Apolipoprotein A-V gene therapy for disease prevention / treatment: a critical analysis

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Abstract

Apolipoprotein (apo) A-V is a novel member of the class of exchangeable apo's involved in triacylglycerol (TG) homeostasis. Whereas a portion of hepatic-derived apoA-V is secreted into plasma and functions to facilitate lipoprotein lipase-mediated TG hydrolysis, another portion is recovered intracellularly, in association with cytosolic lipid droplets. Loss of apoA-V function is positively correlated with elevated plasma TG and increased risk of cardiovascular disease. Single nucleotide polymorphisms (SNP) in the APOA5 locus can affect transcription efficiency or introduce deleterious amino acid substitutions. Likewise, rare mutations in APOA5 that compromise functionality are associated with increased plasma TG and premature myocardial infarction. Genetically engineered mouse models and human population studies suggest that, in certain instances, supplementation with wild type (WT) apoA-V may have therapeutic benefit. It is hypothesized that individuals that manifest elevated plasma TG owing to deleterious APOA5 SNPs or rare mutations would respond to WT apoA-V supplementation with improved plasma TG clearance. On the other hand, subjects with hypertriglyceridemia of independent origin (unrelated to apoA-V function) may not respond to apoA-V augmentation in this manner. Improvement in the ability to identify individuals predicted to benefit, advances in gene transfer technology and the strong connection between HTG and heart disease, point to apoA-V supplementation as a viable disease prevention / therapeutic strategy. Candidates would include individuals that manifest chronic TG elevation, have low plasma apoA-V due to an APOA5 mutation/polymorphism and not have deleterious mutations/polymorphisms in other genes known to influence plasma TG levels.

Keywords: apolipoprotein A-V, adeno-associated virus, triacylglycerol, lipoprotein lipase, atherosclerosis, single nucleotide polymorphism, gene therapy

Apolipoprotein A-V dependent modulation of plasma triacylglycerol

Apolipoprotein (apo) A-V is an enigmatic modulator of plasma triacylglycerol (TG) homeostasis. ApoA-V is expressed solely in the liver and, following secretion, circulates at extremely low concentrations (~150-400 ng/mL plasma). This value is approximately 10,000 fold lower than that of apoA-I, the major apo of high density lipoprotein (HDL). Given the paucity of circulating apoA-V, it is logical to consider that it does not function as a typical member of the apolipoprotein class. Despite this supposition, apoA-V possesses sequence homology with other apo's, is recovered in association with cytosolic lipid droplets.
lipoproteins and displays high lipid-surface seeking activity. Using recombinant protein, it has been demonstrated that apoA-V binds heparan sulfate proteoglycans (HSPG)\(^1-2\), members of the low-density lipoprotein receptor (LDLR) family\(^3\) and the endothelial cell surface protein, glycosylphosphatidylinositol-anchored-high-density lipoprotein binding protein 1 (GPIH-BP1)\(^4\). This latter function has been invoked to explain the ability of apoA-V to enhance lipoprotein lipase (LPL) mediated hydrolysis of lipoprotein associated TG\(^\text{[3]}\). What is not explained, however, is how this can be achieved at such low circulating apoA-V concentrations. For example, it has been estimated that, in the postprandial state, there is only enough apoA-V present to associate with ~1 in 24 apoB containing lipoproteins\(^6\). Thus, it would appear that apoA-V is either an exceptionally potent apolipoprotein or it possesses additional functionality that has yet to be revealed.

Genome wide association studies (GWAS) have identified APOA5 as a determinant of plasma TG concentrations\(^7\). There is also strong evidence that abnormally high concentrations of TG are associated with atherosclerosis\(^8-12\). Given this connection, it is important to consider whether APOA5 may be a risk factor for disease processes that develop from chronic elevation of plasma TG. The fact that ~40 million adults in the United States have high TG (≥200 mg/dL) and ~4 million of these have hypertriglyceridemia (HTG; ≥500 mg/dL)\(^12-13\) indicates the scale of this health problem. In the Prospective Cardiovascular Munster Study, a six-fold increase in coronary heart disease (CHD) risk was measured in subjects with TG values >200 mg/dL\(^14\). Likewise, in the Scandinavian Simvastatin Survival Study, the authors reported increased risk of coronary events as TG levels increased above 220 mg/dL\(^15-16\). In this study the 5-year event rate was significantly increased in untreated patients with mixed dyslipidemia (including those with HTG) compared to those with elevated LDL-cholesterol alone. Finally, the Bezafibrate Infarction Prevention study found that, in men and women with established CHD, elevated plasma TG increased the risk of cardiovascular mortality, stroke and transient ischemic attacks\(^17\).

