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Recent developments in the genetics of LDL deficiency

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Recent developments in the genetics of LDL deficiency

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Purpose of review

Inherited diseases of lipoprotein metabolism may give rise to marked hypocholesterolemia with low or absent levels of low density lipoprotein (LDL), depending on the gene involved and mode of inheritance of the condition, together with the severity of the mutation or mutations present. In this review, we discuss the recent developments in the genetics of LDL deficiency.

Recent findings

Carriers of a single loss-of-function variant in *ANGPTL3* have reduced LDL-cholesterol and triglyceride concentrations, whereas homozygotes have markedly reduced LDL-cholesterol, triglyceride and high density lipoprotein (HDL)-cholesterol concentrations, a recessive form of hypocholesterolemia designated as familial combined hypolipidemia.

Summary

The identification of loss-of-function *ANGPTL3* mutations as a cause of familial combined hypolipidemia suggests a new mechanism for the regulation of LDL metabolism in humans, thereby making *ANGPTL3* an attractive protein to target for therapeutics.

Keywords

Familial hypobetalipoproteinemia, combined hypolipidemia, genetics

KEY POINTS

- Loss-of-function mutations in *ANGPTL3* are associated with a recessive familial combined hypolipidemia characterized by a reduction in all plasma lipids, including HDL-cholesterol.
- *ANGPTL3* is absent in plasma of the patients with homozygous or compound heterozygous *ANGPTL3* mutations.
- The prevalence of *ANGPTL3* mutations giving rise to a combined hypolipidemia phenotype in subjects with severe primary hypobetalipoproteinaemia is about 10%.

INTRODUCTION

Inherited diseases of lipoprotein metabolism may give rise to marked hypocholesterolemia with low or absent levels of low-density lipoprotein (LDL), depending on the gene involved and mode of inheritance of the condition, together with the severity of the mutation or mutations present. The most extreme form of these disorders is abetalipoproteinemia, characterised by the absence of apolipoprotein (apo) B-containing lipoproteins in plasma [1, 2]. Abetalipoproteinemia is caused by mutations in the microsomal triglyceride transfer protein gene (*MTTP*), presents in early childhood with fat malabsorption, steatorrhea and failure to thrive, and may include progressive neurological and ophthalmological abnormalities as the patient ages.

Low plasma concentrations (usually <50% of normal levels for age and sex) of LDL-cholesterol and apoB are observed in familial hypobetalipoproteinaemia, a co-dominant disorder of lipoprotein metabolism caused by the inheritance of a mutation in *APOB*, usually giving rise to a truncated apoB protein [3]. Patients are generally asymptomatic, but may be at increased risk of fatty liver disease. Inheritance of two such mutations in *APOB* is known as homozygous familial hypobetalipoproteinemia and is clinically indistinguishable from abetalipoproteinemia.

In recent years, variants in the proprotein convertase subtilisin kexin 9 gene (*PCSK9*) have been shown to be associated with hypobetalipoproteinemia, although these have not been associated with any clinical symptoms. These loss-of-function mutations in *PCSK9* result in reduced concentrations of LDL-cholesterol in a gene dose-dependent manner, leading to a lifetime low risk of cardiovascular disease [4].

A major recent finding has been the association between mutations in *ANGPTL3* and recessive familial combined hypolipidemia, which is characterised by a reduction in all plasma

lipids, including high density lipoprotein (HDL)-cholesterol. This review summarises the recent advances in the field of genetic causes of LDL-deficiency, focusing on *ANGPTL3*.

ANGPTL3: A MODULATOR OF LIPOPROTEIN LIPASE ACTIVITY AND CAUSE OF HYPOLIPIDEMIA IN MICE

In 2002, it was reported that one strain of the KK obese mouse displayed abnormally low plasma lipid levels, inherited in a recessive manner. Mapping of the hypolipidemia trait to chromosome 4 and positional cloning identified a locus encoding a unique angiopoietin-like lipoprotein modulator, *Angptl3*, for which there is a highly conserved counterpart in humans [5]. As in mice, the majority of expression of *ANGPTL3* in humans occurs in the liver [6]. *ANGPTL3*, along with *ANGPTL4*, modulates lipolysis via the post-transcriptional inhibition of lipoprotein lipase (LPL) [7, 8]; mice lacking *Angptl3* expression have increased LPL activity and reduced plasma triglyceride concentrations [5]. In addition, *Angptl3*-deficient mice show low plasma HDL-cholesterol and HDL phospholipid; *in vitro* studies suggest that *ANGPTL3* inhibits the phospholipase activity of endothelial lipase [9].

