Molecular Genetics and Metabolism Reports 40 (2024) 101100

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Germline variant analysis from a cohort of patients with severe hypertriglyceridemia in Brazil

Camila Mendes, Thereza Loureiro *, Darine Villela, Marcelo Imbroinise Bittencourt, Joselito Sobreira, Diana Bermeo, Mireille Gomes, Dayse Alencar, Luciana Santos Serrao de Castro, Rodrigo Ambrosio Fock, Maria Luisa Tinoco, Henrique Galvão, Cristovam Scapulatempo-Neto, Katia Schiavetti, Andreza A. Senerchia, Maria Helane Costa Gurgel

Diagnósticos da América S.A., DASA, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Severe hypertriglyceridemia
Triglycerides
Dyslipidemia
Genetic testing
Germline variant analysis
Diagnostic yield
Brazilian population

ABSTRACT

Hypertriglyceridemia (HTG) is a common dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. It is well stablished that the severe cases of disease often present with an underlying genetic cause. In this study, we determined the frequency and variation spectrum of genes involved in the triglyceride metabolism in a series of Brazilian patients with severe HTG. A total of 212 patients with very high HTG, defined with fasting triglycerides (TG) ≥ 880 mg/ dL, that underwent a multi-gene panel testing were included in this research. Germline deleterious variants (i.e. Pathogenic/Likely Pathogenic (P/LP) variants) were identified in 28 out of 212 patients, reflecting an overall diagnostic yield of 13% in our cohort. Variants of unknown significance (VUS) were identified in 87 patients, and represent 80% of detected variants in this dataset. We confirm the *LPL* as the most frequently mutated gene in patients with severe HTG, and we had only one suspected case of familial chylomicronemia syndrome, caused by a homozygous variant in *LMF1,* in our cohort. Notably, we report 16 distinct and novel variants (P/LP and VUS), each of them representing a single case, not previously reported in any public databases or other studies. Our data expand our knowledge of genetic variation spectrum in patients with severe HTG in the Brazilian population, often underrepresented in public genomic databases, being also a valuable clinical resource for genetic counseling and healthcare programs in the country.

1. Introduction

Hypertriglyceridemia (HTG) is the most common form of dyslipidemia that result in increased plasma triglyceride (TG) levels, being a complex phenotypic trait with a significant genetic component [1–3]. It is expected that the disease affects nearly 10% of the adult population [4]; chronically elevated TG levels are frequently associated with several metabolic alterations including obesity, diabetes, atherosclerotic cardiovascular disease, and severe cases often lead to chylomicronemia, which is an important risk factor for acute pancreatitis. In particular, severe HTG, defined as fasting TG ≥ 880 mg/ dL [5], has been linked to multiple variants in genes involved in triglyceride metabolism; such variants comprise rare, heterozygous, germline deleterious variants (i.e. Pathogenic/Likely Pathogenic (P/LP) variants) with a large effect size, and may contribute to clustering of certain HTG phenotypes in families [6,7]. It is relevant to mention though that very rare variants, specifically in five genes (*LPL*, *LMF1*, *GPIHBP1*, *APOC2*, *APOA5*), were identified as the basis of familial chylomicronemia syndrome, in which homozygous, compound heterozygous, or double heterozygous loss-of-function variants of these genes, involved in the lipoprotein lipase (LPL) pathway, result in a monogenic disease with an autosomal recessive pattern of inheritance [6,7]. Nonetheless, it is assumed that in most cases of HTG, the genetic basis is highly polygenic, driven by the cumulative effect of several common and rare variants with modest to small effect sizes that could be quantified using a polygenic risk score [7]. Current genetic testing generally screens for variants by analyzing a multi-gene panel through next generation sequencing (NGS) technology and may aid in diagnosis of patients with dyslipidemias [8,9].

* Corresponding author at: Diagnósticos da América S.A., DASA, Av. Divino Salvador, 876 – Terceiro Andar, 04078-013 Indianópolis, SP, Brazil.
E-mail address: darinevillela@gmail.com (T. Loureiro).

https://doi.org/10.1016/j.ymgmr.2024.101100
Received 30 December 2023; Received in revised form 26 May 2024; Accepted 28 May 2024

Despite recent advances in genomic medicine that have increased our knowledge about the genetic causes of dyslipidemia, the clinical utility of genetic testing to identify deleterious variants as a risk factor in predicting acute pancreatitis and severe HTG remains limited. In accordance with the National Lipid Association statement, molecular investigation is considered primarily for evaluating suspected cases of familial chylomicronemia syndrome and cascade screening in first-degree relatives of patients with the monogenic form of the disease [10]. However, considering the rare heterozygous deleterious variants that are frequently associated with the vast majority of severe cases of HTG, their clinical interpretation can pose a significant challenge. Whereas some variants can be confidently predicted to be pathogenic because they affect the structure and function of the gene, a great number of detected variants that result in missense (amino acid substitutions) with unknown functional consequences. In addition, the frequency and spectrum of the disease-causing variants vary among populations and ethnic groups. Although some studies were performed in different populations to estimate the prevalence of rare variants related to HTG, in Brazil the landscape of genetic variation in genes involved in the triglyceride metabolism remains to a great extent unrecognized. Thus, in this study we aimed to determine the frequency and spectrum of rare variants detected in Brazilian patients with severe HTG during routine clinical diagnostics, which could significantly aid in their diagnostic process. This is the first research in Brazil and provides a comprehensive analysis of germline variants identified using a multi-gene panel testing and it is valuable resource for genetic counseling and healthcare programs in the country.

2. Patients and methods

2.1. Study design

This is a retrospective observational study that compiled clinical and molecular data from patients investigated routinely in a private laboratory from Brazil (Diagnósticos da América S.A., DASA), between June 2021 and May 2022. A total of 212 patients aged 18 years or older, with very high hypertriglyceridemia (HTG), defined with fasting triglycerides (TG) $\geq$ 880 mg/ dL, as proposed by the European Atherosclerosis Society [5], were included in our analysis. Patients with severe HTG were defined as having at least 3 triglycerides (TG) measures, with two dosages above 1000 mg/ dL, and none below 170 mg/ dL. The clinical features of all patients included in this research, comprising sex, age at sample collection and levels of TG are shown in Table 1.

