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MINIREVIEW

Apolipoprotein A5 in Health and Disease

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Summary

High plasma levels of triglycerides (TG) are an independent risk factor in the development of cardiovascular disease, with about 50 % of the final levels being determined genetically. Apolipoprotein A5 (*APOA5*) is the last discovered member of the apolipoprotein *APOA1/C3/A4* gene cluster, found by comparative sequencing analysis. The importance of *APOA5* gene for determination of plasma triglyceride levels has been suggested after development of transgenic and knock-out mice (transgenic mice displayed significantly reduced TG, whereas knock-out mice had high TG). In Czech population, alleles C-1131 and Trp19 are associated with elevated levels of plasma TG and higher risk of myocardial infarction development. These alleles also play some role in nutrigenetics and actigenetics of lifestyle interventions leading to the plasma cholesterol changes as well as in the pharmacogenetics of statin treatment. On the contrary, *APOA5* mutations detected in Czech population did not show strict effect on plasma TG levels. Val153 → Met variant exhibit the sex-specific effect of HDL-cholesterol levels. The suggested roles of *APOA5* variants in determination of the plasma remnant particles, plasma concentrations of C-reactive protein or some anthropometrical parameters were excluded.

Key words

Apolipoprotein A5 • Triglycerides • Polymorphism • Myocardial infarction

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Introduction

Cardiovascular disease is the most common cause of death in industrialized countries, with high plasma triglyceride (TG) levels being suggested to be independent risk factor (Austin *et al*. 1987, Forester 2001). It is known, that plasma levels of TG are influenced by dietary composition, smoking, body weight and genetic factors. Similarly to the other risk factors, it is estimated that the contribution of genetic and environmental factors on plasma levels of TG is roughly the same.

The genetic predisposition to a high level of plasma TG levels has been intensively analyzed in last 15 years. Dozens of polymorphisms in different genes that could have some effect on plasma TG levels have been analyzed so far (for example Gehrisch 1999, Cohen *et al.* 1999, Talmud and Humphries 2001).

The most promising results are connected with variants within the apolipoproteins (*APO*) *APOA1/APOC3/APOA4* gene cluster. Especially the *APOC3* SstI (rs5128) polymorphism (Talmud and Humphries 2001) is known as the important genetic determinant of plasma TG levels. However the described results are not consistent (Buzza *et al.* 2001, Hubáček *et al.* 2001, Russo *et al.* 2001). Additionally, this polymorphism is located in the 3‘ untranslated sequence of the *APOC3* gene – thus the mechanism of its influence is not clear. One of the possibilities is that this variant is in linkage disequilibrium with another functional variant in the same region.

This lead to intensive investigation of the DNA sequence around the *APOA1/APOC3/APOA4* gene cluster. The available mice and human sequences around this locus were completed by sequencing and compared. About 200 000 bp of mouse and human sequences were analyzed and this comparison leads to the identification of evolutionary highly conserved sequence that contained a putative lipid-binding apolipoprotein gene. Matching this sequence with mouse expressed sequences suggested the presence of the new apolipoprotein – and apolipoprotein *A5* gene (*APOA5*) in *APOA1/APOC3/APOA4* gene cluster was defined (Pennacchio *et al.* 2001).

Construction of transgenic and knock-out mice definitely assessed the importance of this gene for plasma TG determination. The transgenic mice exhibited diminished, and the knock out mice elevated levels of plasma TG, while the plasma cholesterol levels were not influenced significantly (Pennacchio *et al.* 2001).

Description and function of apolipoprotein A5 gene

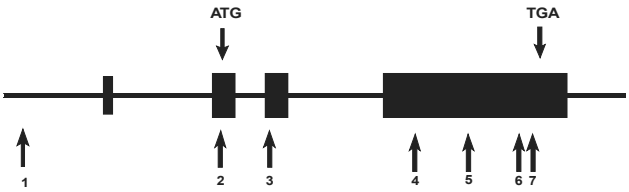
The whole sequence of the human *APOA5* gene was analyzed in details. The human *APOA5* gene consists of 4 exons and codes 369 aminoacid protein, which is expressed almost exclusively in the liver (Pennacchio *et al.* 2001).

