Lipoprotein Lipase Deficiency: heterozygotes match homozygotes in severity

Abstract

Introduction

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Material and methods

Genetic data derived from the Polish cohort of 5623 whole exome sequenced patients. In 52 cases the indication for WES genetic testing was "hypertriglyceridemia '' and for 5571 there was another clinical indication, mainly autism spectrum disorder, dysmorphia and neurodegenerative diseases.

Results

We present 22 heterozygous and 2 homozygous/compound heterozygotes individuals for the pathogenic/likely pathogenic LPL variant and describe HTG levels, phenotypic manifestations and age of onset in the context of molecular findings where available. We report for the first time heterozygous LPL individuals with a very severe TG (TG ≥ 22.6 mmol/L; >2000 mg/dL) and additional symptoms such as pancreatitis and recurrent abdominal pain.

Conclusions

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Introduction

Hypertriglyceridemia (HTG) is the most common lipid disorder that affects approximately 10% of the world's population [1,2]. The diagnosis of HTG depends on the fasting triglyceride levels. Based on the Endocrine Society Clinical Practice Guideline, HTG is classified as mild (triglycerides (TG) 1.7–2.2 mmol/L; 150–199 mg/dL), moderate (TG 2.3–11.2 mmol/L; 200–999 mg/dL), severe (TG 11.3–22.5 mmol/L; 1000–1999 mg/dL), and very severe (TG ≥ 22.6 mmol/L; >2000 mg/dL) [1,2]. Familiar non-syndromic HTG is caused nearly exclusively by biallelic defects of the following five major genes: *LPL, APOC2, APOA5, LMF1*, and *GPIHBP1*. Familial LPL deficiency is the most common form of familial hypertriglyceridemia and accounts for nearly 95% of all cases [3,4,5,6,7].

Biallelic pathogenic variants in the *LPL* gene are associated with familial lipoprotein lipase (LPL) deficiency (MIM #238600) [8], a disease manifesting with recurrent pancreatitis, abdominal pain, cutaneous xanthomata, and hepatosplenomegaly. On the molecular level, biallelic pathogenic variants in the *LPL* gene impair clearance from the chylomicrons to the plasma. The inheritance pattern of the disease is in general autosomal recessive. Heterozygotes are mostly asymptomatic but may have mild to moderate hypertriglyceridemia and may develop atherosclerosis at a younger age. The inheritance pattern of the familial lipoprotein lipase deficiency is also sometimes described as a codominant inheritance mode, where carriers of the one allele of the recessively inherited gene display only mild, often nonspecific symptoms and usually a late disease onset, whereas homozygous for the pathogenic *LPL* variant present with a full disease phenotype [9]. On the contrary, some diseases show both recessive and dominant inheritance, such as myotonia congenita (Thomsen myotonia with *CLCN1* pathogenic variants dominant inheritance; MIM #160800 and Becker myotonia with *CLCN1* pathogenic variants recessive inheritance, MIM #255700) [10] or calpainopathy (traditional recessive inheritance MIM #253600 and recently described dominant inheritance; MIM #618129) [11].

From the beginning, the carrier status of the pathogenic/likely pathogenic *LPL* variants has been widely discussed. In one of the first manuscripts investigating large pedigrees with

heterozygous carriers, the LPL level, although decreased by about 50%, did not allow to differentiate between carriers and non-carriers with similar phenotypes and the same lipid levels occurring in both groups [12]. Carriers presented pathogenic plasma lipid profile and lipid changes including increased TG, Very-low-density lipoprotein (VLDL), apolipoprotein B (apoB), decreased low density lipoprotein (LDL) and high-density lipoprotein (HDL). Interestingly, these differences have been mainly observed only in the probands older than 40 years, suggesting a contributory role of the secondary and environmental factors, such as obesity and insulin resistance. Available studies have not been conclusive regarding the role of the pathogenic variants associated with monoallelic hypertriglyceridemia and intermediate phenotype [13,14].

Some studies reported that heterozygous carriers may present a range of HTG levels rather than a binary affected/non-affected status. In the cohort of 15 *LPL* pathogenic heterozygotes, the HTG level varied greatly and 14.8%, 67.0% and 18.2% were in the normal, mild-to-moderate and severe HTG ranges, respectively in the 1,5-year longitudinal studies [13]. In our group of Polish patients we confirm not only the range of phenotypes but also a near to the biallelic phenotype in heterozygotes and their parents suggesting a dominant inheritance pattern.