2.2. Genetic testing and variant analysis

Genomic DNA samples were extracted from peripheral blood cells following standard procedures. All patients were subjected to a comprehensive chylomicronemia multi-gene panel that include the following genes: *ABCA1, AGPAT2, AKT2, APOA5, APOC2, BSCL2, CAV1, CFTR, CIDEC, CTRC, CYP27A1, GPIHBP1, LIPA, LIPE, LMF1, LMNA, LMNB2, LPL, PLIN1, POLD1, PPARG, PRSS1, PSMB8, SMPD1, SPINK1, and ZMPSTE24*. The genetic testing was conducted using next generation sequencing (NGS)-based capture method for detection of single nucleotide variants (SNVs), small deletions (InDels), and copy number variants (CNVs). DNA enrichment was achieved using the xGen Exome Research Panel v2 and xGen CNV Backbone Panel kits (IDT DNA, USA) for target region capture, which encompasses the entire coding sequences and splicing sties of the genes. Sequencing was performed either on Illumina NextSeq or NovaSeq system (Illumina, USA); the targeted mean vertical coverage was 100×, horizontal coverage $\geq$97.5% at 10× and $\geq$ 95% at 20×. Sanger sequencing (3500 Series Genetic Analyzers, Applied Biosystems) was used to validate variants suspected of false positives, i.e., variants with a vertical coverage <20× or when an allelic imbalance was observed.

Bioinformatic analysis was carried out using the Dragen Enrichment and Emedgene platforms from Illumina, USA. Variant call format files were used for annotation and filtering of genetic variants. Visual verification of the findings was made using data from the Binary Alignment Map (BAM) files with Integrative Genomics Viewer. The ClinVar database (www.ncbi.nlm.nih.gov/clinvar/) was used to determine the biological significance of all reported variants. Detected variants were classified according to the *American College of Medical Genetics and Genomics* (ACMG) guidelines as Pathogenic (P), Likely Pathogenic (LP), Benign (B), Likely Benign (LB), or Variants of Unknown Significance (VUS) [11]. The common variants (B/LB), i.e., those frequently reported in public genomic databases were disregarded from this study. Importantly, for supporting evidence of pathogenicity of novel variants and VUS, we used the Alamut™ Visual Plus and SOPHiADDM™ software (SOPHiA GENETICS, Switzerland) that assesses both the probability of protein sequence damage, and de novo creation of splice sites, based on NNSplice and MaxEnt algorithms. Further, to estimate the impact of novel variants and VUS on protein structure we also specify three evidence categories (population frequency data, variant type and location, and case-level data) as recommended by Harrison 2019; lastly, to compare the frequency of detected variants with those in the general population, we consulted the gnomAD (https://gnomad.broadinstitute.org/) and AbraOM (https://abraom.ib.usp.br/) public databases.

Table 1
Clinical characteristcs of the patients with severe hypertriglyceridemia investigated in this study.

Characteristics	N (%)
Number of patients	212
Sex	
Female	44 (21%)
Male	168 (79%)
Patient age at sample collection, yrs. (Median, range)	46 (26–79)
Triglycerides, mg/dL (Median, range)	1806,5 (1005,0-12,421,0)

3. Results

We performed genetic testing in 212 patients with severe hypertriglyceridemia over a period of 1 year. The largest proportion of patients investigated were males (79%), and the median age during sample collection was 46 years old (age range: 26 to 79 years old). Moreover, the median level of triglycerides was 1806,5 mg/dL, ranging from 1005,0 to 12,421,0 mg/dL (Table 1). Out of the 212 patients, 97 had a negative result, and the remaining 115 individuals were either positive for a clinically relevant variant (P/LP) or VUS in one of the 26 genes that comprise the multi-gene panel testing. Taking into account only the P/LP variants, the overall diagnostic yield in our cohort was 13% (Fig. 1A), with the corresponding frequency of P/LP variants and VUS being 20% and 80%, respectively (Fig. 1B); particulary, 17% of the investigated patients had at least two detected variants in the targeted genes (Supplementary Table 1 and 2). Among the 156 detected variants, 36 (23,2%) were present on *LPL*, which was the most frequently mutated gene in our cohort. Fig. 1C shows the frequency distribution of each gene according to the number of detected variants.

As expected, different types of variants were identified in our analysis, with missense variants being the most common among the P/LP variants and VUS (Fig. 2A and B). Focusing on the P/LP variants, the majority are exonic: 32% are missense variants, 13% are stop codons, 10% are frameshift mutations. Additionally, 3% are predicted to either disrupt splicing or be deletions (intronic or in-frame). The detailed listing and spectrum of all variants detected in our cohort is presented in Table 2 and 3. Importantly, the most frequent P/LP variants, found in three or more unrelated individuals, were *LPL* c.701C > T and *CFTR* c.1210-11 T > G. Five distict and novel presumably disease-causing variants were detected, each of them representing a single case. Those novel P/LP variants are highlighted in bold in Table 2. It is relevant to mention that we detected only one case of familial chylomicronemia

