/dL).

2.3. Mutation screening

The mutation screening strategy used was described previously [2]. Briefly, we amplified all 18 exons of the *LDLR* gene, all 12 exons of the *PCSK9* gene and a segment from the *APOB* gene containing the site of the known mutation c.10580G > A from genomic DNA extracted from peripheral blood by standard procedures. PCR products were screened for mutations by direct DNA Sanger sequencing. Multiplex Ligation-dependent Probe Amplification (MPLA) was performed to detect larger *LDLR* gene rearrangements. All detected sequence variants were compared to HGMD database, Leiden Open Variation Database and UCL *LDLR* variant database. Novel sequence variants were assessed using the prediction tools *PolyPhen2* and *Mutation Taster* [27,28] and their frequency in the ExAC database (http://exac.broadinstitute.org/gene/ENSG00000130164). New variants rated as probably damaging or disease causing by the prediction tools were subsequently rated