Sequencing for LIPA mutations in patients with a clinical diagnosis of familial hypercholesterolemia

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Background and aims: We recently identified lysosomal acid lipase (LAL) deficiency, a recessive disease caused by mutations in LIPA, in 3 patients with a clinical diagnosis of familial hypercholesterolemia (FH). We aimed to determine the prevalence of LIPA mutations among individuals with a clinical FH diagnosis.

Methods: In 276 patients with phenotypic FH, in whom no genetic basis for their phenotype was found, LAL was sequenced. All variants were assessed for pathogenicity using a literature search and in silico prediction models.

Results: We included 213 adults and 63 children with mean (±SD) LDL-C levels of 7.8 ± 1.3 and 4.4 ± 1.5 mmol/L, respectively. Twenty-one variants were identified. Six patients were heterozygous for a (potentially) pathogenic mutation. No homozygous LIPA mutation carriers were identified.

Conclusions: Our data show that LAL deficiency was not missed as diagnosis in our study population but the frequency of heterozygous LIPA mutations implies that the FH population might be relatively enriched with LIPA mutation carriers.

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1. Introduction

Lysosomal acid lipase (LAL) deficiency is a rare recessive lysosomal storage disease caused by mutations in LIPA (OMIM #613497). LAL hydrolyses cholesteryl esters and triglycerides in lysosomes and, as a consequence, dysfunction of this enzyme leads to accumulation of cholesteryl esters and triglycerides in, among others, hepatocytes, intestines and adrenal glands [1]. Depending on the residual LAL activity, LAL deficiency can result in the very severe and lethal Wolman disease or the late onset cholesteryl ester storage disease (CESD) [1]. CESD is typically characterized by hepatosplenomegaly and type IIb hyperlipidemia [2]. Recently, we identified 3 siblings with a clinical diagnosis of familial hypercholesterolemia (FH; type IIa hyperlipidemia) that were homozygous for a mutation in LIPA [3,4]. At physical examination hepatosplenomegaly was not observed. It has been suggested that CESD should be considered as a differential diagnosis in patients with clinical FH in whom no FH mutation is identified [5]. Based on the potential overlap, CESD might be underdiagnosed [4,6]. The current standard of care for dyslipidemia in patients with CESD is treatment with 3-hydroxy-3-methyl-glutaryl-CoA-reductase inhibitors (statins) [6]. However, reduction of plasma LDL-C levels in these patients might potentially lead to increased intra-cellular levels of cholesteryl esters, which may worsen the effects on liver function [6]. Recently, Burton and co-workers showed that enzyme replacement therapy (ERT; sebelipase alpha; Kanuma®) normalized alanine aminotransferase levels and decreased hepatic fat content in CESD patients, compared with placebo [7]. Since December 2015, sebelipase alfa has been approved by the US Food and Drug Administration (FDA) for treatment of patients with LAL deficiency. It has been suggested that screening for LIPA mutations in phenotypically diagnosed FH patients without mutations in LDLR, APOB, and PCSK9, might identify CESD patients who might be eligible for ERT by sebelipase alfa. Therefore, we aimed to establish the prevalence of LIPA mutations in patients with clinically, but not genetically defined FH.
1.1. Patients and methods

The study cohort comprised patients with a clinical FH phenotype in whom, after genetic analysis by Sanger sequencing, no mutations had been identified in LDLR (OMIM #606945) or APOB (OMIM #107730). LDLR was also analysed by MLPA (Multiplex ligation-dependent probe amplification) to detect major gene rearrangements. The genetic studies were performed by the national referral laboratory for DNA diagnostics of inherited dyslipidaemias at the Academic Medical Center of the University of Amsterdam, the Netherlands. Given the low prevalence of mutations in LDLRAP (#605747), leading to Autosomal Recessive Hypercholesterolemia, and PCSK9 (OMIM #607786), causing an FH-like phenotype termed Autosomal Dominant Hypercholesterolemia, sequencing of these genes was only performed when homozygous FH was suspected (for LDLRAP) or when patients fulfilled the criteria for FH as published by Civeira and co-workers[8]. Individuals with a secondary form of dyslipidemia or an LDL-C level below the 95th percentile for age and gender were excluded. Genotypes in which the LDLRAP mutation had been identified in phenotypically diagnosed FH patients, but not genetically confirmed, were carriers of c.683 T > C in exon 7, encoding a phenylalanine to serine amino acid substitution (p.Phe228Ser) that was predicted to be pathogenic by all in silico prediction models used. Two patients were carriers of the c.894G > A mutation in exon 8 (p.Gln298Gln) which is a common synonymous but pathogenic variant among LAL deficiency patients, affecting the splice site at the end of exon 8 (ClinVar RV000185528.3) [9]. One patient was carrier of c.913 T > A in exon 9, causing a phenylalanine to isoleucine substitution (p.Phe305Ile) that was predicted to be potentially pathogenic by PolyPhen2, and one patient was carrier of c.966 + 3A > T, affecting a splice site, to a similar extent as is predicted for the known functional p.Gln298Gln variant in exon 8. Homozygous or compound heterozygous carriers of (potentially) pathogenic LIPA mutations were not identified.