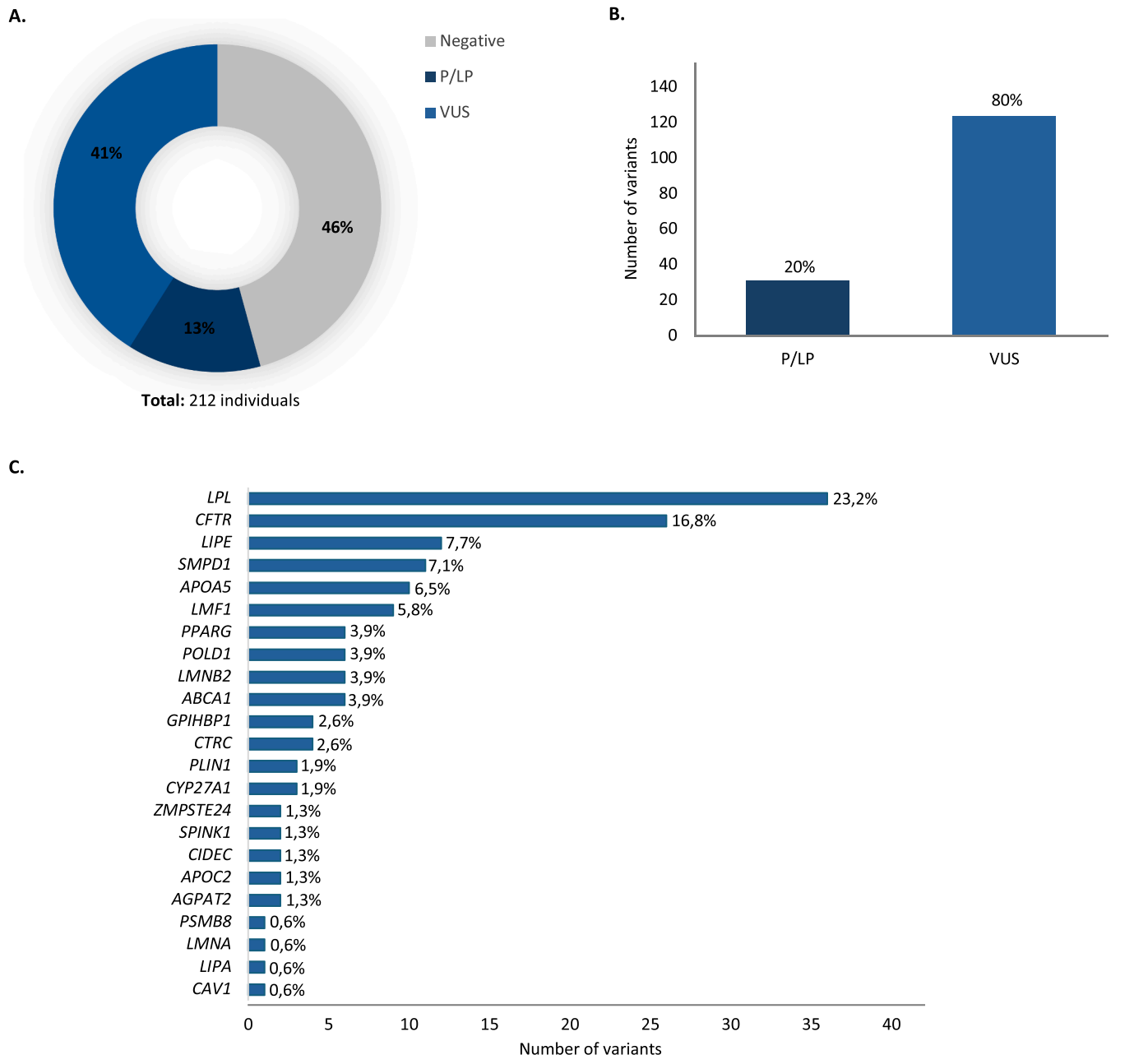

Fig. 1. An overview of the frequency of germline variants detected in patients with severe hypertriglyceridemia. (A) Diagnostic yield in our cohort, showing the frequency of negative results, Pathogenic/Likley Pathogenic (P/LP) variants, and Variants of Unknown Signficance (VUS) (46%, 13%, and 41%, respectively). (B) Frequency of P/LP variants and VUS (20% and 80%, respectively). (C) Frequency distribution of each gene that comprise the multi-gene panel testig, according to the number of detected variants.

syndrome in our cohort, due to a homozygous variant in the *LMF1* gene (c.895C > T). Regarding VUS, eight frequent variants were observed among *LPL*, *CFTR*, *LIPE*, *GPIHBP1*, and *CTRC*, in which were found in three or more individuals in our cohort; further, 11 additional novel variants were also detected, highlighted in bold in Table 3.

4. Discussion

In the present study, we determined the spectrum of frequency and variation in genes involved in the triglyceride metabolism in a series of patients with severe HTG following a diagnostic routine in Brazil. This is the first study in the country using a multi-gene panel testing aiming to screen for disease-causative variants in selected patients. The overall diagnostic yield in our cohort was 13%, with 28/212 patients carrying a P/LP variant and 87/212 carrying a VUS. Among them, 17% (36/212) had at least two detected variants in the targeted genes, including both P/LP variants and VUS. For most cases of HTG, it is assumed that the genetic basis is highly polygenic, resulting from the cumulative effect of several common and rare variants with modest to small effect sizes that can be quantified using a polygenic risk score. Even though our analysis did not allow for a genome-wide association study, the fact that many patients in our cohort present more than one variant in different genes supports a polygenic inheritance, as previously demonstrated in the literature [12–14]. In particular, out of 27 genes included in the multi-gene panel tested, 23 genes had at least one variant detected. Our data confirm previous reports that *LPL* is the most frequently mutated gene in patients with severe HTG [15–17], accounting for 23,2% (36/156) of all detected variants in this dataset. Nonetheless, it is worth mentioning

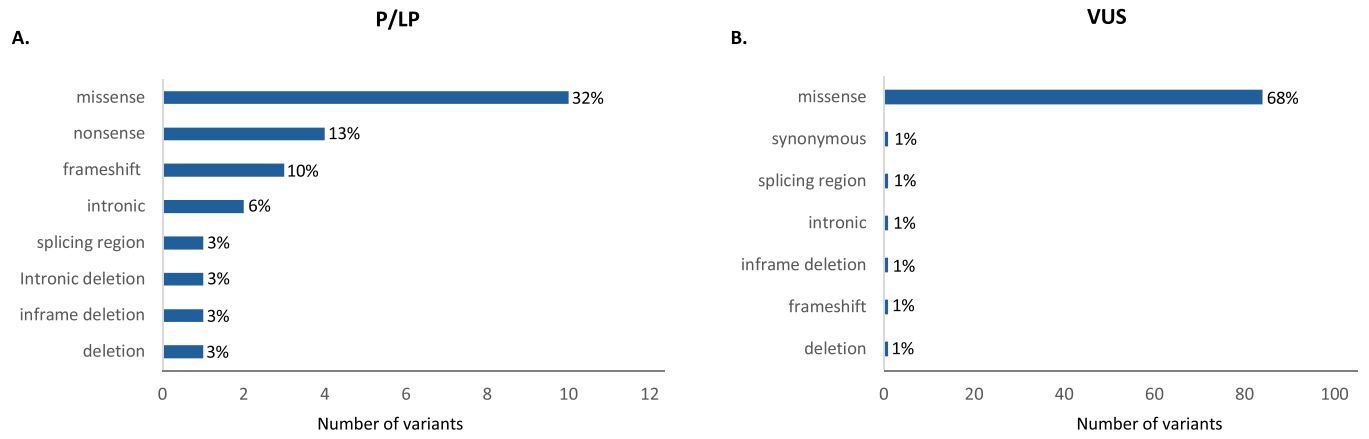

Fig. 2. Characteristic of the germline variants detected in patients with severe hypertriglyceridemia. Distribution of the different types of variants among Pathogenic/Likely Pathogenic (P/LP) variants and Variants of Unknown Signficance (VUS), displayed in the descending order of frequency.

Table 2
Spectrum of all Pathogenic/Likely Pathogenic (P/LP) germline variants detected in patients with severe hypertriglyceridemia.