**Multiple genes can impact plasma TG levels**

It is widely recognized that numerous genes play a role in determining plasma TG levels. In addition to APOA5, well-studied modulators of plasma TG homeostasis include LPL, APOC2, APOC3, GPIHBP1 and others. According to prevailing models, plasma apoA-V functions to facilitate lipoprotein binding to GPIHBP1\(^\text{[3]}\). As a component of TG-rich lipoproteins, apoA-V binding to GPIHBP1 coordinates LPL and apoC-II interactions in a manner that promotes efficient TG hydrolysis. Indeed, when any one of these proteins is missing or defective, HTG ensues. Further complexity is introduced when the effect of apoA-V on plasma TG is examined in different physiological settings. In mice, for example, an inverse correlation between apoA-V and TG is well documented\(^18\). These authors showed that gene disruption of apoa5 leads to a marked increase in plasma TG while transgenic overexpression of APOA5 induces a significant decline. By contrast, in human populations with HTG\(^19\), plasma apoA-V and TG are oftentimes positively correlated! Thus, it is likely that, depending on the genetic background/physiological conditions that lead to HTG, apoA-V may actually accumulate along with TG.

**APOA5 variants and heart disease**

In a recent study, Do et al.\(^20\) investigated the correlation between rare APOA5 variants and early onset myocardial infarction (MI). In this tour de force study, the authors conducted exome sequencing on over 9,700 human subjects with premature MI (≤50 years of age in males and ≤60 years in females) along with MI-free controls. They sought to identify genes in which rare mutations contribute to the risk of early-onset MI in the population. Two genes (LDLR and APOA5) were identified in which rare coding mutations were more frequent in MI cases compared to controls. Approximately 2% of early MI cases harbor a rare, damaging mutation in LDLR. Likewise, in APOA5, those subjects with rare non-synonymous mutations were at 2.2-fold increased risk for MI. When compared to non-carriers, LDLR mutation carriers had higher plasma LDL cholesterol while APOA5 mutation carriers had higher plasma TG. It may be anticipated that subjects identified to be at risk for premature MI as a result of harboring these rare APOA5 mutations could improve or normalize their plasma TG concentration, and reduce the risk of MI, by augmentation with wild type (WT) apoA-V.

A basic question emerging from the above investigation relates to the underlying mechanism whereby elevated plasma TG increases the risk of heart disease. Certainly the correlation between chronic elevated plasma TG and atherosclerosis is less clear than that for cholesterol. While the TG content of plaque is far less prominent, evidence suggests that, in cells located in and around plaque deposits, TG-rich lipoproteins induce inflammation and related atherogenic processes\(^21-23\). Elevated TG also correlates with the formation of small dense LDL, lipoprotein particles that are positively associated with CHD risk and inflammation\(^24\). Given the very low concentration of apoA-V in plasma under normal circumstances, any
decrease will likely impact lipoprotein-associated TG hydrolysis by LPL, thereby interfering with TG-rich lipoprotein clearance. The resulting increase in circulating TG-rich lipoproteins will promote inflammatory cytokine release, contributing to endothelial injury[25].

In vivo evidence for an athero-protective effect of apoA-V

Studies in mouse models of dyslipidemia have provided evidence that apoA-V is athero-protective. For example, Mansouri et al.[20] crossed APOA5 transgenic mice with APOE2 knock in (KI) mice (deficient in apoae and transgenic for APOE2). Compared to control APOE2 KI mice, plasma TG levels were lower and atherosclerotic lesion size was reduced when apoA-V levels were increased. Subsequently, Grosskopf et al.[21] crossed APOA5 transgenic mice with apoE (-/-) mice. In this study a significant decrease in VLDL and remnant lipoproteins, together with a 70% reduction in aortic lesion area, was noted. In both of these studies, the apoA-V concentration in plasma was increased by expression of the transgene. Of interest, however, is the manner in which HTG was induced. In the APOE2 KI model, a dysfunctional apoE protein is present while in apoE (-/-) mice no apoE is present. It is conceivable that, since apoA-V and apoE share the ability to bind HSPGs and members of the LDL receptor family, augmenting apoA-V levels in the absence of functional apoE compensates for the missing or defective apoE.