RARE VARIANTS IN *ANGPTL3* ARE ASSOCIATED WITH HYPOTRIGLYCERIDEMIA IN HUMANS

In humans, *ANGPTL3* consists of 7 exons, encoding a 460 amino acid product. Romeo et al used a reverse genetic strategy to determine whether four members of the angiopoietin-like proteins (*ANGPTL3*, -4, -5 and -6) played important roles in triglyceride metabolism in humans [6]. Resequencing of the coding regions of these genes in the multiethnic Dallas Heart Study population (over 3500 subjects) identified multiple rare nonsynonymous sequence variants associated with low plasma triglyceride concentrations. Overall, 1% of the study population, and 4% of those in the lowest quartile of triglycerides, carried a loss-of-

function variant in *ANGPTL3*, -4 or -5. This included 35 sequence variants in *ANGPTL3*, with 14 in the lower quartile for plasma triglycerides compared to five in the upper quartile. While some of the variants were nonsense, frameshift or splicing mutations, others were missense mutations of unknown effect. *In vitro* expression studies of the mutant proteins in HEK293A cells showed that five of nine missense variants from the lowest triglyceride quartile group abolished secretion of ANGPTL3 from cells compared to none of the five missense variants from the highest triglyceride quartile group. The variants from the lowest quartile of triglyceride group also failed to suppress LPL activity *in vitro*. In addition, the *ANGPTL3* M259T single nucleotide polymorphism (SNP) was present in 5% of African Americans and was significantly associated with plasma triglycerides, with TT homozygotes showing plasma triglyceride levels ~20% lower than those with the wild-type genotype MM.

While deletion of *Angptl3* in mice is associated with a 50% reduction in plasma HDL-cholesterol levels [9], the Dallas Heart Study cohort did not show an association of *ANGPTL3* loss-of-function variants with HDL-cholesterol.

COMBINED HYPOLIPIDEMIA IS CAUSED BY MUTATIONS IN *ANGPTL3*

In a landmark publication in 2010, Musunuru et al. used exome sequencing to identify mutations in *ANGPTL3* as the cause of hypobetalipoproteinemia in two siblings with extremely low plasma levels of LDL-cholesterol, HDL-cholesterol and triglyceride [10, 11●●]. The individuals were from a 38-member, three-generation family, which included four people with combined hypolipidemia and several with hypobetalipoproteinemia that was not linked to the *APOB* gene. Exome sequencing analysis showed that *ANGPTL3* was the only gene that had novel variants in both alleles in both siblings. Both mutations were nonsense mutations, S17X and E129X, occurring in the first exon of *ANGPTL3*. Heterozygotes for either mutation had intermediate levels of LDL-cholesterol and

triglycerides, consistent with a codominant mode of inheritance for these phenotypes.

However, only the compound heterozygotes showed a marked reduction in HDL-cholesterol, indicating that this segregates as a recessive trait. This discrepancy suggests there are distinct mechanisms of action of *ANGPTL3* on different lipoprotein classes.