ID	Gene	HGVS Nomenclature	Protein effect	Zygosity	Type of variation	Variant classification	dbSNP	Frequency in this cohort	Frequency in gnomAD
1	*LPL*	NM_000237.3:c.701C > T	p.(Pro234Leu)	HET	missense	P	rs118204060	0,0189	0,0000424
2	*LPL*	NM_000237.3:c.809G > A	p.(Arg270His)	HET	missense	P	rs118204062	0,0094	0,0000040
3	*LPL*	NM_000237.3:c.337 T > C	p.Trp113Arg	HET	missense	P	rs118204069	0,0047	0,0000319
4	*LPL*	NM_000237.3:c.840del	p.(Asn281Metfs*23)	HET	frameshift	LP	.	.	.
5	*LPL*	NM_000237.3:c.808C > T	p.(Arg270Cys)	HET	missense	P	rs118204077	0,0047	0,0000080
6	*LPL*	NM_000237.3:c.272G > A	p.(Trp91Ter)	HET	nonsense	P	rs118204070	0,0047	0,0000071
7	*LPL*	NM_000237.3:c.644G > A	p.(Gly215Glu)	HET	missense	P	rs118204057	0,0047	0,0001768
8	*LPL*	NM_000237.3:c.755 T > C	p.(Ile252Thr)	HET	missense	P	rs118204080	0,0047	0,0000199
9	*CFTR*	NM_000492.4:c.1210-11 T > G	p.(?)	HET	intronic	P	rs73715573	0,0142	0,0084950
10	*CFTR*	NM_000492.4: c.1520_1522delTCT	p.(Phe508del)	HET	In-frame deletion	P	rs113993960	0,0094	0,0071720
11	*CFTR*	NM_000492.4:c.1558G > A	p.(Val520Ile)	HET	missense	LP	rs77646904	0,0047	0,0001522
12	*CFTR*	NM_000492.4:c.3140-26 A > G	p.(?)	HET	intronic	P	rs76151804	0,0047	0,0000485
13	*CFTR*	NM_000492.4:c.1210-7_1210-6del	p.(?)	HET	Intronic deletion	P	rs1805177	0,0047	0,0246900
14	*CFTR*	NM_000492.4:c.3484C > T	p.(Arg1162Ter)	HET	nonsense	P	rs74767530	0,0047	0,0000567
15	*CFTR*	NM_000492.4:c.14C > T	p.(Pro5Leu)	HET	missense	P	rs193922501	0,0047	0,0000248
16	*SMPD1*	NM_000543.5:c.1451C > A	p.(Ala484Glu)	HET	missense	LP	rs267607075	0,0047	0,0000040
17	*SMPD1*	NM_000543.5:c.319-1G > C	p.(?)	HET	splicing region	LP	.	.	.
18	*SMPD1*	NM_000543.5: c.1829_1831del	p.(Arg610del)	HET	deletion	P	rs120074118	0,0047	0,0002181
19	*APOA5*	NM_052968.5:c.379C > T	p.(Gln127Ter)	HET	nonsense	P	.	.	.
20	*APOA5*	NM_052968.5:c.124del	p.(Glu42Serfs*15)	HET	frameshift	LP	.	.	.
21	*APOA5*	NM_052968.5: c.990_993del	p.(Asp332Valfs*5)	HET	frameshift	P	rs774150500	0,0094	0,0000080
22	*LMF1*	NM_022773.4:c.895C > T	p.(Gln299Ter)	HOM	nonsense	P	rs554054538	0,0047	0,0000362
23	*PPARG*	NM_015869.5:c.634C > T	p.(Arg212Trp)	HET	missense	LP	.	.	.

HET: heterozygosity; HOM: homozygosity; P: pathogenic; LP: likely pathogenic. Highlighted in bold are the novel variants, not reported in any public genomic database. Calculated frequency in this cohort, considering the number of individuals with the detected variant in relation to the total number of individuals: 4/212 = 0,0189; 2/212 = 0,0094; 1/212 = 0,0047.

that for the Brazilian population it will be necessary broad genomic associations studies to evaluate polygenic risk scores for HTG, but the present data will certainly be a valuable resource in future research.

In our cohort, the most frequent P/LP variant was the c.701C > T (p. Pro234Leu) in the *LPL* gene, with four unrelated patients carrying it. This is a well-known deleterious variant with multiple submissions in the ClinVar database, also seen in different populations and ethnic groups. Interestingly, the second most frequent P/LP variant was the c.1210-11 T > G (p.?) in the *CFTR* gene. Most publications associate this variant with cystic fibrosis, yet there also have been multiple associations with a high risk of pancreatitis [18–21]. Whereas severe HTG has historically been linked to deleterious variants, in our analysis, missense represents the most common type of heterozygous variants among the patients. It is worth mentioning that we had only one suspected case of familial chylomicronemia syndrome caused by a homozygous nonsense variant, c.895C > T (p. Gln299Ter), in the *LMF1*. The latter is a recognized gene in which mutations are associated with lipoprotein lipase deficiency. In its classical form, familial chylomicronemia syndrome results from monogenic homozygous or compound-heterozygous variants mainly in five genes (*LPL*, *LMF1*, *GPIHBP1*, *APOC2*, *APOA5*), or its various modulator cofactors. Studies and case reports related to familial chylomicronemia syndrome and its genetic predisposition within the Brazilian population are scarce, with more information available in regions where there is a founder effect [22,23]. Regarding our patient,

Table 3
Spectrum of all germline Variants of Unknown Significance (VUS) detected in patients with severe hypertriglyceridemia.