Another well established model of HTG is the APOC3 transgenic mouse. These mice manifest delayed catabolism of VLDL and chylomicrons owing to an abundance of apoC-III bound to the surface of these particles[28-30]. To study the effect of apoA-V augmentation on apoC-III overexpression-dependent HTG, Qu et al.[31] used adenovirus mediated gene transfer to increase apoA-V production in APOC3 transgenic mice. ApoA-V gene transfer into APOC3 transgenic mice caused a reduction in apoC-III content on VLDL that, in turn, led to an increase in LPL-mediated TG hydrolysis.

Individuals predicted to benefit from increased WT apoA-V expression

As discussed above, and previously by Sharma et al.[3], relatively common APOA5 SNPs are associated with increased plasma TG. For example, individuals homozygous for the c.553G > T APOA5 SNP (rs2075291) have extremely elevated plasma TG levels[32]. Recent studies have shown that HTG in these subjects is due, at least in part, to production of a dysfunctional apoA-V protein[33]. It may be anticipated that an increase in circulating levels of WT apoA-V in homozygous carriers of this SNP will induce TG lowering, thereby reducing the risk of related disease processes. Another coding SNP strongly associated with elevated plasma TG is c.56C>G APOA5 (rs3135506)[19]. This SNP, which introduces an amino acid substitution (Ser19Trp) in the signal sequence of apoA-V, is thought to impede processing/secretion of the mature protein[34]. Along the same lines, a rare homozygous APOA5 deletion mutation has been identified in the signal sequence (c.16_39del; p.Ala6_Ala13del), generating a variant apoA-V protein that is not secreted[35]. The missing amino acids are required for translocation of nascent apoA-V to the endoplasmic reticulum and, as a result, this protein accumulates in the cytoplasm in association with lipid droplets. In both c.56C>G and c.16_39del, the mature protein is predicted to be identical to WT apoA-V. Mutation- and SNP-induced effects on signal sequence function or cleavage decrease secretion efficiency resulting in diminished, or complete lack of, circulating apoA-V. However, despite the fact that individuals harboring the c.56C>G SNP may secrete less apoA-V, if this polymorphism is not the sole or primary underlying cause of HTG, then apoA-V levels will likely accumulate in plasma along with TG[39] and it is doubtful augmentation with apoA-V will induce TG lowering.

Non-coding SNPs located within the APOA5 gene locus are also associated with elevated plasma TG, most likely due to effects on APOA5 gene expression. For example, the -1131T>C SNP (rs662799), located upstream of the transcription start site, has been proposed to reduce transcription efficiency[36]. Likewise, an IVS3+3G > C mutation causes a frameshift in the donor splice site of intron 3 creating a premature stop codon that results in a nonsense protein[37]. Other rare mutations in APOA5 result in severe truncation of apoA-V and abolish function altogether[38-39].

Given this, it may be anticipated that individuals with HTG caused by deleterious APOA5 SNPs or mutations would benefit from increased circulating levels of WT apoA-V (Table 1). Likewise, as seen above, if HTG is caused by enhanced apoC-III production, it may be anticipated that increased circulating levels of apoA-V would be beneficial. On the other hand, if HTG is associated with a diagnosis of metabolic syndrome, for example, simply adding to the pool of apoA-V is not expected to improve the TG profile owing to the multifactorial causation of this disorder. From a practical standpoint, if HTG is observed, then the APOA5 gene should be examined for the presence of deleterious SNPs or loss of function mutations. If present, then WT apoA-V augmentation may induce TG lowering. On the other hand, subjects with HTG unrelated to APOA5 or from a combination of APOA5 variation and other genes, would be expected
to see a lesser or no benefit. This predicted differential response provides a plausible explanation for the conundrum emerging from analysis of apoA-V genetically engineered mouse models (wherein a clear inverse correlation between apoA-V and plasma TG exists) and human population studies that reveal a positive correlation between apoA-V and TG [19]. The importance of discriminating between predicted responders and non-responders is illustrated below.

