Genetic variation in apolipoprotein A-V in hypertriglyceridemia

Shehan D. Perera and Robert A. Hegele

Purpose of review
While biallelic rare *APOA5* pathogenic loss-of-function (LOF) variants cause familial chylomicronemia syndrome, heterozygosity for such variants is associated with highly variable triglyceride phenotypes ranging from normal to severe hypertriglyceridemia, often in the same individual at different time points. Here we provide an updated overview of rare *APOA5* variants in hypertriglyceridemia.

Recent findings
Currently, most variants in *APOA5* that are considered to be pathogenic according to guidelines of the American College of Medical Genetics and Genomics are those resulting in premature termination codons. There are minimal high quality functional data on the impact of most rare *APOA5* missense variants; many are considered as variants of unknown or uncertain significance. Furthermore, particular common polymorphisms of *APOA5*, such as p.Ser19Trp and p.Gly185Cys in Caucasian and Asian populations, respectively, are statistically overrepresented in hypertriglyceridemia cohorts and are sometimes misattributed as being causal for chylomicronemia, when they are merely risk alleles for hypertriglyceridemia.

Summary
Both biallelic and monoallelic LOF variants in *APOA5* are associated with severe hypertriglyceridemia, although the biochemical phenotype in the monoallelic state is highly variable and is often exacerbated by secondary factors. Currently, with few exceptions, the principal definitive mechanism for *APOA5* pathogenicity is through premature truncation. The pathogenic mechanisms of most missense variants in *APOA5* remain unclear and require additional functional experiments or family studies.

Keywords
apolipoprotein, chylomicronemia, complex trait, DNA sequencing, human genetics, hypertriglyceridemia, polygenic trait

INTRODUCTION

Apolipoprotein (apo) A-V is a key regulator of plasma triglyceride (TG) levels [1]. Biallelic loss-of-function (LOF) variants in the *APOA5* gene are a well-documented cause of familial chylomicronemia syndrome (FCS) [2], which is characterized by severely compromised plasma lipolysis resulting in pathogenic elevation predominantly of intestinally-derived chylomicrons, refractory hypertriglyceridemia (HTG), characteristic physical findings, abdominal pain with failure to thrive, and high lifetime risk of pancreatitis. The consequences of heterozygosity – that is, a monoallelic pathogenic variant of *APOA5* – is less well appreciated. We recently found that the phenotype associated with monoallelic variants in *APOA5* is highly variable both within and between patients over time, associated with normal TG, mild-to-moderate and severe HTG phenotypes, with secondary factors playing an important modulatory role [3▪]. This was perhaps counterintuitive to preconceived assumptions that heterozygosity for LOF variants in *APOA5* would be associated with an intermediate HTG phenotype, following an incorrect analogy with familial hypercholesterolemia.

Departments of Biochemistry and Medicine, Schulich School of Medicine and Dentistry, Western University, 1151 Richmond Street North, London, Ontario, Canada

Correspondence to Robert A. Hegele, MD, FRCPC, FACP, Robarts Research Institute, Western University, 4288A-1151 Richmond Street North, London, Ontario N6A 5B7, Canada. Tel: +1 519 931 5271; fax: +1 519 931 5218; e-mail: hegele@robarts.ca

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KEY POINTS

- The primary mechanism of *APOA5* loss-of-function is from premature stop codons leading to truncated protein.
- Both biallelic and monoallelic *APOA5* loss-of-function variants are associated with severe hypertriglyceridemia (HTG), although the phenotype in the heterozygous state is highly variable.
- The clinical consequences of most *APOA5* missense variants is unclear, given minimal available functional evidence.
- Common polymorphisms in *APOA5* are not sufficient in isolation to induce HTG but instead increase the risk of developing HTG in concert with multiple other factors ranging from polygenic predisposition to secondary factors such as age, obesity and diabetes.

Here, we synthesize this new understanding with previous information to provide an up-to-date characterization of *APOA5* variants in HTG.

EXPRESSION AND PHYSIOLOGICAL ROLES OF APOLIPOPROTEIN A5 IN LIPOPROTEIN METABOLISM

Apo A-V is one of the first human proteins identified using a primordial artificial intelligence approach leveraging comparative DNA sequence analysis [4]. It was initially determined to be expressed almost exclusively in the liver and secreted with TG-rich lipoproteins, primarily very-low density lipoprotein (VLDL) [4]. Subsequently, expression of apo A-V in the small intestine was detected [5]. Intestine-derived apo A-V is presumed to circulate in plasma with lipoproteins of intestinal origin, i.e., chylomicrons. Interestingly, apo A-V of hepatic origin is also found in the bile, technically making it an exocrine secretion that may reach the intestinal lumen to possible exert effects there [6]. Despite its very low absolute plasma concentration relative to other apolipoproteins (i.e. $<1\,\mu g/ml$) [7], apo A-V is a potent regulator of plasma TG concentrations [8].