2. Results

We selected 276 patients (213 adults and 63 children) with a phenotypic diagnosis of FH of whom 105 (38.0%) were male. The mean age (±SD) of included patients was 53.3 ± 11.4 and 10.6 ± 3.7, for adults and children, respectively (Table 1). Mean LDL-C levels were 7.8 ± 1.3 mmol/L and 4.4 ± 1.5 mmol/L, respectively. A total of 21 variants were identified of which 16 were previously described (see rs number in Table 2). Six patients were found to be heterozygous carriers of a potentially pathogenic mutation. Two patients were carriers of c.683 T > C in exon 7, encoding a phenylalanine to serine amino acid substitution (p.Phe228Ser) that was predicted to be pathogenic by all in silico prediction models used. Two patients were carriers of the c.894G > A mutation in exon 8 (p.Gln298Gln) which is a common synonymous but pathogenic variant among LAL deficiency patients, affecting the splice site at the end of exon 8 (ClinVar RV000185528.3) [9]. One patient was carrier of c.913 T > A in exon 9, causing a phenylalanine to isoleucine substitution (p.Phe305Ile) that was predicted to be potentially pathogenic by PolyPhen2, and one patient was carrier of c.966 + 3A > T, affecting a splice site, to a similar extent as is predicted for the known functional p.Gln298Gln variant in exon 8. Homozygous or compound heterozygous carriers of (potentially) pathogenic LIPA mutations were not identified.

3. Discussion

In this study we aimed to determine the prevalence of homozygosity for LIPA mutations among individuals who were clinically diagnosed with FH, but not genetically confirmed. We identified 5
heterozygous \textit{LIPA} mutation carriers. No homozygous or compound heterozygous carriers of (potentially) pathogenic mutations were identified, which shows that LAL deficiency was not missed as diagnosis in our study population.

Recently, Pullinger and co-workers investigated the frequency of a common \textit{LIPA} mutation in 1357 patients with various types of dyslipidemia. In this study, six individuals were found to be heterozygous carriers of c.894G > A of whom two had isolated elevated LDL-C levels. After sequencing the heterozygous individuals for other \textit{LIPA} mutations, one patient with combined dyslipidaemia (elevated LDL-C, elevated triglycerides, and low density lipoprotein cholesterol levels) was found to be a compound heterozygous \textit{LIPA} mutation carrier. In line with our results, no homozygous \textit{LIPA} mutation carriers were identified among individuals with isolated elevated LDL-C levels \[10\].

The overall prevalence of LAL deficiency has been estimated to vary between 1 in 40,000 to 1 in 300,000 individuals \[6\]. While applying the Hardy Weinberg Equilibrium, one would expect to identify at least one heterozygous \textit{LIPA} mutation carrier in 100–274 individuals. The fact that we identified 5 heterozygous carriers in 276 individuals with heterozygous FH might imply that the FH population is relatively enriched with \textit{LIPA} mutation carriers and we might have missed LAL deficiency patients due to the relatively small study population. This would be in line with the previous identification of a family with three LAL deficiency patients with a clear FH phenotype \[4\] and underlines the importance of genetic testing for rare diseases in case clinical phenotypes may overlap the phenotype of more prevalent disorders. While considering the diagnosis of LAL deficiency it is, however, of great importance to notice that LAL deficiency patients due to the autosomal recessive disease while FH due to mutations in \textit{LDLR}, \textit{APOB} or \textit{PCSK9} is autosomal dominant. Moreover, it has been shown that heterozygous \textit{LIPA} mutation carriers (eg. the parents of a patient with LAL deficiency) generally do not demonstrate a hypercholesterolemic phenotype \[3\]. Therefore, it seems reasonable to make a distinction between the diseases based on a thorough clinical assessment, including an extensive family history and determination of the lipid profiles of the parents or children of an index case, rather than performing genetic testing for \textit{LIPA} mutations in all patients with an FH phenotype without a mutation in \textit{LDLR}, \textit{APOB} or \textit{PCSK9}.

\textbf{Conflicts of interest}

GKH or his institution received honoraria for consultancy, ad boards, and/or conduct of clinical trials from: AMGEN, Aegerion, Pfizer, Astra Zeneca, Sanofi, Regeneron, KOWA, Ionis pharmaceuticals and Cerenis, none of which are related to the contents of this manuscript. G.K.H. received research support from Aegerion, AMGEN, Sanofi, Astra Zeneca, and Synageva. B.S. or the foundation for PhD students in vascular research of the department of vascular medicine of the Academic Medical Center received lecture and/or advisory board fees from Synageva BioPharma and the Karolinska University, Stockholm, Sweden, none of which are related to the contents of this manuscript. All other authors declare that they have no conflicts of interest and no relationships with industry relevant for this study.

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