**ApoA-V as a TG lowering therapy**

Based on the above discussion, it is conceivable that deleterious APOA5 SNPs or mutations (e.g. interfere with apoA-V secretion efficiency or produce an apoA-V protein with compromised function) may increase the risk of atherosclerosis due to chronically elevated plasma TG levels. To investigate the potential benefit of direct addition of apoA-V, Shu et al. [40] administered recombinant human apoA-V intravenously to hypertriglyceridemic apoA5(-/-) mice. Administration of apoA-V–containing reconstituted HDL induced a 60% decline in plasma TG after 4 h that was attributed to enhanced catabolism and clearance of VLDL. Despite the reduction in plasma TG observed in this experiment, the effect was short-lived, suggesting that this approach may not represent a feasible therapeutic strategy. In an effort to increase the duration of TG-lowering induced by apoA-V, AA V2/8-mediated gene transfer of WT apoA-V was performed in apoA5(-/-) mice [41]. In this study, apoA-V expression lasted at least 8 weeks and induced a 50% decline in plasma TG levels, suggesting that gene therapy may be beneficial in some instances.

It is noteworthy that recent advances suggest gene transfer technology may constitute a feasible therapeutic option for a subset of individuals with chronic HTG. For this purpose, the viral vector must display sustained transgene expression in the absence of toxicity. Vectors most commonly used for treatment of metabolic disorders are adeno-associated virus (AAV) and lenti-virus. Among these, AAV has a number of clinically favorable attributes. First, it lacks both parental agent pathogenicity and vector related cytotoxicity. Furthermore, compared to other viral vectors, AAV induces a minimal host immune response. In fact, AAV vectors have been used to deliver genes to over 500 study subjects by various routes of administration for potential treatment of genetic disorders including cystic fibrosis, hemophilia and Canavan, Batten, Parkinson’s and Alzheimer’s diseases, without significant safety concerns. Among approved AAV-based therapies, Glybera (UniQure) is used for treatment of LPL deficiency [42-43]. Long-term gene expression (>1.5 years) has been demonstrated after AAV transduction in animal models including canine, murine and hamster [44-50]. Moreover, it has been demonstrated that AAV can successfully be transduced into a variety of cell and tissue types, including brain, liver and muscle [51-54]. An advantage of the AAV serotype 2/8 is that it efficiently targets liver [41,55]. Given that apoA-V is expressed solely in liver, AAV2/8 is a strong candidate vector for studies involving apoA-V gene transfer.

Given the role of APOA5 in modulation of plasma TG documented in studies of genetically engineered mice, GWAS, human population loss of function studies and large-scale exome sequencing, therapies designed to promote its athero-protective effects are very attractive. Whereas it may be possible to develop a small molecule therapeutic capable of inducing endogenous apoA-V expression, individuals harboring common SNPs or rare mutations in APOA5 may not benefit from this approach. On the other hand, gene therapy represents a safe, robust and efficient means to achieve sustained expression of WT apoA-V. By controlling plasma TG homeostasis in this manner, the risk of heart disease and atherosclerosis can be reduced.

### Table 1: Anticipated effect of apoA-V gene therapy in individuals with high plasma TG

| Underlying cause of plasma TG elevation | Reference | Predicted outcome of WT apoA-V gene therapy |
|----------------------------------------|-----------|-------------------------------------------|
| APOA5 IVS3+3G→C, frameshift            | [37]      | decreased TG levels                        |
| APOA5 premature stop (Q95X, Q97X, E116X, Q139X, Q148X, Q295X, Q313X) | [20,38-39] | decreased TG levels                        |
| APOA5 frameshift (M92fs, R143fs)       | [20]      | decreased TG levels                        |
| APOA5 deletion (c.16_39del)            | [35]      | decreased TG levels                        |
| LPL deficiency                         | [42]      | no effect                                  |
| GPIHBP1 deficiency                     | [38]      | no effect                                  |
| Type II diabetes                       |           | no effect                                  |
| High fat diet                          |           | no effect                                  |

1For subjects harboring APOA5 mutations, specific criteria must be met prior to consideration for supplementation with WT apoA-V. These include persistent elevated plasma TG, continuing low plasma apoA-V protein levels and a lack of polymorphisms / mutations in other known TG modulating genes. TG: triglyceridemia, WT: wild type, LPL: lipoprotein lipase.
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