Apo A-V reduces plasma TG concentrations via multiple mechanisms. First, apo A-V plays an intracellular role whereby it interferes with hepatic synthesis of VLDL particles, averting their secretion into the circulation, by associating with cellular membrane components and various lipid species within hepatocytes [9–11]. Second, apo A-V is bound to TG-rich lipoproteins (TGRLs) in plasma [7] and directly enhances the activity of lipoprotein lipase (LPL) to clear TG from circulation, although this also depends on the concurrent presence of apo C-II [7,11,12] and glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) [13–15]. Specifically, this function of apo A-V is most likely due to its ability to bind to and/or interact with heparan sulfate proteoglycans (HSPGs) [13] and GPIHBP1 [14,15] on endothelial cell surfaces, which enhance the association of apo A-V-containing lipoproteins with endothelial cell surface features associated with LPL. GPIHBP1 is a major capillary lumen binding site and anchor for LPL [16] and is also the platform upon which LPL-mediated lipolysis occurs [17,18]. Third, there is some evidence that apo A-V mediates hepatic uptake of TRL remnants through interaction with members of the low-density lipoprotein (LDL) receptor family [19].

Recently, a novel role for apo A-V was described by which it indirectly enhances LPL activity by competing with LPL for binding to a unique inhibitory epitope present in the ANGPTL3/8 complex, thereby suppressing the LPL-inhibitory effects of the complex [20,21▪▪]. Additionally, this function of apo A-V plays a role in regulating selective tissue uptake of circulating TG in the fed versus fasted state [20,21▪▪,22–26]. Specifically, in the fed state, increased insulin signaling induces the expression of ANGPTL8 in liver and adipose tissue while downregulating hepatic apo A-V production and adipose ANGPTL4 expression. The net effect is an increase in circulating ANGPTL3/8 complex uninhibited by apo A-V, which suppresses LPL activity in oxidative tissues such as skeletal muscle. TG hydrolysis in adipose tissue in turn increases because ANGPTL8 expressed in adipose tissue complexes with ANGPTL4, and the ANGPTL4/8 complex has reduced LPL-inhibitory activity compared to ANGPTL4 alone. Additionally, binding of the ANGPTL4/8 complex to adipose tissue LPL blocks interaction with circulating ANGPTL3/8 and ANGPTL4, which results in most circulating TG in the fed state being hydrolyzed in adipose tissue, where adipocytes take up the released fatty acids and store them for energy. The opposite occurs in the fasted state. Finally, recent tantalizing experimental data suggest that the modulating influence of ANGPTL4/8 includes effects that link the coagulation system with lipolysis [27▪], an interaction that requires further exploration. These functions are summarized in Table 1.

Thus, integrating the above multiple effects suggests that apo A-V promotes lipolysis and thus its plasma levels should be inversely related to TG levels. However, it has long been appreciated that there is a somewhat counterintuitive positive correlation between plasma levels of apo A-V and TG [28]. This is the same direction of correlation seen between TG and apo C-III, an inhibitor of LPL activity that counteracts the effect of apo A-V [29]. One possible explanation for this direct correlation is that apo A-V circulates on TGRL and thus within the macro-biochemical context,

Table 1. Known and putative roles of apolipoprotein A-V in regulating plasma triglyceride levels

Location of action	Description
Intracellular, within hepatocytes	Interferes with hepatic synthesis of very low-density lipoprotein (VLDL) particles, averting their secretion into circulation. May also lead to hepatic lipid accumulation
Plasma, bound to circulating triglyceride-rich lipoproteins	Directly enhances the activity of lipoprotein lipase (LPL) to clear triglycerides from circulation by strengthening the association of apo A-V-containing lipoproteins with endothelial cell surface features associated with LPL
	Mediates hepatic uptake of triglyceride-rich lipoprotein remnants via interaction with members of the low-density lipoprotein (LDL) receptor family
	Indirectly enhances LPL activity by competing with LPL for binding to a unique LPL-inhibitory epitope present in the angiopoietin-like protein 3/8 (ANGPTL3/8) complex. This functionality also plays a role in fed versus fasted state selective tissue uptake of circulating triglycerides

is positively correlated with TG levels. However, at the micro-biochemical regional level in close proximity to LPL, such as the endothelial cell or adipocyte, apo A-V enhances lipolysis, eventually resulting in TG reduction, but not at an immediate or sufficiently large scale as to be reflected in total plasma TG concentration, whose decline would be delayed and reactive, adhering to a slower time course.

PROTEIN STRUCTURE OF APO A-V

After cleavage of the 23 amino acid signal peptide, the mature apo A-V protein consists of an ~39 kDa protein composed of 343 amino acids [4,30]. It is secreted as a component of high density lipoprotein (HDL), VLDL and chylomicrons [4,9,30]. Apo A-V has two coiled-coil domains and a large α-helical content with most recent predictions of structure, indicating an α-helical content ~35% in the lipid-free state and increasing to ~45% upon association with lipid, corresponding to elongation and stabilization of α-helix segments [31].