ID	Gene	HGVS Nomenclature	Protein effect	Zygosity	Type of variantion	Variant classification	dbSNP	Frequency in this cohort	Frequency in gnomAD or AbraoM
1	*LPL*	NM_000237.3:c.953 A > G	p.(Asn318Ser)	HET	missense	VUS	rs268	0,0519	0,01297
2	*LPL*	NM_000237.3:c.373G > A	p.(Ala125Thr)	HET	missense	VUS	rs199675233	0,0236	0,00004375
3	*LPL*	NM_000237.3:c.734 T > C	p.(Ile245Thr)	HET	missense	VUS	rs114792062	0,0094	0,00001193
4	*LPL*	NM_000237.3:c.998G > A	p.(Arg333His)	HET	missense	VUS	rs144466625	0,0094	0,00007075
5	*LPL*	NM_000237.3:c.134C > A	p.(Thr45Asn)	HET	missense	VUS	rs143944126	0,0047	0,00007555
6	*LPL*	NM_000237.3:c.1420 T > C	p.(Ser474Pro)	HET	missense	VUS	rs759923339	0,0047	0,00000398
7	*LPL*	NM_000237.3:c.249G > A	p.(Thr83=)	HET	synonymous	VUS	rs544872445	0,0047	0,00009956
8	*LPL*	NM_000237.3:c.858 T > A	p.(Ser286Arg)	HET	missense	VUS	.	.	0
9	*CFTR*	NM_000492.4: c.1727G > C	p.(Gly576Ala)	HET	missense	VUS	rs1800098	0,0142	0,005042
10	*CFTR*	NM_000492.4: c.2002C > T	p.(Arg668Cys)	HET	missense	VUS	rs1800100	0,0142	0,005979
11	*CFTR*	NM_000492.4: c.3468G > T	p.(Leu1156Phe)	HET	missense	VUS	rs139729994	0,0047	0,0001316
12	*CFTR*	NM_000492.4: c.2991G > C	p.(Leu997Phe)	HET	missense	VUS	rs1800111	0,0047	0,002222
13	*CFTR*	NM_000492.4: c.2057C > A	p.(Ser686Tyr)	HET	missense	VUS	rs201444561	0,0047	0,00006779
14	*CFTR*	NM_000492.4:c.958 T > G	p.(Leu320Val)	HET	missense	VUS	rs144476686	0,0047	0,0005766
15	*CFTR*	NM_000492.4:c.1043 T > A	p.(Met348Lys)	HET	missense	VUS	rs142920240	0,0047	0,0001274
16	*CFTR*	NM_000492.4: c.1684G > A	p.(Val562Ile)	HET	missense	VUS	rs1800097	0,0047	0,000142
17	*CFTR*	NM_000492.4: c.2252G > A	p.(Arg751His)	HET	missense	VUS	rs397508357	0,0047	0,00001203
18	*CFTR*	NM_000492.4: c.4048C > T	p.(His1350Tyr)	HET	missense	VUS	rs955306189	0,0047	0,00001315
19	*CFTR*	NM_000492.4:c.1244 A > C	p.(Asn415Thr)	HET	missense	VUS	.	.	0
20	*CFTR*	NM_000492.4:c.850 A > G	p.(Met284Val)	HET	missense	VUS	.	.	0
21	*LIPE*	NM_005357.4: c.1540G > A	p.(Gly514Ser)	HET	missense	VUS	rs201302932	0,0189	0,0001027
22	*LIPE*	NM_005357.4: c.3040G > A	p.(Val1014Met)	HET	missense	VUS	rs193061079	0,0142	0,0007731
23	*LIPE*	NM_005357.4: c.2813G > A	p.(Arg938His)	HET	missense	VUS	rs776959885	0,0047	0,00002088
24	*LIPE*	NM_005357.4: c.2888C > G	p.(Pro963Arg)	HET	missense	VUS	rs137885656	0,0047	0,0004805
25	*LIPE*	NM_005357.4: c.3203_3221del	p.(Val1068Glyfs*102)	HET	frameshift	VUS	rs587777699	0,0047	0,0006509
26	*LIPE*	NM_005357.4:c.956 T > C	p.(Ile319Thr)	HET	missense	VUS	rs1419855904	0,0047	0,000004089
27	*LIPE*	NM_005357.4: c.1798C > G	p.(Pro600Ala)	HET	missense	VUS	.	.	0
28	*SMPD1*	NM_000543.5:c.1550 A > T	p.(Glu517Val)	HET	missense	VUS	rs142787001	0,0094	0,002469
29	*SMPD1*	NM_000543.5: c.1022G > C	p.(Arg341Pro)	HET	missense	VUS	rs200242334	0,0047	0,00002785
30	*SMPD1*	NM_000543.5:c.1094 T > C	p.(Ile365Thr)	HET	missense	VUS	rs1387046059	0,0047	0,000008033
31	*SMPD1*	NM_000543.5:c.73G > A	p.(Ala25Thr)	HET	missense	VUS	rs758894722	0,0047	0
32	*SMPD1*	NM_000543.5:c.1471 A > C	p.(Ile491Leu)	HET	missense	VUS	rs1429766647	0,0047	0,000007954
33	*SMPD1*	NM_000543.5: c.1474G > A	p.(Gly492Ser)	HET	missense	VUS	rs144873307	0,0047	0,0009229
34	*SMPD1*	NM_000543.5:c.631 T > C	p.(Trp211Arg)	HET	missense	VUS	.	.	0
35	*APOA5*	NM_052968.5:c.607G > T	p.(Gly203Trp)	HET	missense	VUS	rs778493133	0,0094	0 (gnomAD) AbraoM: 0.0016
36	*APOA5*	NM_052968.5:c.956C > T	p.(Pro319Leu)	HET	missense	VUS	rs781438417	0,0047	0,00001061

(*continued on next page*)

Table 3 (*continued*)