ApoA5 is located on TG rich particles (chylomicrones and very low density lipoproteins – VLDL) and high density lipoprotein (HDL) particles. In comparison to other apolipoproteins, the plasma concentration of apoA5 is low in human – about 100 μg/l (O`Brien *et al.* 2005). Others detected that apoA5 binds to and enhances the activity of lipoprotein lipase (LPL) (Fruchart-Najib *et al.* 2004, Schaap *et al.* 2004). This lead in mice expressing human apoA5 to reduction of TG levels in VLDL particles. Additionally, the treatment with apoA5 lead in mice to a reduction of VLDL-TG production rate, but the concentration of the VLDL particles was the same as in normal mice (Fruchart-Najib *et al.* 2004, Schaap *et al.* 2004). This result was confirmed recently by Priore-Oliva *et al.* (2005) on patients with *APOA5* mutations resulting in premature stop codons (Gln145 → Stop and Gln 139 → Stop). The mutation Gln145 → Stop was present in homozygous form and plasma of this patient was found to activate LPL in vitro less efficiently than plasma from control subjects. These results confirmed that apoA5 plays a role in activation of LPL. Very recently, Dorfmeister *et al.* (2008) have demonstrated that recombinant APOA5 interacts with high affinity with the LDL receptor family members. This result suggests that plasma concentration of APOA5 could not be necessarily a reliable marker of its function and will not be of major importance.

Inside the *APOA5* gene more than 15 different variants have been detected (Pennacchio *et al.* 2002, summarized by Hubáček 2004, 2005), the majority of them were also detected in Czech population (Table 1).

Table 1. Summary of the apolipoprotein A5 gene variants analyzed in more details in the Czech population. A-3>C variant is in almost complete linkage disequilibrium with T-1131>C variant, thus, was not associated with details separately. N refers to the position on the figure.

N	*APOA5* variant	Population frequencies of variant allele	Association with TG levels
1	*T-1131>C*	~ 8.5 %	+++
2	*A-3>C*	~ 8.5 %	+++
3	*Ser19>Trp (C56>G)*	~ 7.2 %	+++
4	*Val153>Met (G457>A)*	~ 3.8 %	-
5	*Gly185>Cys (G553>C)*	Not detected	
6	*Ala315>Val (C944>T)*	< 1 %	?
7	*His321>Leu (A962>T)*	< 1 %	?



ATG – start codon, TGA, stop codon.

In collaborations with other groups we have screened the entire gene sequence for DNA variants in more than one hundred individuals and if the detected *APOA5* variants (both polymorphisms and mutations; mostly mismatched variants and variants located in promoter of the gene) are associated with or have effect on

1) plasma lipid levels (triglycerides, remnant particles, LDL cholesterol, HDL cholesterol, non-HDL cholesterol) in different groups of individuals of both genders
2) development of extreme hypertriglyceridemia

3) development of myocardial infarction
4) response to environmental changes – live style and pharmacological interventions in different population cohort, i.e. nutrigenetics, actigenetics and pharmacogenetics.
5) confirmatory analysis of the relationships between *APOA5* variants and different biochemical/ anthropometrical parameters reported by other research groups.

Additionally, we have analyzed apoA5 gene organisation, sequence and expression in Prague hypertriglyceridemic rats.

Association between *APOA5* and plasma lipid levels in general population

When 501 individuals (European-Americans, healthy non-smokers, without lipid lowering medications) were originally genotyped, an association has been found between T-1131 → C (rs662799) polymorphism as well as between Ser19 → Trp (C56 → G, rs3135506) variant and plasma levels of TG on random, high-fat as well as on low-fat diet (Pennacchio *et al.* 2001, 2002). In both cases, individuals with at least one less common allele had significantly higher TG levels than the others.

We have confirmed the original finding on representatively selected ethnically homogenous group of Czechs (1191 males and 1368 females, mean age 48.0±10.7 years), where plasma TG levels were analyzed twice independently in two different examinations (years 1997/8 and 2000/1) (Hubáček *et al.* 2003, 2004a).