Apo A-V has several main functional domains. First, the N-terminal region spanning residues 1 to 146 of the mature protein is the likely hydrophilic domain of the protein with the α-helices in this region adopting a water-soluble helix bundle configuration [31]. Second, the C-terminal region spanning residues 295–343 of mature apo A-V is highly hydrophobic [10,30,31] and has lipid binding properties [30–33]. Finally, the central intervening region between the terminal regions spanning residues 147–294 contains a string of residues associated with enhancement of LPL activity by interacting with GPIHBP1. Specifically, a positively charged region spanning residues 186–227 is involved in binding to HSPG [13], LDL receptor family members [19] and GPIHBP1 [18]. There is strong evidence that this positively charged region on apo A-V and the acidic domain of GPIHBP1 are both required for interaction [14], which likely facilitates the LPL-enhancing function. Thus, enhancement of efficient LPL-mediated lipolysis of TGRLs requires coordination between GPIHBP1, apo A-V and LPL [30]. Another region of note within the central intervening region of apo A-V is the hydrophobic region spanning residues 161 to 181 preceding the positively charged region; this region has been implicated in enabling apo A-V to bind to the surface of intracellular lipid droplets [34]. This may explain the function of apo A-V to reduce hepatic VLDL secretion, although this may also result in concurrent hepatic lipid accumulation [30].

Currently, the exact region of apo A-V associated with its ability to bind the ANGPTL3/8 complex is unknown [20,21▪▪], although some inferences might be drawn based on properties of the apo A-V-interacting epitope of the ANGPTL3/8 complex. Specifically, it seems that the apo A-V-interacting epitope is a hydrophilic leucine zipper-like motif [21▪▪]. Thus, apo A-V might interact with this motif either through its N-terminal domain, which is hydrophilic [31], or via residues in the intervening region between its terminal domains [32], since the C-terminal region of apo A-V is highly hydrophobic [10,31] and might thus be unlikely to be involved directly in this interaction.

GENOMIC STRUCTURE OF *APOA5*

The gene encoding apo A-V in humans, namely *APOA5*, is located on chromosome 11q23.3 within the apolipoprotein gene cluster that also includes *APOA1*, *APOC3*, and *APOA4* [2,4]. The *APOA5* gene is composed of four exons and three introns spanning roughly 3.05 kb on the reverse strand. As mentioned above, the gene is expressed primarily in the liver and secondarily in the small intestine.

BIALLELIC *APOA5* VARIANTS AND FAMILIAL CHYLOMICRONEMIA SYNDROME

APOA5 is one of the five canonical genes in which presence of biallelic LOF variants underlies an

extremely rare – prevalence of ~1–10 in a million [35] – condition known as familial chylomicronemia syndrome (FCS) [36]. FCS is characterized by sustained, refractory severe HTG due to essentially complete loss of LPL-mediated lipolysis of TGRLs, leading to excessive accumulation of chylomicrons in circulation [37]. Clinically, this manifests as severe HTG that is resistant to treatment, potentially resulting in several other systemic manifestations such as lipemia retinalis, eruptive xanthomatosis, hepatosplenomegaly, abdominal and acute pancreatitis, which can be fatal [1,36].

Loss of LPL-mediated lipolytic activity is caused primarily by biallelic LOF variants (i.e. homozygous or compound heterozygous) in one of the following genes; *LPL* in 70–80% of all cases, with the remaining 20–30% of cases caused by variants in *APOA5*, *APOC2*, *GPIHBP1* or *LMF1* genes [1,2,36]. These genes comprise those encoding lipoprotein lipase (*LPL* gene), and its four essential co-factors, apo A-V (*APOA5* gene), apo C-II (*APOC2* gene), glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (*GPIHBP1* gene), and lipase maturation factor 1 (*LMF1* gene), respectively. Occasional FCS cases are digenic, with affected individuals having single heterozygous variants in two of these genes. In depth discussion of the diagnosis and management of FCS is beyond the scope of this review, and is covered elsewhere [1,37].

PHENOTYPIC IMPACT OF HETEROZYGOUS RARE PATHOGENIC VARIANTS IN *APOA5*

The phenotype associated with biallelic LOF variants in the FCS genes is well understood. However, despite being much more common, the phenotype associated with monoallelic or heterozygous LOF variants in these genes is less well appreciated. We previously showed that ~3–4% of the general population with a normal lipid profile are heterozygous for rare pathogenic variants in one of the five FCS genes [38]. Furthermore, heterozygosity for rare variants in these genes is statistically overrepresented in mild-to-moderate and severe HTG cohorts, at three- and fivefold enrichment respectively [38,39]. However, the heterozygous state is not 100% causal for HTG – it merely predisposes to increased risk of HTG. In addition, we recently showed that heterozygosity for rare LOF variants in both *LPL* and *APOA5* is associated with highly variable TG levels both within and between patients over time, associated at times with normal TG, mild-to-moderate and severe HTG phenotypes [3▪,40▪].

A CURATED ASSEMBLY OF *APOA5* VARIANTS

With respect to definite or likely disease-causing variants of *APOA5*, to the best of our knowledge, there are currently at least 118 unique rare variants reported in the literature and/or databases as being associated or with suggestive evidence of possible associations with phenotypes such as FCS and HTG, but also with atherosclerotic cardiovascular disease (ASCVD). We have summarized the coding sequence variants resulting in amino acid changes alongside the relative positions of the major functional domains of apo A-V in Fig. 1. We have also summarized outlined the nucleic acid changes for noncoding region variants in Fig. 2. Finally, we have curated and annotated some details of these variants in Table 2. Full details and notes related to molecular defect (where applicable) and exact citations are in Table 1, Supplemental Digital Content, http://links.lww.com/COL/A28.