ID	Gene	HGVS Nomenclature	Protein effect	Zygosity	Type of variantion	Variant classification	dbSNP	Frequency in this cohort	Frequency in gnomAD or AbraoM
37	*APOA5*	NM_052968.5:c.551C > G	p.(Thr184Ser)	HET	missense	VUS	rs201229911	0,0047	0,0001128
38	*APOA5*	NM_052968.5:c.106G > A	p.(Gly36Arg)	HET	missense	VUS	rs146323308	0,0047	0
39	*APOA5*	NM_052968.5:c.547C > G	p.(His183Asp)	HET	missense	VUS	rs978043764	0,0047	0
40	*LMF1*	NM_022773.4:c.800C > T	p.(Thr267Met)	HET	missense	VUS	rs754428234	0,0047	0,00008433
41	*LMF1*	NM_022773.4:c.176C > T	p.(Ala59Val)	HET	missense	VUS	rs759181295	0,0047	0,0002938
42	*LMF1*	NM_022773.4:c.796G > A	p.(Glu266Lys)	HET	missense	VUS	rs778529081	0,0047	0,00001665
43	*LMF1*	NM_001352018.2: c.67G > A	p.(Gly23Arg)	HET	missense	VUS	rs569261180	0,0047	0,00007969
44	*LMF1*	NM_022773.4: c.1138G > A	p.(Val380Met)	HET	missense	VUS	rs201734228	0,0047	0,0002259
45	*LMF1*	NM_022773.4:c.145 T > G	p.(Phe49Val)	HET	missense	VUS	rs965954981	0,0047	0,00001314
46	*LMF1*	NM_022773.4:c.1189 T > A	p.(Phe397Ile)	HET	missense	VUS	.	.	0
47	*PPARG*	NM_001354669.2: c.14 T > C	p.(Ile5Thr)	HET	missense	VUS	rs1469268585	0,0047	0
48	*PPARG*	NM_015869.5:c.826G > A	p.(Val276Ile)	HET	missense	VUS	rs147996578	0,0047	0
49	*PPARG*	NM_015869.5:c.635G > A	p.(Arg212Gln)	HET	missense	VUS	rs1553647989	0,0047	0
50	*PPARG*	NM_015869.5:c.449 A > T	p.(His150Leu)	HET	missense	VUS	.	.	0
51	*PPARG*	NM_015869.5: c.1274G > A	p.(Arg425His)	HET	missense	VUS	.	.	0
52	*POLD1*	NM_002691.4: c.2926G > A	p.(Glu976Lys)	HET	missense	VUS	rs750457028	0,0047	0,00006321
53	*POLD1*	NM_002691.4: c.2224G > C	p.(Glu742Gln)	HET	missense	VUS	rs752937018	0,0047	0
54	*POLD1*	NM_002691.4: c.2475C > G	p.(Asp825Glu)	HET	missense	VUS	rs973646375	0,0047	0,000004024
55	*POLD1*	NM_002691.4:c.353C > T	p.(Ser118Phe)	HET	missense	VUS	rs780604625	0,0047	0,00008554
56	*POLD1*	NM_002691.4: c.2023G > A	p.(Ala675Thr)	HET	missense	VUS	rs753870000	0,0047	0
57	*POLD1*	NM_002691.4: c.3236del	p. (Phe1079Serfs*45)	HET	deletion	VUS	.	.	0
58	*LMNB2*	NM_032737.4:c.11C > T	p.(Pro4Leu)	HET	missense	VUS	rs1255813804	0,0047	0,0002907
59	*LMNB2*	NM_032737.4: c.1048C > T	p.(Arg350Trp)	HET	missense	VUS	rs780748266	0,0047	0,00001198
60	*LMNB2*	NM_032737.4:c.1821 + 4G > A	p.(?)	HET	splicing region	VUS	rs779811801	0,0047	0,00002854
61	*LMNB2*	NM_032737.4: c.1432_1434del	p.(Glu478del)	HET	in-frame deletion	VUS	rs775270360	0,0047	0,000004081
62	*LMNB2*	NM_032737.4:c.1264 A > G	p.(Ser422Gly)	HET	missense	VUS	rs751986614	0,0047	0,0000045
63	*LMNB2*	NM_032737.4:c.35 A > G	p.(Gln12Arg)	HET	missense	VUS	.	.	0
64	*ABCA1*	NM_005502.4: c.4031G > A	p.(Arg1344Gln)	HET	missense	VUS	rs1188153505	0,0047	0,000003977
65	*ABCA1*	NM_005502.4: c.4196C > T	p.(Thr1399Met)	HET	missense	VUS	rs199668464	0,0047	0,00001591
66	*ABCA1*	NM_005502.4: c.2419G > A	p.(Asp807Asn)	HET	missense	VUS	rs563665817	0,0047	0,00004596
67	*ABCA1*	NM_005502.4: c.2471C > T	p.(Ser824Leu)	HET	missense	VUS	rs551884479	0,0047	0,00002784
68	*ABCA1*	NM_005502.4: c.4250G > A	p.(Arg1417His)	HET	missense	VUS	rs116034780	0,0047	0,0002192
69	*ABCA1*	NM_005502.4:c.4629 A > T	p.(Gln1543His)	HET	missense	VUS	rs1195526722	0,0047	0
70	*GPIHBP1*	NM_178172.6:c.523G > C	p.(Gly175Arg)	HET	missense	VUS	rs145844329	0,0189	0,0009734
71	*CTRC*	NM_007272.3:c.761G > A	p.(Arg254Gln)	HET	missense	VUS	rs755811899	0,0142	0,00001592
72	*CTRC*	NM_007272.3:c.640-12G > A	p.(?)	HET	intronic	VUS	rs183053579	0,0047	0,0006976
73	*PLIN1*	NM_002666.5:c.97 A > C	p.(Thr33Pro)	HET	missense	VUS	rs962003217	0,0047	0

(*continued on next page*)

Table 3 (*continued*)

ID	Gene	HGVS Nomenclature	Protein effect	Zygosity	Type of variantion	Variant classification	dbSNP	Frequency in this cohort	Frequency in gnomAD or AbraoM
74	*PLIN1*	NM_002666.5:c.358 A > G	p.(Ile120Val)	HET	missense	VUS	rs148785094	0,0047	0,0001289
75	*PLIN1*	NM_002666.5:c.644 A > G	p.(Lys215Arg)	HET	missense	VUS	rs150086924	0,0047	0,0001451
76	*CYP27A1*	NM_000784.4:c.871G > A	p.(Glu291Lys)	HET	missense	VUS	rs190012697	0,0094	0,00006365
77	*CYP27A1*	NM_000784.4:c.1181 T > C	p.(Leu394Pro)	HET	missense	VUS	rs1406298698	0,0047	0,000003979
78	*ZMPSTE24*	NM_005857.5:c.845C > T	p.(Thr282Ile)	HET	missense	VUS	rs1232186729	0,0047	0,000003978
79	*ZMPSTE24*	NM_005857.5:c.395 T > C	p.(Leu132Pro)	HET	missense	VUS	rs762325459	0,0047	0,000003988
80	*SPINK1*	NM_003122.4:c.101 A > G	p.(Asn34Ser)	HET	missense	VUS	rs17107315	0,0094	0,00902800
81	*CIDEC*	NM_022094.3:c.481G > A	p.(Val161Met)	HET	missense	VUS	rs145323356	0,0047	0,0005974
82	*CIDEC*	NM_022094.3:c.628C > G	p.(Gln210Glu)	HET	missense	VUS	.	.	0
83	*APOC2*	NM_000483.5:c.196G > A	p.(Ala66Thr)	HET	missense	VUS	rs770092327	0,0047	0,000016
84	*APOC2*	NM_000483.5:c.8C > T	p.(Thr3Ile)	HET	missense	VUS	rs148343756	0,0047	0,0003783
85	*AGPAT2*	NM_006412.4:c.598G > C	p.(Val200Leu)	HET	missense	VUS	rs17855341	0,0047	0,00000503
86	*AGPAT2*	NM_006412.4:c.359 A > G	p.(Lys120Arg)	HET	missense	VUS	rs114782902	0,0047	0,0004646
87	*PSMB8*	NM_148919.4:c.701 A > G	p.(Tyr234Cys)	HET	missense	VUS	rs55853041	0,0047	0,0006152
88	*LMNA*	NM_170707.4: c.1718C > T	p.(Ser573Leu)	HET	missense	VUS	rs60890628	0,0047	0,00014010
89	*LIPA*	NM_000235.4:c.877 A > G	p.(Met293Val)	HET	missense	VUS	rs764343762	0,0047	0,00000398
90	*CAV1*	NM_001753.5:c.172C > T	p.(His58Tyr)	HET	missense	VUS	rs777929541	0,0047	0,00002388

