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Original article

Exonic variants of *GPIHBP1* gene in Thai subjects with severe hypertriglyceridemia

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Background: Hypertriglyceridemia (HTG) is one of the risk factors for cardiovascular disease and acute pancreatitis. It is associated with genetic variations in various genes involved in triglyceride metabolism. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*) encodes a membrane protein involved in an intravascular triglyceride hydrolysis.
Objective: The purpose of this study was to examine the genetic variants in the exons of *GPIHBP1* gene in Thai subjects with severe HTG.
Methods: All 4 exons of the *GPIHBP1* gene were sequenced in 101 Thai subjects with severe HTG. All subjects had triglyceride levels e" 10 mmol/L or 886 mg/dL. Subjects with normolipidemia (n = 111) were used as controls.
Results: The allele frequency of the common p.Cys14Phe variant (rs11538389) in control group was higher than in severe HTG group (0.523 *vs.* 0.386, $P = 0.11$). Interestingly, 2 rare missense variants were identified in 3 HTG patients. A homozygous p.Ser107Cys (rs587777643) was found in 1 patient and a heterozygous p.Arg16Gln (rs748509621) was found in 2 patients. These two rare variants were not observed in the normolipidemic controls.
Conclusion: Our study demonstrated that p.Ser107Cys and p.Arg16Gln variants were exclusively found in HTG patients. The finding suggested that these 2 variations in *GPIHBP1* gene might be a rare genetic cause of severe HTG among Thai population.

Keywords: *GPIHBP1*, genetics, hypertriglyceridemia, triglyceride, variants.

Hypertriglyceridemia (HTG) has recently emerged as a risk factor for cardiovascular disease and severe HTG is associated with an increased risk of acute pancreatitis. [1, 2] In the circulation, triglycerides are carried in triglyceride-rich lipoproteins, mainly chylomicron and very low-density lipoprotein (VLDL). Triglyceride hydrolysis is a critical step in the turnover of triglycerides in these triglyceride-rich lipoproteins. This process occurs at the vascular surface of endothelial cells and is mediated by lipoprotein lipase (LPL). LPL is not synthesized by endothelial cells but mainly by parenchymal cells, such as adipocytes and myocytes.Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*), a membrane protein of vascular endothelial cells, is crucial for the transport of LPL across endothelial cells and the proper localization of LPL on the luminal surface of the endothelial cells. Once LPL is inside the vascular lumen, *GPIHBP1* acts as a platform for triglyceride hydrolysis. Apolipoprotein (apo) C2 and apo A5 are also necessary for LPL-mediated triglyceride hydrolysis. As a result, defects in any of these key proteins in triglyceride hydrolysis, namely LPL, apo C2, apo A5 or *GPIHBP1*, could lead to HTG due to accumulation of triglyceride-rich lipoproteins in the circulation.

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In severe HTG, excess amount of chylomicron is usually present in the circulation, resulting in chylomicronemia. Two forms of chylomicronemia exist, familial chylommicronemia syndrome (FCS) and multifactorial chylomicronemia syndrome (MCS). While FCS, the monogenic form of chylomicronemia due to the presence of bialleic pathogenic variants in the genes involved in triglyceride hydrolysis, is rare, MCS, an oligogenic or polygenic form of chylomicronemia due to heterozygous variants in the candidate genes along with predisposing environmental factors, is more common and has been associated with an increased risk for cardiovascular disease.[3 - 5] Our previous works in a cohort of Thai subjects with severe HTG have revealed the genetic architecture underlying severe HTG. Approximately one-third of our subjects, most likely a mixture of FCS and MCS, displayed common and rare variants in the *LPL* and/or *APOA5* genes. No pathogenic variant in the *APOC2* or *LMF1* genes were found.[6] Therefore, the objective of the present study was to examine the contribution of the last remaining candidate gene, *GPIHBP1*, in our cohort using a resequencing approach.

Materials and methods

Subjects

One hundred and one subjects with severe HTG, defined as a fasting triglyceride level ≥ 10 mM or 886 mg/dL on at least two occasions, were enrolled as previously described. In addition, 111 subjects with triglyceride level < 1.7 mM or 150 mg/dL served as a control group. Subjects in this study, in both the case and control groups, were mean age and gender matched. There are mean ages of 48.0 ± 11.0 and 54.0 ± 12.0, respectively. and percentages of females of 37.0% and 40.0%, respectively (P = 0.38). This study has been approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB no. 471/52). Written informed consent was obtained from each subject.