We obtained this list of variants by first compiling *APOA5* variants listed in three databases as disease-causing or associated variants, namely the Human Gene Mutation Database (HGMD) [41], ClinVar [42], and Leiden Open Variation Database 3.0 (LOVD3) [43] and then double-checking their reporting in the literature when possible. We independently assessed the pathogenicity of these variants using our laboratory pipeline as we have previously reported [3▪,40▪,44] using the Franklin by Genoox tool (https://franklin.genoox.com) paired with manual curation, to determine the pathogenicity classifications of these variants according to the American College of Medical Genetics and Genomics (ACMG) guidelines [45]. We also included variants found in our own clinical testing at the Lipid Genetics Clinic, London, Ontario, Canada, if they were considered pathogenic or likely pathogenic under the ACMG guidelines. We note that eight variants reported in ClinVar, namely p.Arg40Trpfs*16, p.Glu52Ter, p.Glu156Ter, p.Val166Argfs*102, p.Arg259Ter, p.Thr266Leufs*31, p.Gln283Ter, and p.Trp348Ter, nine reported in LOVD3, namely p.Gly26Glufs*37, p.Gln46Hisfs*11, p.Glu102del, p.Arg126Gln, p.Gln161Arg, p.Gln161Leu, p.Thr184Asnfs*84, p.Ala219Profs*79, and p.Gln229Ter, and the two novel variants found in our clinic, p.Ala20Profs*37 and p.Asp37Thrfs*20, have been included without any prior literature citations associated with them. At the time of writing, these variants have only been reported as clinical testing results. However, we feel that including these variants is warranted given that most predict premature stop codons (Table 2) in a gene for which LOF due to premature protein truncation is an accepted mechanism of disease [2].

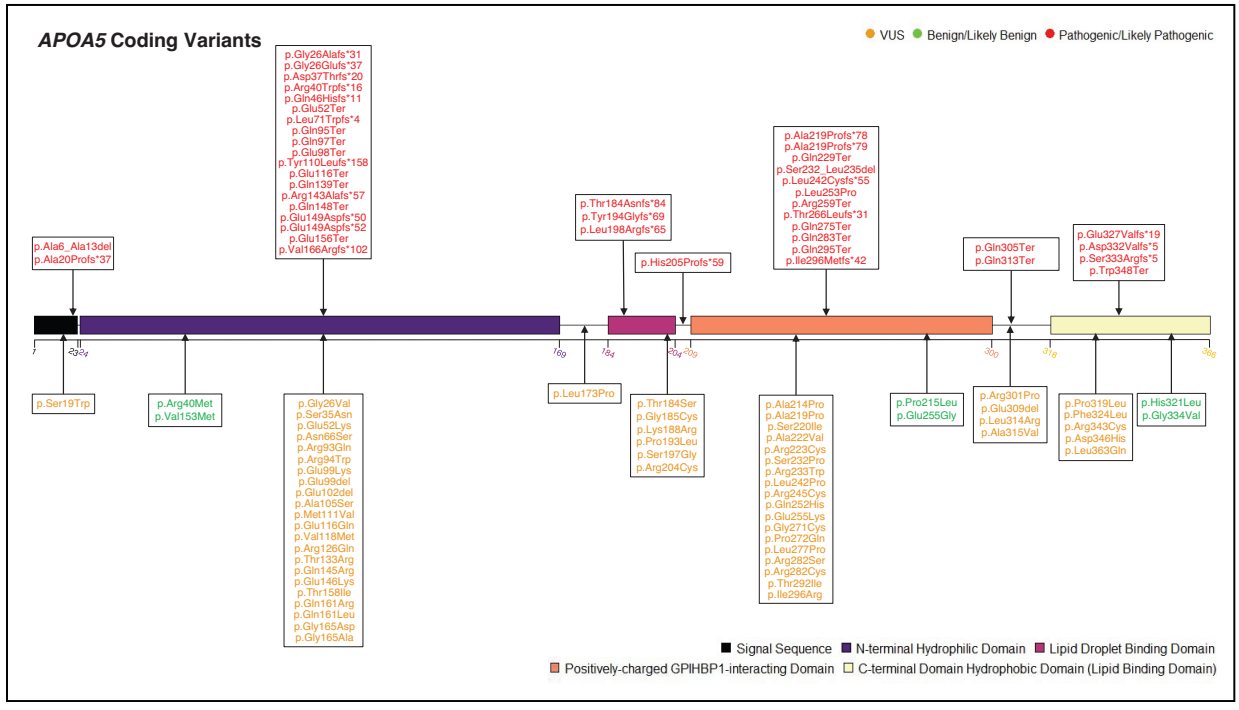

FIGURE 1. Map of reported *APOA5* coding sequence variants. Boxes represent functional domains of the apo A-V peptide: Black represents the signal peptide, purple represents the N-terminal hydrophilic domain, magenta represents the lipid droplet binding domain, orange represents the positively charged GPIHBP1-interacting domain, and the yellow represents the C-terminal hydrophobic domain (lipid binding domain). Axis numbering represents the amino acid residue number in the primary structure of the newly synthesized apo A-V peptide and the specific residues indicated represent the first and last residues of the domains they share color with. All variants are color-coded according to pathogenicity classification according to ACMG guidelines: red indicates pathogenic or likely pathogenic, orange indicates a variant of uncertain significance (VUS), and green indicates benign or likely benign.