Materials and methods

Whole exome sequencing (WES) has been performed on 5623 Polish individuals (as of 23.05.2024) In 52 cases the indication for WES genetic testing was "hypertriglyceridemia '' and for 5571 there was another clinical indication, mainly autism spectrum disorder, dysmorphia and neurodegenerative diseases. Technical sequencing was performed by CeGaT (Tübingen, Germany), bioinformatic analysis and scientific and diagnostic interpretation of variants was performed by MEDGEN laboratory (Warsaw, Poland). The genomic DNA of patients was extracted from a whole blood sample using Prepito. The sequencing exome library was prepared according to Twist Exome Enrichment (Twist Bioscience). The enriched DNA libraries were sequenced by the Illumina NovaSeq 6000 instrument, 2 x 100 bp.
. Raw sequencing reads were mapped to the human reference genome GRCh37 and GrCh38 assembly using BWA MEM (bwa-mem2.avx2 mem 0.7.17-r1188) [15]. Duplicates were removed using biobambam2 version 2.0.183 [16]. Variants were called using HaplotypeCaller

(GATK v4.2.6.1 [17,18]), FreeBayes v1.3.2 [19], and named using Variant Effect Predictor (VEP109) [20]. The variants were classified according to ACMG recommendations [21]. The presence of the variant in control populations was checked in 1000 Genomes [22] and gnomAD (v4) (Broad Institute) databases [23]. The in silico splicing analysis was performed using algorithms embedded in Alamut Visual Plus software (Sophia Genetics), i.e. SpliceSiteFinder-like, MaxEntScam, NNSPLICE and GeneSplicer and SpliceAI [24]. The presence of the detected pathogenic/likely variants was confirmed by Sanger sequencing. The XHMMv1.0 algorithm and in-house scripts were used to search for small, rare, hemizygous and homozygous CNVs.

All exomes have been searched first for variants in the *LPL* gene reported in the ClinVar database as "pathogenic/potentially pathogenic" regardless of reported clinical manifestations. Then, our entire WES database has been searched for rare nonsense variants, frameshifts and canonical splicing, and large deletions and duplications not reported in the ClinVar database. For patients included in our study, pathogenic rare variants were only found in *LPL* and none of the other canonical familial chylomicronemia syndrome (FCS) genes (i.e. *APOC2*, *APOA5*, *GPIHBP1* and *LMF1*) [25].

Results

Genetic findings in the Polish cohort
The tested individuals were 1-76 years old (Table 1). In the WES analysis of individuals with the "hypertriglyceridemia '' as an indication for testing, there was a pathogenic/likely pathogenic *LPL* variant present in 23% (12 out of 52). In the WES analysis of individuals with the "other (not hypertriglyceridemia)" as an indication for testing there was a pathogenic/likely pathogenic variant in 0.18% (10/5571) (p<0.05).

In 17 individuals the variant(s) in the *LPL* gene have been reported as pathogenic in ClinVar and in 7 cases the variants have not been reported as pathogenic in ClinVar, but fulfilled the pathogenicity criteria according to the ACMG classification (Table 1; Figure 1). One patient was homozygous and one compound heterozygous for the pathogenic LPL variants. Interestingly in two cases there was a copy number variant (CNV) in the *LPL* gene causing

duplication of the whole exome 6 and predicted to be pathogenic according to ACMG criteria. 13 individuals were diagnosed with HTG whereas 11 were not, despite carrying a pathogenic variant.

In the group of patients with a known pathogenic variant, 15 were heterozygous, 1 was homozygous, and 1 was a compound heterozygous.

Figure 1. Distribution of the pathogenic/likely pathogenic (according to the ACMG criteria) ClinVar and non-ClinVar variants in the heterozygotes with and without HTG.