Laboratory determinations

*Ethylenediamine tetraacetic acid (*EDTA) plasma and serum samples were collected after 10 - 12 hours of fasting. Lipid levels were measured using enzymatic methods in an automated system by Roche.

Genetic analysis

DNA from blood leukocytes was extracted by phenol-chloroform. Coding regions and intron-exon boundaries of the *GPIHBP1* gene was amplified by PCR and purified by ExoSap-IT (Amersham Biosciences). PCR products were sequenced using an ABI 3730XL DNA Analyzer (Applied Biosystems) at Macrogen (South Korea). The rs number of each variant was checked in the dbSNP154 database (https://www-ncbinlm-nih-gov.gate2.inist.fr/snp/). The allele frequency of *GPIHBP1* variants was evaluated with genome aggregation database (https://gnomad.broadinstitute.org) version 2.1.1 (all-population databases).

Bioinformatic studies

Both the PolyPhen (http://genetics.bwh.harvard.edu/pph2/: version 2.2.2) and Protein Analysis Through Evolutionary Relationships (PANTHER; www.pantherdb.org:version 15 released 2020-02-14) programs were used to determine dysfunction of the variants. The PolyPhen program predicts the impact of non-synonymous single nucleotide polymorphisms (SNPs) according to a position-specific independent counts (PSIC) score difference. The results are classified into three types, “probably damaging”, “possibly damaging” and “benign”. The PANTHER program determines the non-synonymous coding SNP to cause a functional impact on the protein. The PANTHER-PSEP (position-specific evolutionary preservation) calculates the length of time (in millions of years or my) a given amino acid has been preserved by tracking back to its reconstructed direct ancestors. The longer a position has been preserved, the more likely that the change will have a deleterious effect. The thresholds were: “probably damaging” (time > 450 my), “possibly damaging” (450 my > time > 200 my) and probably benign (time < 200 my).

Statistical analysis

Data were presented as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) with posthoc analyses was used to compare data among multiple groups. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the statistical package for the social sciences (SPSS) software program (version 22, Chicago, IL).

Results

Among 101 subjects with severe HTG, 37.0% were female, the mean age was 48 years, and the mean triglyceride level was 1,944 mg/dL as previously described. Approximately one-third of subjects had common and/or rare variants in the *LPL* and *APOA5* genes that could explain the phenotype. In the current study, we further examined the genetic contribution of *GPIHBP1* variants in these subjects. We found three common variants in the *GPIHBP1* gene, two synonymous variants [p.Leu4Leu (rs61747644) and p.Val46Val (rs11538388)] and one known missense variant [p.Cys14Phe (rs11538389)], as shown in Table 1. The allele frequency of the p.Cys14Phe variant was, however, higher in the control group (0.523 *vs.* 0.386 in the severe HTG groups, $P = 0.11$) and both the Polyphen and the PANTHER programs predicted that this variant was benign, suggesting that this variant did not significantly contribute to the severe HTG phenotype.

We also found two rare variants, p.Arg16Gln (rs748509621) and p.Ser107Cys (rs587777643), both in the severe HTG group. Although the heterozygous p.Arg16Gln variant was found in two patients in the severe HTG group and none in the control group, both the PolyPhen and PANTHER programs predicted that this variant was benign. In contrast, the Ser107Cys variant was predicted to be damaging by both programs and our previous study on this patient confirmed that this homozygous was pathogenic and contributed to severe HTG
in this patient.

Collectively, our result suggested that the variant in the *GPIHBP1* gene contributing to severe HTG was relatively rare in Thai subjects (Table 1).

Discussion

In our current study, we examined the variants in the *GPIHBP1* gene in a cohort of 101 Thai subjects with severe HTG. We found that the pathogenic *GPIHBP1* variant was rare as only one subject was definitely confirmed to harbor the pathogenic variant that contributed to the HTG phenotype. In addition, the common p.Cys14Phe variant was rather unlikely to play a significant role in the pathogenesis of severe HTG.

Genetic variations in various genes differ among different races and ethnicities. Our previous studies in Thai subjects with various types of dyslipidemia, representing Asians, clearly illustrate certain similarities and differences from those performed in subjects of European descents. For example, in subjects with very high levels of high-density lipoprotein-cholesterol (HDL-C), our studies in the Thai subjects have shown that variants in the *CETP* gene are common. This result is in contrast to that from studies in Caucasians in which the variants in this gene are rare. For severe HTG, our own studies in Thai subjects have shown that rare variants in the *LPL* are more common than those in the *APOC2* and *APOA5* genes, similar to what have been reported from studies in European descents. However, certain common variants are ethnic-specific. Collectively, both rare and common variants in the *LPL* and/or *APOA5* genes were found in approximately one-third of subjects, which could potentially explain the phenotype. In addition, we have previously identified a novel rare variant in the *GPIHBP1* gene in one subject with severe HTG and pancreatitis. In this study, we further extended the investigation and demonstrated that pathogenic variants in the *GPIHBP1* gene contributing to severe HTG in our population were indeed rare. Except for the p.Ser107Cys pathogenic variant identified in one subject , we found only one other heterozygous missense variant, p.Arg16Gln, which was predicted to be benign. Additional works are needed to explore the functionality of p.Arg16Gln variant and its association with HTG.