Interestingly, our curation indicates that only 46 of the 118 variants in our compiled list are considered pathogenic or likely pathogenic according to the ACMG guidelines. This is because of a lack of functional research demonstrating the pathogenicity of most of these variants. To the best of our knowledge, nonsense-mediated mRNA decay is not a prevalent mechanism by which LOF occurs with regards to *APOA5* variants. It has been previously shown that even severely prematurely truncated *APOA5* variants are synthesized and secreted [46]. Premature truncation leads to disruption and/or elimination of key functional domains and misfolding of the truncated peptide. These deficits in the homozygous and compound heterozygous states lead to severe loss of apo A-V mass and activity in plasma [47], resulting in FCS, as discussed above. Indeed, as mentioned above, premature protein truncation is the primary mechanism by which LOF occurs in *APOA5*.

Currently, 47 of 50 variants classified as pathogenic predict premature protein truncations of varying severities (Figs. 1 and 2). Even small truncations eliminating small portions of the protein are deleterious as the lipid binding properties of apo A-V are enabled by the C-terminal lipid binding domain [30–33]. The remaining three pathogenic LOF variants in *APOA5* are also inferred to have marked functional compromise. These are namely: (i) *APOA5* p.Ala6_Ala13del, which codes for a gross deletion of eight amino acids from the signal sequence resulting in missorting and impaired secretion [48]; (ii) *APOA5* p.Ser232_Leu235del, which codes for a deletion of 4 amino acids from the GPIHBP1-interacting domain [14,18,49]; and (iii) *APOA5* p.Leu253Pro, a missense variant that results in decreased liposome binding and loss of binding to sortilin, which is thought to affect both VLDL secretion and LPL activity [49,50].

However, the picture is less clear regarding the monoallelic LOF condition. As discussed earlier [3▪], previous descriptions of heterozygous carriers of LOF variants in *APOA5* found considerable variability in baseline TG levels, ranging from normal TG [12,51] to mild-to-moderate HTG [12,52] to severe HTG [46,49,52–54]. This is consistent with our recent findings of extensive baseline and longitudinal TG level variability [3▪]. Taken together,

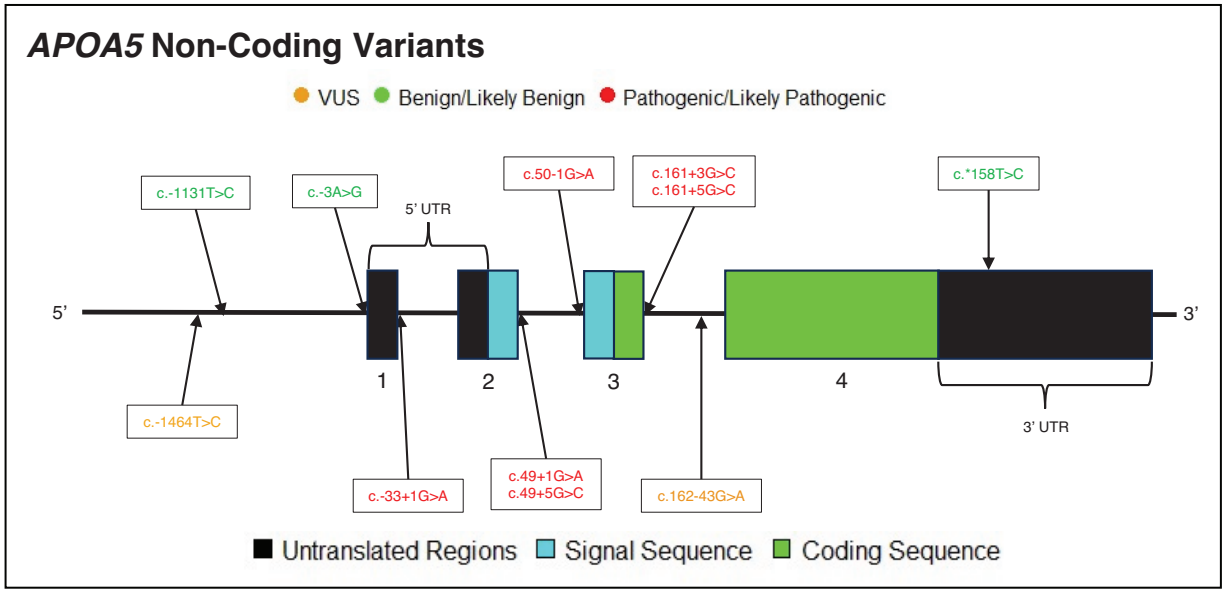

FIGURE 2. Map of reported *APOA5* noncoding variants. Gene map of *APOA5* annotated with variants discovered in the regulatory regions [5′ and 3′ untranslated regions (UTRs), promoter region, etc.], splice donor and acceptor sites, and introns. Numbering underneath boxes indicates exons. Major structural features are color-coded. Black boxes indicate untranslated sequences, blue boxes indicate sequences coding for the apo A-V signal peptide, and green boxes indicate sequences coding for the mature protein. Variants are presented using nucleic acid changes. All variants are color-coded according to pathogenicity classification according to ACMG guidelines: Red indicates pathogenic or likely pathogenic, orange indicates a variant of uncertain significance (VUS), and green indicates benign or likely benign.