HET: heterozygosity; VUS: variant of unknown significance. Highlighted in bold are the novel variants, not reported in any public genomic database. Calculated frequency in this cohort, considering the number of individuals with the detected variant in relation to the total number of individuals: 1/212 = 0,0047; 2/212 = 0,0094; 3/212 = 0,0142; 4/212 = 0,0189; 5/212 = 0,0236; 11/212 = 0,0519.

follow-up was not possible and we were unable to perform segregation analysis in the family to determine if there was a compound heterozygous alteration. Notably, we identified five novel P/LP variants that were not found in any public genomic database, each of them representing a single case. These novel variants were detected in the *APOA5*, *LPL*, *PPARG*, and *SMPD1* genes (see Table 2), and represent different types of variants resulting either in frameshift, missense, nonsense or disrupting splicing, each present in an exon expect to affect the transcript.

A significant number of VUS stands out in our analysis and represent 80% (124/156) of all detected variants in this dataset. A total of 90 unique VUS were identified among the investigated patients, of which 11 were novel, not found in any public genomic database (see Table 3). The vast majority of VUS were missense variants (68%), with similar percentages for other variant types. Indeed, missense are the most common type of variants detected in multi-gene panels. To classify these variants according to the ACMG criteria, in the absence of well-established in vivo or in vitro studies, we assigned pathogenicity or benignity scores using in silico predictors. We employed the REVEL (Rare Exome Variant Ensemble Learner) metapredictor, which is an ensemble method for predicting the pathogenicity of missense variants. It integrates various tools such as MutPred, FATHMM, VEST, PolyPhen, SIFT, PROVEAN, MutationAssessor, MutationTaster, LRT, GERP, SiPhy, phyloP, and phastCons [24]. Important to note that the use of multi-gene panel testing considerably increases the likelihood and number of VUS per test, as demonstrated by several studies. These variants of uncertain significance frequently pose significant challenges when determining their clinical relevance without proper genetic counseling. For that reason, it is imperative to conduct both pre- and post-test genetic counseling for patients undergoing genetic testing, underscoring the importance of vigilance in variant reclassification when a VUS is encountered. In the context of clinical care, it is important a long term follow-up of patients with reported VUS considering a potential reclassification. However, it is relevant to mention that the heterogeneity in the variants observed in this study may reflect the diverse ethnic backgrounds of the Brazilian population. Also, our population is often underrepresented in public genomic databases, reflecting the necessity of more investigations and data sharing related to the Brazilian population to ensure its accurate representation. It is expected that most of these detected VUS would be reclassified as benign if more individuals in Brazil would be sequenced and its genomic data available for sharing and comparison across research as well as diagnostic studies.

Limitations of this study include the inability to assess the clinical validity and utility of the genetic testing in selected patients with severe HTG which was beyond the scope of this work. However, studies have demonstrated that molecular investigation can identify patients with P/LP variants at higher risk for pancreatitis, who are likely to benefit from triglyceride-lowering therapies, and not necessarily correspond to the very rare individuals with the monogenic disease (i.e. the familial chylomicronemia syndrome). In addition, studies conducted in other countries involving Latin populations and patients with HTG, noteworthy findings have emerged. An Italian study found that 37.5% of their subjects with chylomicronemia were classified as having familial chylomicronemia due to the presence of biallelic, rare mutations, while 59.4% were identified as heterozygous or homozygous for non-pathogenic variants [25]. In another study, conducted in Spain, with a cohort of 23,310 subjects exhibiting high levels of HTG (aged 18 to 80 years old), 194 subjects were initially selected as suspected cases of

primary HTG (1.04%). Among these, 90 individuals (46.4%) met the inclusion criteria for primary HTG and underwent genetic analysis. The genes evaluated in this study included *LPL, LMF1, APOC2, APOA5, APOE*, and *GPIHBP1*. The analysis revealed that 9 out of the 73 subjects (12.3%) carried 7 disease-causing variants in these genes [26]. In other populations, such as in the study conducted by Wang et al. in European individuals, they examined 110 non-diabetic subjects with severe HTG and observed a rare variant frequency of 10.9% in the *LPL*, *APOA5*, and *APOC2* genes [27]. Moreover, important to acknowledge that applying a multi-gene panel testing strategy in clinical care, it is also possible to detect heterozygous variants in *LPL* and *APOA5,* known to cause familial combined hyperlipidaemia and type V hyperlipoproteimia, respectively [28–30].

In summary, our study determined the frequency and variation spectrum of genes involved in the triglyceride metabolism in a series of Brazilian patients with severe HTG. This is the first research in Brazil aiming to screen for disease-causative variants in selected patients and provides a comprehensive analysis of germline variants identified using a multi-gene panel testing, being also a valuable resource for genetic counseling and healthcare programs in the country. Nearly all genes included in the multi-gene panel presented at least one detected variant, with some patients having at least two detected variants in the targeted genes. Such evidence reveals that an NGS-based approach considering the simultaneous investigation of multiple genes is the ideal alternative to screening for variants considering a disease that it is expected to have a polygenic basis.

Ethics approval

This study was approved by the Ethics Committee from Hospital 9 de Julho (CAAE: 67222623.3.0000.5455).

Funding statement

This research was supported by PTC Therapeutics, Brasil.

CRediT authorship contribution statement

Camila Mendes: Methodology, Validation, Formal analysis, Data curation. **Thereza Loureiro:** Methodology, Validation. **Darine Villela:** Writing – review & editing. **Marcelo Imbroinise Bittencourt:** Methodology. **Joselito Sobreira:** Methodology, Formal analysis, Data curation. **Diana Bermeo:** Methodology. **Mireille Gomes:** Methodology. **Dayse Alencar:** Methodology. **Luciana Santos Serrao de Castro:** Methodology. **Rodrigo Ambrosio Fock:** Methodology. **Maria Luisa Tinoco:** Methodology, Formal analysis, Data curation. **Henrique Galvão:** Methodology. **Cristovam Scapulatempo-Neto:** Methodology. **Katia Schiavetti:** Funding acquisition. **Andreza A. Senerchia:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Maria Helane Costa Gurgel:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

M.H.C.G. reported that she received payment from PTC Therapeutics, Brasil for teaching activities. All other authors report no conflict of interest relevant to this article.

