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Review

Apolipoprotein A-V; a potent triglyceride reducer

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ABSTRACT

Since its discovery, apolipoprotein A-V has been considered to be a potent factor affecting plasma triglycerides (TG) in humans and mice. Several single nucleotide polymorphisms in the *APOA5* gene are associated with increased TG levels in humans, and some nonsense mutations affecting protein structure predispose for familial hypertriglyceridemia and late onset chylomicronemia.

It is not clear, how apoA-V decreases plasma TG. There are three major hypotheses: apolipoprotein A-V could work through (1) an intracellular mechanism affecting VLDL production in the liver, (2) stimulation of proteoglycan-bound lipoprotein lipase at the endothelium of capillaries in peripheral organs, or (3) enhancing the clearance of TG-rich lipoproteins via lipoprotein receptors in the liver. There is good evidence for a role of apoA-V in extracellular TG metabolism and increasing support for an additional function of ApoA-V as a receptor ligand. The intracellular role of apoA-V for lipoprotein assembly and secretion is still speculative. This review discusses these possible mechanisms.

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1. Introduction

Apolipoprotein (apo) A-V was first described in 2001 as a result of comparative genome sequencing between mouse and man [1]. Another group identified apoA-V as a protein upregulated during liver regeneration after partial hepatectomy in rats [2]. Located on chromosome 11q23 and present in the *APOA1-C3-A4* gene cluster, apoA-V got its name from homology with apoA-IV and from binding to HDL and other lipoproteins. Human and mouse data consistently show that apoA-V is a factor that strongly reduces plasma triglyceride (TG) levels [1]. However, the mechanism of its action is still not completely understood.

ApoA-V is predominantly produced in the liver and can be found both intracellularly and associated with cell membrane structures. Compared to other apolipoproteins, apoA-V is present in human plasma at extremely low concentrations ranging from about 20 to 500 ng/ml [3]. On a molar basis this is approximately 1000-fold lower than the concentration of $apoB_{100}$, meaning that only one in 24 VLDL particles can carry an apoA-V molecule [4]. In plasma, apoA-V is found mainly as a monomer on chylomicrons, VLDL and HDL [5].

Abbreviations: TG, triglycerides; LPL, lipoprotein lipase; LDLr, LDL receptor; LRP1, LDLr-related protein 1; HSPG, heparin sulfate proteoglycans.

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Based on experimental data, an inverse relation between apoA-V protein and plasma TG levels was expected. In fact, such an association has been reported in Asian subjects [6,7]. However, most studies found a positive correlation between apoA-V and TG, particularly in hypertriglyceridemicpatients [5,8–12]. Chan et al. [13] modeled lipoprotein kinetics in human subjects using stable isotopes and found no correlation between apoA-V plasma levels and the production rate or the fractional catabolic rate of VLDL. Since the plasma levels are dependent on both production and decay rates, it had been suggested that the relatively strong binding of apoA-V to lipoproteins and its transfer between lipoprotein classes could result in a slower apoA-V turnover. This could prevent its decay, especially in hypertriglyeridemia, as is known for apoE. It has been shown that HDL may act as a reservoir of apoA-V [5], and that apoA-V is transferred to VLDL during lipoprotein lipase (LPL) mediated lipolysis [14]. Therefore, the lack of an inverse correlation between TG and total plasma apoA-V levels does not argue against a role of apoA-V in TG lowering. Increased apoA-V production by transgenic [1,15] or adenoviral [16] expression lowered plasma TG in mice. On the other hand, *hAPOA5* transgenic mice lacking the murine *APOA5* gene showed a positive correlation between apoA-V plasma levels and plasma TG levels, similar to humans [15]. Later it was shown that many different mice models, with unmodified *APOA5* expression, demonstrated a positive relationship between plasma apoA-V and plasma TG, just like humans [17]. So although increased production of apoA-V lowers plasma TG levels, there is still a positive correlation between apoA-V protein and TG levels in plasma.

2. ApoA-V variants in human disease

Elevated TG levels are an independent risk factor for cardiovascular disease [18]. It has been shown in humans, over a large number of ethnicities, that variations in the *APOA5* gene are associated with increased plasma TG levels [19–24]. Furthermore, mutations in the *APOA5* gene leading to apoA-V deficiency are related to severe hypertriglyceridemia in humans (see below) [25]. In addition, *APOA5* gene variants have been associated to coronary heart disease and other manifestations of atherosclerosis [26–29], as well as to the metabolic syndrome [30–32]. Some data show that *APOA5* variants may be associated with decreased BMI or weight reduction [33,34]. It should be noted that many SNPs in *APOA5* are in linkage disequilibrium with SNPs in other TG modulating genes, as discussed by van Dijk et al. [35]. Overexpression of human apoA-V in mice did, however, not affect body weight gain or glucose homeostasis [36]. An expected finding was, that overexpression of apoA-V retarded atherosclerosis development. On an apoE2 knock-in background, ApoA-5 resulted in an atheroprotective plasma lipid profile compared to control animals and there was a clear effect on atherosclerotic lesion size by apoA-V overexpression [37].