alongside findings of young, healthy heterozygous carriers of *APOA5* LOF variants [51], this suggests that secondary factors, such as increased age and body mass index (BMI), are likely needed to force expression of clinical HTG in *APOA5* LOF variant carrying heterozygotes. However, the exact molecular mechanism by which the heterozygous state for a pathogenic variant predisposes to HTG is unclear. In the monoallelic state, the wild-type allele should theoretically provide sufficient compensation capacity to offset the LOF due to the variant allele. This is consistent with the findings of normolipidemic heterozygous carriers of APOA5 LOF variants [51] and suggests that haploinsufficiency, where two healthy alleles are required to express a completely normal phenotype, is not a likely mechanism for HTG related to monoallelic *APOA5* LOF variants. Rather, indirect evidence suggests that *APOA5* variants exert a dominant-negative effect, where the variant protein interferes with the normal functionality of the wild-type protein. Specifically, preliminary evidence suggests that certain truncated apo A-V proteins are expressed and can interfere with the ability of wild-type apo A-V protein to associate with lipoprotein particles during their formation in cells [46]. Given that normolipidemic carriers of *APOA5* LOF variants are prevalent in the population [38,39,51], this dominant-negative effect of truncated apo A-V variants and the nonfunctional variant protein alone are insufficient to induce phenotypic HTG. But perhaps the heterozygous state compromises the homeostatic capacity of the lipolytic cascade such that it is more easily saturated in the presence of secondary TG-elevating risk factors, such as elevated age, increased BMI, polygenic risk accumulation, excessive calorie intake, etc. [55]. A more easily saturated lipolytic capacity would more readily precipitate HTG, and even severe HTG if the secondary stresses were large enough. However, this explanation is just speculative at this time and the actual mechanistic picture underlying this dominant-negative interaction requires additional study.

The majority of reported *APOA5* variants are missense variants that have been previously associated with HTG or related comorbidities [49], although according to the ACMG guidelines, many variants lack sufficient functional evidence to be considered pathogenic. Compared to the obvious molecular lesions caused by premature stop codons, the consequences of most missense variants in *APOA5* are less obvious. Dedicated functional, structural, and/or family studies of the protein produced by missense variants are needed to determine the pathogenicity of these variants and the specific molecular defects they may induce.

Table 2. *APOA5* variants reported in literature and/or clinical testing

Variant type	Nucleotide change	Amino acid change[a,b]	ACMG classification[c]
Regulatory	c.-1464T>C	N/A	VUS
Regulatory	c.-1131T>C	N/A	Benign
Splicing	c.-33+1G>A	N/A	Likely pathogenic
Regulatory	c.-3A>G	N/A	Benign
Gross deletion	c.16_39del	p.Ala6_Ala13del	Likely pathogenic
Splicing	c.49+1G>A	N/A	Likely pathogenic
Splicing	c.49+5G>C	N/A	Likely pathogenic
Splicing	c.50-1G>A	N/A	Likely Pathogenic
Missense	c.56C>G	p.Ser19Trp	VUS
Small deletion	c.58delG	p.Ala20Profs*37	Likely pathogenic
Small duplication	c.73_76dup	p.Gly26Glufs*37	Likely pathogenic
Missense	c.77G>T	p.Gly26Val	VUS
Small deletion	c.77delG	p.Gly26Alafs*31	Likely pathogenic
Missense	c.104G>A	p.Ser35Asn	VUS
Small deletion	c.109delG	p.Asp37Thrfs*20	Likely pathogenic
Small deletion	c.117_120del	p.Arg40Trpfs*16	Likely pathogenic
Missense	c.119G>T	p.Arg40Met	Likely benign
Small deletion	c.138del	p.Gln46Hisfs*11	Likely pathogenic
Missense	c.154G>A	p.Glu52Lys	VUS
Nonsense	c.154G>T	p.Glu52Ter	Likely pathogenic
Splicing	c.161+3G>C	N/A	Likely pathogenic
Splicing	c.161+5G>C	N/A	Likely pathogenic
Splicing	c.162-43A>G	N/A	VUS
Missense	c.197A>G	p.Asn66Ser	VUS
Small deletion	c.211delC	p.Leu71Trpfs*4	Likely pathogenic
Missense	c.278G>A	p.Arg93Gln	VUS
Missense	c.280C>T	p.Arg94Trp	VUS
Nonsense	c.283C>T	p.Gln95Ter	Likely pathogenic
Nonsense	c.289C>T	p.Gln97Ter	Likely pathogenic
Nonsense	c.292G>T	p.Glu98Ter	Likely pathogenic
Missense	c.295G>A	p.Glu99Lys	VUS
Small deletion	c.295_297delGAG	p.Glu99del	VUS
Small deletion	c.305_307del	p.Glu102del	VUS
Missense	c.313G>T	p.Ala105Ser	VUS
Small insertion	c.326_327insC	p.Tyr110Leufs*158	Likely pathogenic
Missense	c.331A>G	p.Met111Val	VUS
Missense	c.346G>C	p.Glu116Gln	VUS
Nonsense	c.346G>T	p.Glu116Ter	Likely pathogenic
Missense	c.352G>A	p.Val118Met	VUS
Missense	c.377G>A	p.Arg126Gln	VUS
Missense	c.398C>G	p.Thr133Arg	VUS
Nonsense	c.415C>T	p.Gln139Ter	Likely pathogenic
Small deletion	c.427delC	p.Arg143Alafs*57	Likely pathogenic
Missense	c.434A>G	p.Gln145Arg	VUS
Missense	c.436G>A	p.Glu146Lys	VUS