Heterozygotes with a pathogenic/likely pathogenic variant in the *LPL* gene
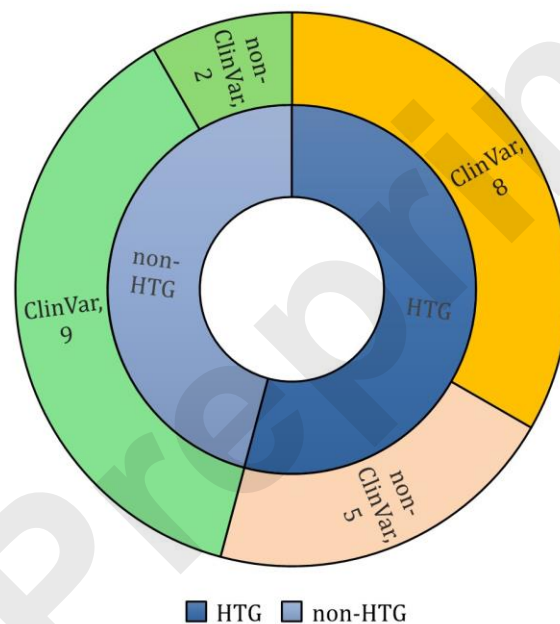
Nr	Variant	allelicity	hypertriglyceridemia	Approximate age in years at the genetic testing	ClinVar (as for 14.05.2024)
1	p.Ile221Thr	het	no	2	Yes
2	p.Trp113Arg	het	no	9	Yes
3	p.Trp113Arg	het	no	9	Yes
4	p.Gly215Glu	het	no	6	Yes
5	p.Gly215Glu	het	no	18	Yes
6	p.Gly215Glu	het	no	11	Yes
7	p.Ile252Thr	het	no	7	Yes
8	p.Arg270Cys	het	no	14	Yes
9	p.Trp113Arg	het	no	1	Yes

10	p.Arg219Ter	het	no	5	No
11	p.Ser17AspfsTer22	het	no	59	No
12	p.Arg270His	het	1000 mg/dl	10	Yes
13	p.Arg333His	het	800 mg/dl	2	Yes
14	Trp113Arg	hom	2000 mg/dl	11	Yes
15	p.Ile221Thr	het	1000 mg/dl	2	Yes
16	p.Trp113Arg/Arg197Leu	comp het	2500 mg/dl	38	Yes
17	p.Trp113Arg	het	800 mg/dl	76	Yes
18	p.Asp183Asn	het	500 mg/dl	15	Yes
19	p.Gly215Glu	het	600 mg/dl	1	Yes
20	p.Gln240Ter	het	3500mg/dl	50	No
21	c.1018+1G>A	het	800mg/dl	3	No
22	Duplication ex 6	het	728 mg/dl	14	No
23	Duplication ex 6	het	2000 md/dl	45	No
24	p.Arg270Ser	het	3000 mg/dl	52	No

Table 1. Variants, zygosity, TG levels, and approximate age at the molecular diagnosis in patients with pathogenic LPL variant.

Phenotype analysis

In 11 cases the heterozygotes for the pathogenic *LPL* variant did not present elevated TG levels, whereas in 11 cases they did. Further, we investigated common *LPL* variants: *LPL* p.Trp113Arg and *LPL* p.Gly215Glu. Two *LPL* p.Trp113Arg carriers had HTG (age 11 and 76) and normal HTG levels (age 9, 9 and 1 year). One *LPL* p.Gly215Glu carrier showed HTG (age 1 year) and three normal TG levels (age 6, 11 and 18 years) (Table 2).

Interestingly not only HTG has been found in heterozygotes, but also 8 monoallelic patients had clinical symptoms, including non-specific symptoms such as abdominal pain and periodic nausea (Supplementary Table 1). 3 out of 8 patients had at least 1 episode of acute pancreatitis in the history, 3 had enlargement of the liver and/or spleen. None of these symptomatic patients drank alcohol. One patient had only moderately high triglyceride levels without additional symptoms typical of *LPL* deficits. There was a correlation between the presence of the HTG and age in the group of heterozygous *LPL* individuals. The medium age in the heterozygotes with HTG was 24.5 years (2-76 years), whereas in the heterozygotes without HTG it was 12.8 years (1-59 years).

variant	TG normal	HTG
For all pathogenic variants	11	11
p.Gly215Glu	3	1
p.Trp113Arg	3	2

Table 2. TG levels divided by variants.