3. Lessons from apoA-V deficient patients

Type 1 chylomicronemia is typically caused by mutations in the LPL or the *APOC2* genes; and as described more recently also by mutations in the genes for GPI-anchored HDL-binding protein 1 (*GPIHBP1*) [38–41] or lipase maturation factor 1 (*LMF1*) [42]. GPIHBP1 is an endothelial membrane protein, which is important for the stability of LPL and for its transport to the luminal side of the capillary endothelium [39,43]. LMF1 is a chaperon localized in the endoplasmatic reticulum, which is necessary for proper folding of LPL after synthesis. In patients with chylomicronemia, fasting plasma TG levels above 10 mmol/l are found, and onset is usually during childhood.

APOA5 was analyzed as a possible candidate gene in patients with chylomicronemia, in whom mutations in LPL and *APOC2* had been excluded. This lead in one patient to the detection of the homozygous state of Q148X (c.442 C > T) (originally mislabelled as Q145X; c.433 C > T). This mutation causes a premature termination of transcription and a protein with reduced size [44]. Ten out of fourteen family members were heterozygous carriers for the same Q148X, but only half of them had mild hypertriglyceridemia, indicating that this mutation requires additional factors to raise plasma TG. In fact, in that study, all subjects carrying the Q148X mutation also had the common variant S19W (c.56 C > G) at the same *APOA5* allele.

A second mutation causing premature truncation of apoA-V, Q139X (c.415 C > T), was identified in a pedigree of late-onset chylomicronemia [45]. Out of 140 other independent cases with chylomicronemia, two individuals were also Q139X positive. In a family study, five out of nine Q139X carriers had chylomicronemia, while only one individual was homozygote. As in the of the Q148X mutation, the phenotype was strictly associated with the presence of one other TG-raising *APOA5* haplotype (either APOA5*2 or APOA5*3 [19]) on the second allele. Plasma apoB kinetic studies in three patients with the Q139X mutation showed a severe defect in TG lipolysis, while VLDL-apoB production rates were normal.

After that, additional *APOA5* mutations have been identified which possibly cause severe hypertriglyceridemia. As with the above mentioned mutations, in all cases, other factors were necessary to yield in the full hypertriglyceridemic phenotype. These could be age, additional *APOA5* or *APOC3* aberrations (reported with G271C [46], c.161 + 3G > C [47] and c.49 + 1G > A [48]), LPL mutations (reported with E255G and H321L [46]), reduced LPL activity (c.161 + 3G > C [47] and Q97X [49]), or pregnancy (c.161 + 5G > C [50] and E255G [46]). In Asians, c.553 G > T (G185C) is common, and may cause severe hypertriglyceridemia, e.g. in patients receiving HIV protease inhibitors [51]. One mutation, Q97X, was described yielding no apoA-V protein in plasma and defective binding of apoA-V to lipoproteins. It is not clear whether this truncated protein is secreted from the liver at all [49].

Taken together, *APOA5* mutations causing severe structural changes of the ApoA-V protein can trigger severe hypertriglyceridemia. However, the penetrance of these mutations is low and appears to require the presence of other genetic or environmental factors for full phenotypic expression.

4. Expression patterns and regulation

ApoA5 is mainly expressed in the liver [1,2]. Several nuclear receptors have been implicated in upregulation of *APOA5* expression including peroxisome proliferator-activated receptor α (PPARα) in humans [52,53], farnesoid X-activated receptor (FXR) [53], retinoid acid receptor-related orphan receptor α (RORα) [54], hepatocyte nuclear factor 4α (HNF4α) [55], and thyroid receptor β (TRβ) [56]. In contrast, LXR [57], insulin [58] and glucose via the upstream stimulatory factor (USF)1/2 [59] have all been found to downregulate *APOA5* expression. Thus, regulation in the liver occurs through a number of different factors intimately linked to energy homeostasis. Recently it has been reported that nuclear receptor Nur77 binds to a response element in the human *APOA5* promoter region, suggesting a role in energy metabolism for this orphan receptor [60].

5. Structure and molecular interactions

APOA5 encodes a 366 amino acid residue protein in humans [1]. The mature apoA-V contains 343 amino acid residues. ApoA-V is a hydrophobic protein consisting mainly of amphipathic α-helix