Table 2 (*Continued*)

Variant type	Nucleotide change	Amino acid change[a,b]	ACMG classification[c]
Nonsense	c.442C>T	p.Gln148Ter	Likely pathogenic
Small deletion	c.447_450delGCAG	p.Glu149Aspfs∗50	Likely pathogenic
Small insertion-deletion	c.447delGinsCTC	p.Glu149Aspfs∗52	Likely pathogenic
Missense	c.457G>A	p.Val153Met	Benign
Nonsense	c.466G>T	p.Glu156Ter	Likely pathogenic
Missense	c.473C>T	p.Thr158Ile	VUS
Missense	c.482A>G	p.Gln161Arg	VUS
Missense	c.482A>T	p.Gln161Leu	VUS
Missense	c.494G>A	p.Gly165Asp	VUS
Missense	c.494G>C	p.Gly165Ala	VUS
Small duplication	c.494dup	p.Val166Argfs∗102	Likely pathogenic
Missense	c.518T>C	p.Leu173Pro	VUS
Small duplication	c.550dup	p.Thr184Asnfs∗84	Likely pathogenic
Missense	c.551C>G	p.Thr184Ser	VUS
Missense	c.553G>T	p.Gly185Cys	VUS
Missense	c.563A>G	p.Lys188Arg	VUS
Missense	c.578C>T	p.Pro193Leu	VUS
Small deletion	c.579_592del14	p.Tyr194Glyfs∗69	Likely pathogenic
Missense	c.589A>G	p.Ser197Gly	VUS
Small deletion	c.593_606del14	p.Leu198Argfs∗65	Likely pathogenic
Missense	c.610C>T	p.Arg204Cys	VUS
Small deletion	c.614_624del11	p.His205Profs∗59	Likely pathogenic
Missense	c.640G>C	p.Ala214Pro	VUS
Missense	c.644C>T	p.Pro215Leu	Likely benign
Small duplication	c.653_654dup	p.Ala219Profs∗79	Likely pathogenic
Small deletion	c.654delC	p.Ala219Profs∗78	Likely pathogenic
Missense	c.655G>C	p.Ala219Pro	VUS
Missense	c.659G>T	p.Ser220Ile	VUS
Missense	c.665C>T	p.Ala222Val	VUS
Missense	c.667C>T	p.Arg223Cys	VUS
Nonsense	c.685C>T	p.Gln229Ter	Likely pathogenic
Missense	c.694T>C	p.Ser232Pro	VUS
Small deletion	c.694_705del12	p.Ser232_Leu235del	Likely pathogenic
Missense	c.697C>T	p.Arg233Trp	VUS
Small deletion	c.724delC	p.Leu242Cysfs∗55	Likely pathogenic
Missense	c.725T>C	p.Leu242Pro	VUS
Missense	c.733C>T	p.Arg245Cys	VUS
Missense	c.756G>C	p.Gln252His	VUS
Missense	c.758T>C	p.Leu253Pro	Likely pathogenic
Missense	c.763G>A	p.Glu255Lys	VUS
Missense	c.764A>G	p.Glu255Gly	Benign
Nonsense	c.775A>T	p.Arg259Ter	Likely pathogenic
Small deletion	c.795del	p.Thr266Leufs∗31	Likely pathogenic
Missense	c.811G>T	p.Gly271Cys	VUS
Missense	c.815C>A	p.Pro272Gln	VUS

Table 2 (*Continued*)

Variant type	Nucleotide change	Amino acid change[a,b]	ACMG classification[c]
Nonsense	c.823C>T	p.Gln275Ter	Likely pathogenic
Missense	c.830T>C	p.Leu277Pro	VUS
Missense	c.844C>A	p.Arg282Ser	VUS
Missense	c.844C>T	p.Arg282Cys	VUS
Nonsense	c.847C>T	p.Gln283Ter	Likely pathogenic
Missense	c.875C>T	p.Thr292Ile	VUS
Nonsense	c.883C>T	p.Gln295Ter	Likely pathogenic
Missense	c.887T>G	p.Ile296Arg	VUS
Small deletion	c.888delA	p.Ile296Metfs∗42	Likely pathogenic
Missense	c.902G>C	p.Arg301Pro	VUS
Nonsense	c.913C>T	p.Gln305Ter	Likely pathogenic
Small deletion	c.926_928delAGG	p.Glu309del	VUS
Nonsense	c.937C>T	p.Gln313Ter	Likely pathogenic
Missense	c.941T>G	p.Leu314Arg	VUS
Missense	c.944C>T	p.Ala315Val	VUS
Missense	c.956C>T	p.Pro319Leu	VUS
Missense	c.962A>T	p.His321Leu	Benign
Missense	c.972C>G	p.Phe324Leu	VUS
Small deletion	c.980_981delAG	p.Glu327Valfs∗19	Likely pathogenic
Small deletion	c.990_993delAACA	p.Asp332Valfs∗5	Likely pathogenic
Small deletion	c.995_998delACAG	p.Asp332Valfs∗5	Likely pathogenic
Gross insertion	c.999insGGCAAGG TTGTGAGCAAGCT GCAGGCCC	p.Ser333Argfs∗5	Likely pathogenic
Missense	c.1001G>T	p.Gly334Val	Likely benign
Missense	c.1027C>T	p.Arg343Cys	VUS
Missense	c.1036G>C	p.Asp346His	VUS
Nonsense	c.1044G>A	p.Trp348Ter	Likely pathogenic
Missense	c.1088T>A	p.Leu363Gln	VUS
Regulatory	c.∗158C>T	N/A	Benign