Data availability

All data generated or analyzed during this study are included in this published article [and its supplementary information file].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2024.101100.

References

[1] G. Yuan, K.Z. Al-Shali, R.A. Hegele, Hypertriglyceridemia: its etiology, effects and treatment, Canad. Med. Assoc. J. 176 (2007) 1113–1120, https://doi.org/10.1503/cmaj.060963.
[2] P.W.F. Wilson, S.M. Grundy, The metabolic syndrome practical guide to origins and treatment: Part I, Circulation 108 (2003) 1422–1424, https://doi.org/10.1161/01.CIR.0000089505.34741.E5.
[3] R.A. Hegele, R.L. Pollex, Hypertriglyceridemia: phenomics and genomics, Mol. Cell. Biochem. 326 (2009) 35–43.
[4] A. Ruiz-García, et al., Prevalence of hypertriglyceridemia in adults and related cardiometabolic factors. SIMETAP-HTG study, Clinica e Investigacion en Arteriosclerosis 32 (2020) 242–255.
[5] H.N. Ginsberg, et al., Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies-a consensus statement from the European atherosclerosis society, Eur. Heart J. 42 (2021) 4791–4806.
[6] G.D. Carrasquilla, M.R. Christiansen, T.O. Kilpeläinen, The genetic basis of hypertriglyceridemia, Genet. Genom. (2024), https://doi.org/10.1007/s11883-021-00939-y/Published.
[7] J.S. Dron, R.A. Hegele, Genetics of Hypertriglyceridemia, Front. Endocrinol. 11 (2020), https://doi.org/10.3389/fendo.2020.00455.
[8] A.J. Berberich, R.A. Hegele, The role of genetic testing in dyslipidaemia, Pathology 51 (2019) 184–192, https://doi.org/10.1016/j.pathol.2018.10.014.
[9] M.R. Deshotels, et al., Genetic testing for hypertriglyceridemia in academic lipid clinics: implications for precision medicine - brief report, Arterioscler. Thromb. Vasc. Biol. 42 (2022) 1461–1467.
[10] E.E. Brown, et al., Genetic testing in dyslipidemia: a scientific statement from the National Lipid Association, J. Clin. Lipidol. 14 (2020) 398–413.
[11] D.T. Miller, et al., ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG), Genet. Med. 24 (2022) 1407–1414, https://doi.org/10.1016/j.gim.2022.04.006.
[12] J.S. Dron, R.A. Hegele, Genetics of Hypertriglyceridemia, Front. Endocrinol. (Lausanne) 11 (2020).
[13] S. Ying, et al., Polygenic risk for triglyceride levels in the presence of a high impact rare variant, BMC Med. Genet. 16 (2023) 281.
[14] J.S. Dron, et al., Severe hypertriglyceridemia is primarily polygenic, J. Clin. Lipidol. 13 (2019) 80–88.
[15] S. Gehrisch, Common Mutations of the Lipoprotein Lipase Gene and their Clinical Significance, 1999.
[16] D. Evans, J. Arzer, J. Aberle, F.U. Beil, Rare variants in the lipoprotein lipase (LPL) gene are common in hypertriglyceridemia but rare in Type III hyperlipidemia, Atherosclerosis 214 (2011) 386–390.
[17] C. Rabacchi, et al., Spectrum of mutations of the LPL gene identified in Italy in patients with severe hypertriglyceridemia, Atherosclerosis 241 (2015) 79–86.
[18] P.G. Noone, et al., Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations, Gastroenterology 121 (2001) 1310–1319.
[19] S. Werlin, et al., Genetic and electrophysiological characteristics of recurrent acute pancreatitis, J. Pediatr. Gastroenterol. Nutr. 60 (2015) 675–679.
[20] D.D. Ballard, et al., Evaluating adults with idiopathic pancreatitis for genetic predisposition: higher prevalence of abnormal results with use of complete gene sequencing, Pancreas 44 (2015) 116–121.
[21] B. Steiner, et al., Common CFTR haplotypes and susceptibility to chronic pancreatitis and congenital bilateral absence of the vas deferens, Hum. Mutat. 32 (2011) 912–920.
[22] M.C. de Oliveira Izar, et al., Brazilian position statement for familial Chylomicronemia syndrome – 2023, Arq. Bras. Cardiol. 120 (2023).
[23] J.G. Lima, et al., A novel GPIHBP1 mutation related to familial chylomicronemia syndrome: a series of cases, Atherosclerosis 322 (2021) 31–38.
[24] N.M. Ioannidis, et al., REVEL: an ensemble method for predicting the pathogenicity of rare missense variants, Am. J. Hum. Genet. 99 (2016) 877–885.
[25] L. D'Erasmo, et al., Spectrum of mutations and long-term clinical outcomes in genetic Chylomicronemia syndromes, Arterioscler. Thromb. Vasc. Biol. 39 (2019) 2531–2541.
[26] M. Nejati, M.A. Atlasi, M. Karimian, H. Nikzad, A. Azami Tameh, Lipoprotein lipase gene polymorphisms as risk factors for stroke: a computational and meta-analysis, Iran J Basic Med. Sci. 21 (2018) 701–708.
[27] J. Wang, et al., Resequencing genomic DNA of patients with severe hypertriglyceridemia (MIM 144650), Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2450–2455.
[28] T. Gotoda, et al., Diagnosis and management of type and type hyperlipoproteinemia, J. Atheroscler. Thromb. 19 (2012).
[29] M.C.G.J. Brouwers, M.M.J. Van Greevenbroek, C.D.A. Stehouwer, J. De Graaf, A.F. H. Stalenhoef, The genetics of familial combined hyperlipidaemia, Nat. Rev. Endocrinol. 8 (2012) 352–362, https://doi.org/10.1038/nrendo.2012.15.
[30] E. Taghizadeh, et al., Familial combined hyperlipidemia: An overview of the underlying molecular mechanisms and therapeutic strategies, IUBMB Life 71 (2019) 1221–1229, https://doi.org/10.1002/iub.2073.