T-1131 → C variation affects plasma TGs showing a higher level in C-1131 carriers than in T/T-1131 homozygotes. This association has been observed in both years (only the year 2001 is shown) in both males (2.40±1.63 mmol/l vs. 2.06±1.66 mmol/l) ($p<0.001$) and females (1.57±0.85 mmol/l vs. 1.43±0.85 mmol/l) ($p<0.001$). The same variant affects the plasma non-HDL cholesterol showing significantly higher levels in C-1131 carriers than in T/T-1131 homozygotes. This association has been observed in both males (4.61±1.09 mmol/l vs. 4.47±1.07 mmol/l, $p<0.01$) and females (4.46±1.22 mmol/l vs. 4.24±1.17 mmol/l, $p<0.01$). Interestingly, if LDL cholesterol (obtained by Friedewald formula) or HDL cholesterol were analyzed, no significant association was detected (Hubáček *et al.* 2008a). This may be explained by the fact, that the small but significant part of cholesterol is located in VLDL particles.

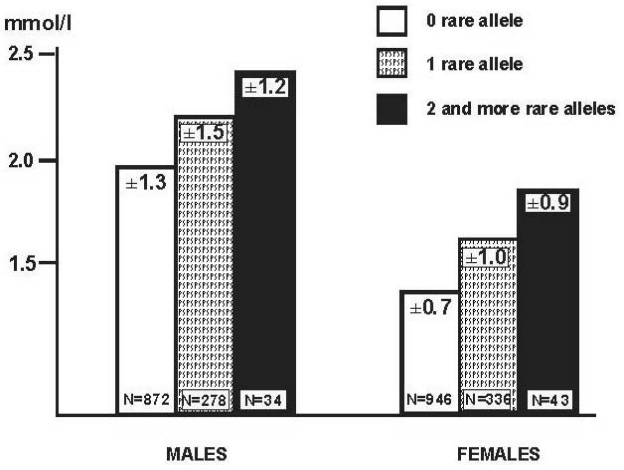


Fig. 1. Schematic summary of the additive effect of two common *APOA5* variants (T-1131 → C and Ser19 → Trp) on plasma triglycerides. Each less common individual allele (C-1131 and Trp19) adds ~ 10 % to the total value.

Plasma TG were also influenced by the Ser19 → Trp *APOA5* genotypes. In both males (2.40±1.97 mmol/l vs. 2.07±1.60 mmol/l) and females (1.65±1.02 mmol/l vs. 1.43±0.82 mmol/l), the Trp19 carriers have triglycerides significantly (both $p<0.001$) higher compared to the Ser19 homozygotes. All these results (Fig. 1) are consistent with the associations detected in all other Caucasian populations, where all studies find significant associations between TG concentrations and *APOA5* polymorphisms, despite the fact, that the strengths of the associations vary between studies (Hubáček 2005, Tai and Ordovas 2008).

Interestingly, the third common *APOA5* variant (Val153 → Met, G457 → A, rs3135507) has no effect on plasma TG in the same population. Nevertheless in females (but not in males) we have detected (Hubáček *et al.* 2005a) an association between this variant and plasma HDL cholesterol levels showing a higher level in Val/Val homozygotes than in Met carriers (1.52±0.37 mmol/l compared to 1.39±0.35 mmol/l, $p<0.01$).

Remnant lipoproteins (RLP) are product of catabolized TG-rich particles. Elevated levels of RLP are associated with atherosclerosis and they are a predictor of coronary events in patients with coronary artery disease, mainly in women (McNamara *et al.* 2001).

We have evaluated the influence of *APOA5* polymorphisms (T-1131 → C, Ser19 → Trp and Val153 → Met) on plasma levels of RLP-cholesterol and RLP-TG in 285 unrelated representative selected individuals (131 men and 154 women) aged 33-72 years. RLP-cholesterol and RLP-TG levels were not significantly

influenced by the *APOA5* variants either in whole population or in males and females, if analyzed separately. We conclude that variations T-1131 → C, Ser19 → Trp and Val153 → Met in the *APOA5* gene have no effect on plasma levels of remnant particles (Hubáček *et al.* 2004b).