[a]For frameshift variants resulting in premature stop codon, the notation 'fs∗(number)' indicates that the frameshift variant results in stop codon at the position (number) residues downstream of the variant site.
[b]N/A, not applicable.
[c]VUS, variant of uncertain significance.

Currently, perhaps the only missense variant that can be definitively labeled as a pathogenic LOF variant is *APOA5* p.Leu253Pro. This variant was observed in the homozygous state in a pediatric female patient at age five years, with severe HTG with no other TG-elevating variants [49]. Functional analysis [49] revealed three consequences of this variant: (i) the secreted abnormal apo A-V had impaired liposome binding ability; (ii) the abnormal apo A-V had complete loss of sortilin and SorLA/LR11 binding ability, which are thought to mediate the ability of apo A-V to reduce hepatic VLDL synthesis and secretion; and finally, (iii) the secreted apo A-V variant potently inhibited LPL activity rather than enhancing it. In silico modeling suggested that the deleterious impact of this variant is because the leucine at residue 253 in wild-type apo A-V interfaces between two α-helices via hydrogen bonding interactions [49]. Therefore, given the high α-helical content of mature apo A-V and the functional importance of these structures [30], it is likely that a missense variant that disrupts the formation and/or interaction of these α-helices would be pathogenic. However, specific functional studies are needed to confirm variant pathogenicity. In summary, while most missense variants in *APOA5* are considered variants of uncertain significance

(VUS), it is likely that at least some are in fact pathogenic variants and one of the potential mechanisms may be via disruption of key α-helical structures.

COMMON POLYMORPHISMS IN *APOA5* MISTAKENLY CONSIDERED AS PATHOGENIC VARIANTS

Many studies on *APOA5* genetics have highlighted the TG-elevating effects of several common polymorphisms, as previously summarized [56]. With respect to HTG, the *APOA5* promoter polymorphism c.-1131T>C [57–59], c.-3A>G [60], c.56C>G, also known as p.Ser19Trp [2,28,58,61], c.553G>T, also known as p.Gly185Cys [2,62,63], c.725C>G, also known as p.Leu242Val [56,64], IVS3 + 476G>A (also known as c.162-43G>A) [56,65,66] and c.∗158T>C [67] polymorphisms have been associated with elevated TG in numerous small case-control association studies, and more recently as small effect signals for slight deviations in TG levels in genome-wide association studies.

These common *APOA5* polymorphisms, especially p.Ser19Trp and p.Gly185Cys, are often interpreted as being major contributors to the large variability in HTG severity observed in heterozygotes for true rare *APOA5* LOF variants, such as truncating variants. They are even sometimes erroneously reported as being causal for FCS on clinical genetic reports of next-generation sequencing analysis. In reality, these are merely risk alleles that raise the probability or odds of developing HTG if the metabolic context allows. But they are not directly causal or pathogenic, as evidenced by their high frequency of 5 to 15% in normolipidemic populations [68]. Furthermore, they are actually components of polygenic risk scores for HTG [69], and contribute to the burden of TG-raising polymorphisms in patients with both mild-to-moderate and severe HTG [38,39]. We suggest that these polymorphisms should be excluded from any experimental analysis of true LOF pathogenic rare variants of *APOA5*.

CONCLUSION

Apo A-V is an important regulator of TG metabolism despite its relatively low plasma concentration and near exclusive expression in hepatic tissue. Here, we have summarized current understanding of the physiological roles of apo A-V related to TG metabolism and have provided a very comprehensive overview of *APOA5* genetic variants. Apo A-V has a wide range of functions related to lowering HTG. Thus, LOF variants in *APOA5* represent an important genetic contributor and sometimes direct cause of HTG. Our recent study [3▪] clarified the TG phenotype associated with heterozygosity for *APOA5* LOF variants and highlighted the highly variable TG phenotypes both between and within patients over time. Our analysis indicates that the primary mode of *APOA5* LOF pathogenicity is through premature stop codons. Furthermore, we have also highlighted a need for improved understanding of the role of missense variants in *APOA5*-associated disease. Currently, minimal in vitro or in vivo functional studies have been conducted to evaluate dysfunction of *APOA5* missense variants. Therefore, future studies using high throughout functional analytic platforms should aim to uncover the molecular and biochemical defects associated with *APOA5*. Greater understanding of the biochemistry underlying these variants may lead to new and better diagnosis and treatment for HTG.

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Conflicts of interest

R.A.H. reports consulting fees from Acasti, Aegerion, Akcea/Ionis, Amgen, Arrowhead, Boston Heart, HLS Therapeutics, Pfizer, Novartis, Regeneron, Sanofi and Ultragenyx. The other authors have no conflicts to disclose.

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