FURTHER CHARACTERIZATION OF *ANGPTL3* MUTATIONS ASSOCIATED WITH FAMILIAL COMBINED HYPOLIPIDEMIA

Pisciotta et al. characterised *ANGPTL3* mutations in three families with combined hypolipidemia [12]. The probands, aged 59, 65 and 85 years, each had a total cholesterol concentration below 2 mmol/L (80 mg/dL), with LDL-cholesterol ~1 mmol/L (40 mg/dL), triglyceride ~0.5 mmol/L (45 mg/dL) and HDL-cholesterol ranging from 0.26–0.70 mmol/L (10–27 mg/dL). DNA sequencing showed that they did not carry a mutation in the *APOB*, *MTTP*, *PCSK9*, *APOA1*, *LCAT* or *ABCA1* genes. One proband was homozygous for an *ANGPTL3* splice donor mutation, c.1198+1G>T, which caused partial retention of intron 6 and introduction of a premature stop codon into the reading frame (p.G400Vfs*5). Screening of 200 healthy individuals identified one heterozygous carrier with a normal lipid profile. The other two probands were compound heterozygotes for the same two deletions in exon 1 of *ANGPTL3*: c.55delA and c.439_442del, frameshift mutations leading to production of truncated proteins (p.I19Lfs*22 and p.N147X, respectively). c.1198+1G>T and c.439_442del mutations in heterozygous subjects were previously described by Romeo et al. [6]. Pisciotta et al. showed that the mean LDL-cholesterol in heterozygotes was below the 25th percentile of population distribution, so unlike carriers of *APOB* truncating mutations, would not be easily discernible in population screening for low LDL-cholesterol [12●].

ANGPTL3 was absent in plasma of the patients with homozygous or compound heterozygous mutations, while heterozygotes showed reduced concentrations of circulating

ANGPTL3 compared to the general population [12●]. While their sera had a reduced capacity *in vitro* to promote cellular cholesterol efflux through the ABCA1, ABCG1 and SR-BI pathways, the *ANGPTL3*^{-/-} subjects did not show any clinical evidence of accelerated atherosclerosis. The ability to promote cholesterol efflux was directly correlated with plasma HDL-cholesterol concentration. However, with the small number of subjects, more studies are needed to firmly establish whether or not the lifelong exposure to low levels of LDL-cholesterol counterbalances the proatherogenic effects of low HDL-cholesterol.

In four unrelated Spanish families meeting the criteria for familial hypobetalipoproteinaemia (apoB and LDL-cholesterol <5th percentile for age and sex), but without *APOB* mutations, two probands were found to be homozygotes for a 5 bp deletion in *ANGPTL3*, p.N121L*2 [13]. These individuals featured combined hypolipidemia including low triglyceride and HDL-cholesterol concentrations.

PREVALENCE OF *ANGPTL3* MUTATIONS IN COMBINED HYPOLIPIDEMIA

Noto et al. determined that the prevalence of *ANGPTL3* mutations giving rise to a combined hypolipidemia phenotype in subjects with severe primary hypobetalipoproteinaemia is about 10% [14●●]. In two cohorts comprising 913 Italian and American subjects with total cholesterol <5th percentile of the general population, they chose total cholesterol and HDL-cholesterol cut-offs of 2nd percentile and the 2nd decile based on data from a previously reported familial combined hypolipidemia kindred [11●●]. This identified 78 subjects with combined hypolipidemia. DNA sequencing identified *ANGPTL3* gene mutations in 8 subjects and no *PCSK9*, *MTP* or *APOB* mutations. Four subjects were homozygous or compound heterozygous for *ANGPTL3* mutations, and four were simple heterozygotes.

Overall, in this population study, *APOB* mutations are found in 7–8% of subjects with total cholesterol <2nd percentile [14●●]. This suggests that in subjects with total cholesterol

<2nd percentile, those with HDL-cholesterol <2nd decile may be carrying *ANGPTL3* mutations, whereas those with higher HDL-cholesterol may be carrying *APOB* mutations. Ultrasonography of three *ANGPTL3* mutation carriers detected severe fatty liver in one subject, suggesting that this complication may not be limited to familial hypobetalipoproteinemia due to *APOB* mutations.