In 369 patients with diabetes mellitus type 1 and 2 (202 males and 167 females), we have analyzed an association between *APOA5* variants T-1131 → C, Ser19 → Trp and Val153 → Met and plasma lipid levels. In contrast to healthy population, T-1131 → C was associated not only with plasma levels of triglycerides, but also with plasma levels of LDL-cholesterol (in these individuals, the LDL-cholesterol was not calculated, but directly measured in plasma) both in males and females. T-1131T homozygotes have significantly elevated plasma levels of LDL cholesterol (3.31±0.07 mmol/l vs. 2.98±0.14 mmol/l in females and 3.21±0.06 mmol/l vs. 2.95±0.16 mmol/l in males, both $p<0.05$) and significantly lower levels of TG (1.78±0.10 mmol/l vs. 2.31±0.14 mmol/l in females and 2.04±0.26 mmol/l vs. 3.03±0.73 mmol/l in females, both $p<0.05$) than carriers of the allele C-1131 (Hubáček, unpublished results).

Association between *APOA5* variants and extreme plasma triglyceride levels

As the common variants within the *APOA5* gene are associated with plasma TG levels, we have also analyzed them in the individuals with extremely high levels (more than 10 mmol/l; mean 20.4±12.8 mmol/l; n=83). Further we have sequenced the *APOA5* gene region in these patients in order to detect some new *APOA5* variants, which could be responsible for the extreme TG levels.

As expected, the frequencies of the carriers of the less common alleles C-1131 and Trp19 were more than twice higher (both $p<0.0001$) in hypertriglyceridemic patients (32.5 % and 30.1 % respectively) than in control population (15.4 % and 14.1 % respectively) (Hořínek *et al.* 2003, Vrablík *et al.* 2003).

No association between the Val153 → Met variant and extreme plasma levels of TG was found in the same group of patients (the frequencies of Met153 carriers were 6.5 % in controls and 9.6 % in patients). This implicated that Val153 → Met polymorphism in the *APOA5* gene does not represent an important risk factor for developing extreme levels of plasma TG (Hubáček *et al.* 2004c).

Additional sequencing of these individuals reveals the presence of the two rare *APOA5* mutations. Firstly, three carriers of the Ala315 → Val (C944 → T) variant have been detected within hypertriglyceridemic patients. However, in 3 302 representatively selected healthy individuals we have found another 22 carriers of the Val315 allele, 19 out of them having normal triglyceride levels and only three elevated triglyceride levels. Therefore, we conclude that this variant itself does not play an important role in genetic determination of hypertriglyceridemia (Hubáček *et al.* 2008b). It may interact with other genetic variants to cause hypertriglyceridemia. Secondly, one hypertriglyceridemic individual with His321 → Leu (A962 →T) mutation (the variant was not found in 282 controls) was detected (Dorfmeister *et al.* 2008), but detailed analysis did not reveal the major importance of this change for TG metabolism.

Independently, the *APOA5* variant Cys185 → Gly has been detected in Chinese population (Kao *et al.* 2003). We did not detect carriers of the Gly185 allele among 83 individuals with high plasma TG levels and 420 healthy individuals. We suppose that this variant is probably not present in Caucasian populations at all or the frequency is too low to have some detectable impact on plasma TG levels (Hubáček *et al.* 2004c).

Association between *APOA5* variants and myocardial infarction development

All together, we have analyzed the genotype frequencies of the three common *APOA5* polymorphisms in male patients under 65 years of age who survived theirs first myocardial infarction (MI) and compared them with the healthy control male population.

In a group of patients with MI (n=435), the frequency of the rare homozygotes for at least one *APOA5* variant (C/C-1131 and/or Trp/Trp19) was significantly (7.4 % vs. 2.0 %, $p<0.00001$) higher than in the population sample (n=1191) (Hubáček *et al.* 2003, 2004a). In contrast, the frequency of the Met153 carriers was not significantly different between these two groups (6.5 % vs. 6.4 %) (Hubáček *et al.* 2005a).

Association between *APOA5* variants and responses to interventions – nutrigenetics, actigenetics and pharmacogenetics

The possible interactions between *APOA5*

variants and environmental factors were analyzed in three studies with different design.

We have evaluated the influence of common variations in the *APOA5* gene on plasma lipid levels in 117 males for whom dietary composition markedly changed and total cholesterol decreased (from 6.21±1.31 mmol/l in 1988 to 5.43±1.06 mmol/l in 1996) over an 8-year follow-up study. *APOA5* T-1131 → C and Val153 → Met variants did not influence the change in these measures over time. In contrast the Ser19 → Trp polymorphism was strongly associated with a decrease in plasma total cholesterol over the 8-year time period. In Ser/Ser19 homozygotes the plasma cholesterol was relatively stable over the years (6.1±1.2 mmol/l in 1988 and 5.6±1.0 mmol/l in 1996, –8 %). In the Trp19 carriers the decrease of the plasma cholesterol was more than 25 % (6.5±1.6 mmol/l in 1988 and 5.1±1.0 mmol/l in 1996) ($p<0.005$). Changes in other analyzed lipid parameters (HDL-cholesterol, LDL-cholesterol, triglycerides) have not been associated with other *APOA5* variants (Hubáček *et al.* 2006, 2007).