We identified 3 individuals with a heterozygous *LPL* pathogenic variant (according to the ACMG criteria) and a very severe TG (TG ≥ 22.6 mmol/L; >2000 mg/dL) (Table 1). In 2 cases HTG level exceeded 3000 mg/dl. All of the individuals presented with pancreatitis- a symptom related to the HTG. One of the patients suffered even from 3 episodes of severe pancreatitis.

One individual with a severe/very severe TG had a normal BMI, one was mildly overweight (BMI 25.3) and one was obese (BMI 36.3) (Supplementary Table 1). All these three individuals were older than 40 at the time of the genetic testing. Two variants were lost of function (LoF), among them one was a duplication of the whole exome 6, and one was classified as a missense. All variants were located in the exons 5 and 6 of the *LPL* gene. None of the variants has been reported in ClinVar and all variants were classified as pathogenic according to the ACMG classification.

The segregation analysis has been performed in all three cases. In two cases nobody from the family inherited the variants. In patient 3 affected daughter (born in 2009) with the same deletion showed TG of more than 700 mg/dl in the 2nd decade of life already.

Discussion

We described the phenotypic and molecular spectrum of a large cohort of homo- and heterozygous individuals for the pathogenic variants in the *LPL* gene. We showed that individuals with a heterozygous *LPL* variant present the whole range of phenotypes, from being asymptomatic to display the severe, fully developed phenotype with a very severe HTG and clinical symptoms. We cannot exclude that the development of the phenotype is influenced by factors such as age and sex, age, environment and comorbidities.

The heterozygotes with HTG were older than those without HTG, however, there was no age threshold for symptoms of heterozygous individuals with very severe HTG presenting already at the age of 2. For example, the variant *LPL* p.Gly215Glu was reported in a young child (2 years old) with severe HTG, whereas older children with the same variant were asymptomatic (Table 1) and rises a question about the influence of environmental exposure. This is in contrast to Perera et al. [13], where only one individual out of 15 had an HTG below the age of 40. There was no correlation between the type of variant (LoF or missense) and disease severity/presence of HTG in the laboratory testing. This is in opposition to some studies that suggested a more severe phenotype in the LoF variants than in missense in the *LPL* gene [13,26,27].Two patients with a very severe HTG presented with a LoF variants. In one case we

report a duplication of exon 6. Heterozygous deletions in the *LPL* gene have been already associated with a severe, but not very severe HTG (28, 29). In a few patients in the literature duplications disrupting exon 6 have been associated with a very severe TG, but without any symptoms related to it, such as pancreatitis, however no genotype-phenotype correlation could be established (29).

We confirm that the heterozygous *LPL* carriers may present with the full range of phenotypes. This is in accordance with the recent hypothesis not to classify the disease as dominant and recessive but rather as a heterozygosity spectrum. Additionally, we show that the *LPL* heterozygotes may exhibit a very severe HTG in laboratory testing and show clinical manifestations and dominant variant inheritance patterns. The inheritance pattern of the *LPL* gene is described either as recessive or codominant, meaning that two pathogenic variants determine a more severe phenotype while heterozygotes for the pathogenic variants in the *LPL* gene have an intermediate phenotype. We argue that *LPL* could also follow a dominant inheritance pattern with affected parents passing the variant related to the phenotype to their offspring. . Not to forget is the fact that the development of the phenotype is certainly influenced by factors such as age and sex and comorbidities (Supplementary Table 1).

For the first time, we report the frequency of the heterozygous pathogenic/likely pathogenic *LPL* variants in the Polish population accounting for 1:1000 individuals. An important fact is that the majority of samples for the WES examinations derive from the MEDGEN laboratory with the indication for intellectual disability, autism, dysmorphia, and neurodegenerative diseases. Because mainly these indications are the focus of referring physicians, they often forget to enter a symptom such as 'hypertriglyceridemia', considering it less clinically relevant. It is therefore possible that there are indeed more of these symptomatic *LPL* heterozygotes as this group is not fully characterised clinically. It is important to remember that because of the indications to testing, including HTG and hypercholesterolaemia, that there is an overrepresentation of HTG in our cohort so it is not representative for the whole Polish population. In the future, it would be advisable to study a random population group.