CLINICAL AND BIOCHEMICAL CHARACTERIZATION OF FAMILIAL COMBINED HYPOLIPIDEMIA CAUSED BY *ANGPTL3* S17X

In 1991, Fazio et al identified a three-generation Italian family in the small Italian town of Campodimele with a dominant form of hypobetalipoproteinaemia that was not due to a mutation in *APOB* [15]. Recently, Minicocci et al. identified the *ANGPTL3* S17X mutation in the proband of this family and also in the probands of an additional eight local families [16●●]. Two of the probands were homozygous for the mutation whereas seven were heterozygotes. Family screening identified 20 further subjects with *ANGPTL3* S17X, including four homozygotes. Compared with noncarrier family members, those subjects heterozygous for *ANGPTL3* S17X had reduced HDL-cholesterol and triglyceride concentrations.

A voluntary screening program in which ~60% of the town residents participated identified 32 other carriers of the *ANGPTL3* mutation out of the 352 subjects screened. Although 46 individuals had LDL-cholesterol <5th percentile for age and sex, only six carried S17X (four heterozygotes and two homozygotes). The remaining population were then screened, identifying a further 26 S17X heterozygotes. The prevalence of *ANGPTL3* variants in the population was estimated to be 9.4%.

Overall, homozygosity for *ANGPTL3* S17X reduced LDL-cholesterol by 48%, triglyceride by 62%, HDL-cholesterol by 46%, apoB by 44% and apoA-I by 48%. Of interest,

heterozygotes only showed a significant reduction in total cholesterol and HDL-cholesterol [16●●], in contrast with earlier findings suggesting that heterozygotes had lower LDL-cholesterol and triglyceride concentrations [11●●]. This may relate to the specific mutation, a partial influence on plasma lipids, or other environmental or genetic differences between the studied individuals.

The prevalence of elevated liver enzymes was not different between *ANGPTL3* S17X carriers and non-carriers, suggesting that familial combined hypolipidemia is not associated with liver abnormalities. Of interest, blood glucose levels were significantly lower in homozygotes than noncarriers. Romeo et al. reported a higher prevalence of *ANGPTL3* nonsynonymous variant carriers in those subjects with the lowest quartile of blood glucose [6]. The mechanism by which *ANGPTL3* affects glucose levels is uncertain but may relate to the reduction in lipolysis and free fatty acid availability, which may improve tissue insulin sensitivity [16●●].

Plasma non-cholesterol sterols and ratios were determined in carriers and noncarriers of *ANGPTL3* mutant alleles. The indices of cholesterol absorption, cholesterol synthesis and bile acid metabolism were not different between groups, except for a higher mean lanosterol to total cholesterol ratio in homozygotes. However, the other marker of cholesterol synthesis, lathosterol to total cholesterol ratio, was not different. This data suggests that *ANGPTL3* mutations do not cause significant alterations of whole-body cholesterol metabolism.

CONCLUSION

ANGPTL3, a protein produced in the liver and found in the circulation, inhibits the activity of lipoprotein and endothelial lipase. *Angptl3* knockout mice have marked recessive hypolipidemia. The identification of loss-of-function *ANGPTL3* mutations as a cause of familial combined hypolipidemia suggests a new mechanism for the regulation of LDL

metabolism in humans, thereby making ANGPTL3 an attractive protein to target for therapeutics.

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Conflicts of interest

There are no conflicts of interest.

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Exome sequencing was used to identify mutations in *ANGPTL3* as the cause of hypobetalipoproteinemia in two siblings with extremely low plasma levels of LDL-cholesterol, HDL-cholesterol and triglyceride.

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This study identified further subjects with familial combined hypolipidemia and showed that while their sera had a reduced capacity *in vitro* to promote cellular cholesterol efflux, they did not appear to show any clinical evidence of accelerated atherosclerosis.

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The authors determined that the prevalence of *ANGPTL3* mutations giving rise to a combined hypolipidemia phenotype in subjects with severe primary hypobetalipoproteinaemia is about 10%, and that HDL-cholesterol may be used to distinguish between *ANGPTL3* and *APOB* mutation related hypocholesterolemia.

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This study identified over 60 subjects in a small Italian town who were heterozygous or homozygous for the *ANGPTL3* S17X mutation. While homozygosity was associated with a combined hypolipidemia, heterozygotes only showed a significant reduction in total cholesterol and HDL-cholesterol compared to noncarriers.

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