Furthermore, 98 unrelated overweight and obese non-diabetic Czech females (BMI over 27.5 kg/m^2) whose underwent 9 weeks of lifestyle modification program consisting of a reduction of energy intake and the aerobic exercise were examined. No significant association between BMI decrease and *APOA5* variants was found, but T-1131T carriers have significantly higher body weight both before and after intervention ($p<0.05$ for BMI). Furthermore, plasma TG levels decreased in Ser19Ser homozygotes but increased in Trp19 carriers (1.42±0.62 mmol/l vs. 1.28±0.48 mmol/l compared to 1.15±0.47 mmol/l vs. 1.41±0.80 mmol/l, $p<0.05$). Similarly, in carriers of at least one less common *APOA5* allele (n=26), plasma LDL-cholesterol levels did not decreased as they did in T-1131T/Ser19Ser carriers (3.11±0.70 mmol/l vs. 3.27±0.81 mmol/l compared to 3.39±0.81 mmol/l vs. 3.16±0.86 mmol/l, $p<0.05$) (Suchánek *et al.* 2008).

Finally, we examined the putative association between *APOA5* SNPs (T-1131 → C, Ser19 → Trp and Val153→ Met) and efficacy of three months of low doses statin treatment in 188 adult Caucasians. Carriers of the *APOA5* genotype TT-1131 benefited more from statin treatment in comparison to the C-1131 allele carriers (Δ LDL-C –36.3±15.1 % vs. Δ LDL-C – 29.9±12.5 %; $p<0.005$, Mann-Whitney test) (Hubáček *et al.* 2009).

Confirmatory analysis of the relationships between *APOA5* variants and different biochemical/anthropometrical parameters reported by other research groups

Associations between C-1131 allele and higher mother's height as well as with longer fetal birth length were suggested by Ward *et al.* (2003). The explanation for this association was the hypothesis that the elevated TG levels could lead to a better intrauterine nutrition of the fetus.

In 1 305 females, aged between 28 and 67 years and having at least one child, we have analyzed a putative association between T-1131 → C *APOA5* variant and body height. Mother's body height did not differ between T/T homozygotes (n = 1 093, 162.5±6.5 cm) and C allele carriers (n = 212, 162.1±6.4 cm). Thus we have failed to confirm, that mothers with *APOA5* C-1131 allele are higher than T/T-1131 homozygotes (Hubáček *et al.* 2004d).

Association between the T-1131 → C *APOA5* variant and plasma concentrations of C-reactive protein in 158 young non-obese Korean males was described by Jang *et al.* (2004). Carriers of at least one allele C-1131 have higher plasma levels of C-reactive protein if compared to carriers of common T-1131T genotype. In 1119 Caucasian males, (49.2±10.8 years), we have analyzed a putative association between common *APOA5* variants and C-reactive protein concentrations (after log transformation). C-reactive protein levels did not differ between T/T-1131 homozygotes (n=946, 0.33±0.24 mg/l) and carriers of the C allele (n=173, 0.33±0.23 mg/l). Thus, in contrast to Korean males, in a large group of Caucasian males, T-1131 → C *APOA5* variant had no effect on plasma concentrations of C-reactive protein and also other *APOA5* variants (Ser19 → Trp and Val153 → Met) did not influence plasma concentrations of C-reactive protein (Hubáček *et al.* 2005b).

In two studies, the interaction between common polymorphism (E2, E3 and E4) in *APOE* gene, *APOA5* variant Ser19 → Trp and the occurrence of hypertriglyceridemia was analyzed.

Schaefer *et al.* (2004) found 7 *APOE2/E2* out of 170 screened individuals with elevated TG levels (over 2.3 mmol/l). Six of them also have Trp at position 19 in the *APOA5* gene. Additionally, they have failed to detect this combination in healthy normolipidemic individuals.