Studies in patients of European descents showed that variants in the *GPIHBP1* gene are rare causes of severe HTG. A few case reports in Asian subjects with severe HTG as well as recent studies in Asians using a next-generation sequencing approach also confirmed this finding. [7 - 9] For example, no rare variants in the *GPIHBP1* gene were found among 11 Chinese patients with severe HTG and acute pancreatitis. Similarly, Matsunaga A, *et al*.[9] failed to identify rare *GPIHBP1* variants among 23 Japanese patients with severe HTG. A study in 103 Chinese subjects with triglyceride level ≥ 500 mg/dL identified three heterozygous rare *GPIHBP1* variants [7] whereas two heterozygous rare variants were found among 26 Korean patients with triglyceride level ≥ 885 mg/dL.[8] It is of note that the functional effects of these rare variants have not yet been investigated, therefore, it is currently unknown whether HTG in these patients was due to these rare *GPIHBP1* variants.

Table 1. Genetic variants identified in the *GPIHBP1* gene.

Rs number	Exon	Variant name	Polyphen prediction/score	PANTHER prediction/ preservation time (my)	Severe HTG (n = 101)		Controls (n = 111)		Allele frequency (genomAD browser)
					Homo zygous	Heteroz ygous	Homo zygous	Heteroz ygous	
Common variants									
rs61747644	1	p.Leu4Leu	-	-	6	31	4	27	1.8×10^{-1}
rs11538389	1	p.Cys14Phe	benign/0.030	probably benign/91	6	27	8	42	1.2×10^{-1}
rs11538388	2	p.Val46Val	-	-	20	32	ND	ND	4.0×10^{-1}
Rare variants									
rs748509621	1	p.Arg16Gln	benign/0.000	probably benign/91	0	2	0	0	3.6×10^{-5}
rs587777643	4	rp.Ser107Cys	probably damaging/1.000	possibly damaging/324	1	0	0	0	8.0×10^{-6}

ND: no data

A mature *GPIHBP1* protein contains three principal domains: an acidic N-terminal domain (amino acid residues 1 - 62), a Ly6 domain (amino acid residues 63 - 139) and a C-terminal domain (amino acid residues 140 - 184) with the amino acid residues 1 - 20 function as a signal peptide. While the N-terminal and the Ly6 domains play a role in the interaction of GPIHBP1 with LPL, the C-terminal domain is important for transfer of GPIHBP1 to the cell surface.

In our study, we found one nonsynonymous common variant, p.Cys14Phe, in both the severe HTG and the control groups. This cysteine-14 residue resides in the signal peptide region of the GPIHBP1 protein. Although amino acid substitution in the signal peptide might reduce protein translocation efficiency into the endoplasmic reticulum, both the Polyphen and the PANTHER programs predicted that this p.Cys14Phe variant was benign. An earlier report has shown a higher prevalence of p.Cys14Phe allele carriers in severe HTG cohort compared with controls, and *in vitro* experiments demonstrated that the level of this variant protein was mildly reduced, suggesting that the p.Cys14Phe variant might contribute to the severe HTG phenotype. However, our current study showed that the p.Cys14Phe allele frequency was, in fact, higher, albeit non-significantly, in controls than in the HTG groups. Hence this particular variant might not necessarily contribute to severe HTG, at least in our population. To mechanistically understand the pathogenesis of hyperlipidemia in Thai people, a functional study of the identified pathogenic variants should be further investigated.

Conclusion

Our results along with others suggest that in the East and Southeast Asian populations, similar to the populations from North America and Europe, the variants in the *GPIHBP1* gene rarely contribute to the severe HTG phenotype.

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Conflict of interest statement

Each of the authors has completed an ICMJE disclosure form. None of the authors declare any potential or actual relationship, activity, or interest related to the content of this article.

Data sharing statement

The present review is based on the reference cited. Further details, opinions, and interpretation are available from the corresponding authors on reasonable request.

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