Our study has several limitations. Some of them are related to the technical limitations of the methodology. Whole exome sequencing can detect only coding sequences, which leaves a

possibility that there is a structural or a splice site variant that has been omitted, especially in severely affected heterozygous individuals. Another technical limitation is that the deletion of exon 6 has not been confirmed by the second molecular method. Secondly, we have only limited phenotypic data. We do not have exact lipids levels for individuals without pathogenic *LPL* variants identified, what may be source of bias. Also, we do not have full data on the environmental and secondary factors, influencing LPL levels. Especially information on the BMI is missing in several cases that can be source of bias. We do not have a longitudinal follow up so we cannot reliably describe the age of onset of the HTG and age when HTG develops, this means we cannot exclude that the asymptomatic heterozygotes will become symptomatic at the later point of life Additionally, we did not investigate a polygenic risk score in the heterozygotes. Polygenic accumulation of genetic risk is associated with a mild [30], but also severe HTG disease form [31]. Common variants account for most of the mild HTG cases [32], but also for around 46% of the severe disease form [30].

Conclusions

We argue that although the individuals carrying the single LPL pathogenic/likely pathogenic variant display the whole disease spectrum, the severe phenotype of heterozygotes dominant LPL-related HTG may also exists. There was no clear association between the type of variant and the onset of the disease. Phenotypic and demographic characterization of further heterozygotes should help to better understand the background of the symptomatic spectrum.
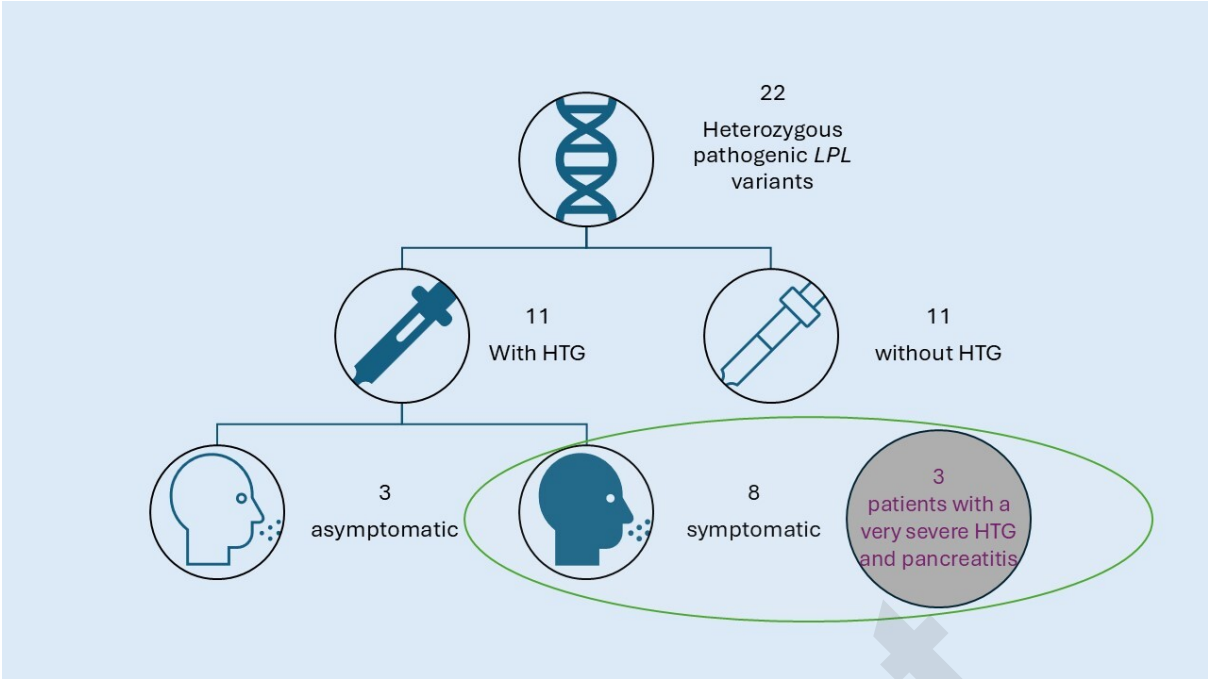
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22
Heterozygous
pathogenic *LPL*
variants

11
With HTG

11
without HTG

3
asymptomatic

8
symptomatic

3
patients with a
very severe HTG
and pancreatitis