In contrast, we did not find significant association between *APOE2/E2* and presence of Trp19

allele in the *APOA5* gene in hypertriglyceridemic individuals (Hubáček *et al.* 2005c). However, in 111 patients with extreme TG levels (>10 mmol/l) more then 50 % of the patients with the Trp19 allele also have *APOE4* allele, in contrast to only 13 % of such individuals in the whole population ($p<0.001$).

We have detected sex-specific interactions between the variants in *APOA5* and *APOE* genes (Hubáček *et al.* 2008c) in the general population of 2500 representatively selected Caucasians (1168 males, 1332 females). In females (but not in males), an association between *APOE* polymorphism and total cholesterol (TC) was observed on the background of the common *APOA5* haplotype (TT-1131/SerSer19) - *APOE2* carriers have the lowest (5.12±1.15 mmol/l) and the *APOE4* carriers the highest (6.05±1.06 mmol/l) levels of plasma TC ($p<0.001$). If at least one *APOA5* C-1131 or Trp19 allele was present, *APOE* exhibited no significant effect on plasma TC. *APOA5* did not affect plasma TG levels, if *APOE4* allele was present. In the presence of *APOE2* or *APOE3*, carriers of the *APOA5* alleles C-1131 and/or Trp19 had higher TG levels (1.64±1.05 mmol/l) than others (1.37±0.75 mmol/l) ($p<0.01$). In males, no similar associations were observed.

ApoA5 gene organization, sequence and expression in Prague hereditary hypertriglyceridemic rat

Prague hereditary hypertriglyceridemic (HTG) rats are a useful model of human hypertriglyceridemia and other symptoms of metabolic syndrome (Vrána and Kazdová 1990). Thus, the variation of apoA5 gene and its expression were studied in this strain under normal conditions and after chronic fructose loading.

We have sequenced the whole *apoA5* gene of these animals and Lewis and Whistar rats served as normotriglyceridemic controls. There were no differences in gene structure or sequences between these strains of rats. Similarly, the apoA5 expression after feeding the animals with fructose diet (which stimulates the development of hypertriglyceridemia) did not display significant differences between the strains (Kadlecová *et al.* 2006). Thus, in this model, apoA5 plays no important role in determination of plasma triglycerides.

Conclusions

Gene for apolipoprotein *A5* and effect of its variants on different lipid parameters under different conditions were analyzed in details on different individuals of the Czech origin. Careful analysis has confirmed that some of its variants have an important effect on plasma triglyceride concentration but that they could also influence the risk of myocardial infarction.

1) C-1131 and Trp19 alleles are associated with elevated levels of plasma TG in both males and females. Similarly to the healthy individuals, plasma TG levels (and LDL-cholesterol) are affected by *APOA5* variant T-1131 → C also in patients with diabetes mellitus. Carriers of the Met153 allele have lower level of plasma HDL cholesterol, but this association is gender specific and valid just in the females. Concentration of remnant particles is not associated with *APOA5* variants in Caucasians.
2) In hypertriglyceridemic patients, the frequencies of C-1131 and Trp19 alleles are significantly higher than in the control population. No association with extreme TG levels and Val153 → Met variant was detected. Cys185 allele was not detected in these patients and theirs controls (~ 500 individuals together) at all – thus the presence of this allele is most probably race specific. The exact role of other identified mutations (Ala315 → Val, and His321 → Leu) in determination of hypertriglyceridemia remains to be clarified.
3) Carriers of at least one genotype C-1131C or Trp19Trp have higher risk of myocardial infarction development.
4) Males carrying at least one allele Trp19 have better response to dietary changes in the population cohort than Ser19Ser homozygotes. Combined dietary/exercise intervention and statin treatment could also be more beneficial for some subgroups defined by *APOA5* variants.
5) We failed to confirm the suggested associations of *APOA5* variants with either height of mothers or with plasma levels of C-reactive protein in males.
6) Certain, but so far not completely understood interaction between development of hypertriglyceridemia and *APOE* and *APOA5* genotypes is possible.

In conclusion, despite its low plasma concentration, *APOA5* gene and its variants is so far the strongest determinant of plasma triglycerides in humans.

Conflict of Interest

There is no conflict of interest.

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