Research article

The APOA5 Trp19 allele is associated with metabolic syndrome via its association with plasma triglycerides

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Abstract

Background: The goal of the present study was to assess the effect of genetic variability at the APOA5/A4/C3/A1 cluster locus on the risk of metabolic syndrome.

Methods: The APOA5 Ser19Trp, APOA5 -12,238T>C, APOA4 Thr347Ser, APOC3 -482C>T and APOC3 3238C>G (Sst1) polymorphisms were analyzed in a representative population sample of 3138 men and women from France, including 932 individuals with metabolic syndrome and 2206 without metabolic syndrome, as defined by the NCEP criteria.

Results: Compared with homozygotes for the common allele, the odds ratio (OR) [95% CI] for metabolic syndrome was 1.30 [1.03–1.66] (p = 0.03) for APOA5 Trp19 carriers, 0.81 [0.69–0.95] (p = 0.01) for APOA5 -12,238C carriers and 0.84 [0.70–0.99] (p = 0.04) for APOA4 Ser347 carriers. Adjustment for plasma triglycerides, (but not for waist girth, HDL, blood pressure or glycemia – the other components of metabolic syndrome) abolished these associations and suggests that triglyceride levels explain the association with metabolic syndrome. There was no association between the APOC3 -482C>T or APOC3 3238C>G polymorphisms and metabolic syndrome. The decreased risk of metabolic syndrome observed in APOA5 -12,238C and APOA4 Ser347 carriers merely reflected the fact that the APOA5 Trp19 allele was in negative linkage disequilibrium with the common alleles of APOA5 -12,238T>C and APOA4 Thr347Ser polymorphisms.

Conclusion: The APOA5 Trp19 allele increased susceptibility to metabolic syndrome via its impact on plasma triglyceride levels.

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Background
Metabolic syndrome is a complex disease characterized by the clustering of several metabolic disorders [1,2]. Excess body weight, insulin resistance, altered plasma lipid levels & glucose homeostasis and increased blood pressure are the principal components of this cluster. Environmental influences (such as low physical activity and an inappropriate diet) play a major role in the development of metabolic syndrome. Furthermore, familial aggregation of metabolic disorders has been reported and suggests a genetic contribution to the etiology of this syndrome [3-5]. Accordingly, it has been reported that genetic variability at several loci is associated with an increased risk of metabolic syndrome [6-12].

The APOA5 gene is located on chromosome 11q23 and codes for a 369 amino-acid protein secreted by the liver [13]. In the plasma, APOA5 is bound to triglyceride-rich and high-density lipoproteins. APOA5 reduces plasma triglyceride levels by inhibiting VLDL-triglyceride production and stimulating LPL-mediated triglyceride hydrolysis [14-18]. In vitro, insulin decreases APOA5 promoter activity – suggesting that the hormone may affect triglyceride levels via an interaction with APOA5 [19]. Given its role in triglyceride metabolism and its interaction with insulin, the APOA5 gene is a candidate gene for metabolic syndrome.

Several common single nucleotide polymorphisms (SNPs) have been described in the APOA5 gene locus; these include a T>C substitution at -12,238 (SNP4), a T>C substitution at -1131 (SNP3; rs662799) and a C>G mutation at the first base of codon 19 (rs3135506; c.56C>G) that changes a serine (Ser) residue into a tryptophan (Trp) [20]. An association between the APOA5 Ser19Trp polymorphism and plasma triglycerides has been reported in several population samples [13,20-23]. In contrast, an association between the APOA5 -12,238T>C polymorphism and plasma lipid variables has not been consistently observed. Together with APOA4, APOC3 and APOA1, the APOA5 gene lies within an expanded gene cluster [13,24]. Strong linkage disequilibrium (LD) has been detected for SNPs in this cluster [24]. The APOA5 Ser19Trp and APOC3 3238C>G SNPs (SstI; rs5128; c.386C>G) both tag common haplotypes in this cluster – haplotypes that may have independent effects on plasma triglycerides.

The goal of the present study was to assess the relative contribution of SNPs in the APOA5/A4/C3/A1 locus to the risk of metabolic syndrome by analyzing a limited number of representative SNPs within the cluster and a large population-based sample from 3 areas of France.

Methods
Subjects
Participants were recruited as part of the WHO-MONICA population survey conducted from 1995 to 1997 in three different parts of France: the Lille Urban Community in northern France (n = 1168), the Bas-Rhin county in eastern France (n = 1117) and the Haute-Garonne county in southern France (n = 1176). The protocol was approved by the appropriate independent ethics committee in each center. Subjects (aged 35–64) were randomly selected from electoral rolls after stratification by town size, gender and age in order to obtain 200 participants for each gender and each 10-year age group (WHO-MONICA Project protocol) [25]. A total of 1746 men and 1715 women completed the recruitment procedure. Subsequently, 323 subjects were excluded due to missing data on at least one criterion for metabolic syndrome.

After providing written informed consent, participants filled out a standard questionnaire and physical measurements were taken by a specially trained nurse. The questionnaire covered questions on socioeconomic factors, physical activity, alcohol consumption, smoking status, personal & family medical history and current medications taken (if any). The level of leisure-time physical activity during was categorized as follows: none, light (light physical activity almost every week), intense (at least 20 minutes of intense physical activity more than once a week). In terms of smoking exposure, subjects were categorized as never-smokers, former smokers and current smokers (i.e. subjects reporting at least one cigarette per day). Total alcohol intake was expressed as the sum of ml alcohol per week from wine, beer, cider and spirits. Alcohol consumption was categorized in 4 Q1-Q4 classes for men [≤100 ml/week; 100–250 ml/week; 250–500 ml/week; >500 ml/week] and for women [0 ml/week; 0–60 ml/week; 60–190 ml/week; >190 ml/week]. Educational level was assessed by counting the number of years of schooling and classifying the values into 3 categories: primary, secondary/technical and university. Anthropometric measurements included body weight (rounded to the nearest even decimal), waist girth (at a level midway between the lower rib margin and the iliac crest, to the nearest 0.5 cm) and were performed on subjects in light clothing without shoes. Body mass index was calculated according to the Quetelet equation.

Diabetic subjects were identified by fasting glycemia ≥126 mg/dl or antidiabetic treatment. Abdominal obesity was defined by waist girth ≥102 cm in men and ≥88 cm in women. Metabolic syndrome was defined (according to the NCEP III recommendations [26]) by the presence of at least 3 or more of the following abnormalities: waist girth...
was undetermined. The
GCACTGAGAATACTGTCCCTTT-3'. In the sample, 3.5%
GCTACCTGCCTATCCATCCTGCG-3' and APOC3-R: 5'-
CTGCC-3', APOC3-F2: 5'-GAAGGTGACCAAGTTCAT-
GAAGGTCGGAGTCAACGGATTGAGTAAAGGCACAGAAGACCA-3'
using the following probes: APOC3-F1: 5'-

Laboratory methods

A 20 mL blood sample was drawn into a disodium EDTA
tube (after the subjects had fasted for at least 10 hours),
stored at room temperature and centrifuged within 4
hours. All measurements were performed in a central lab-
oratory (Purpan Hospital, Toulouse, France). Cholesterol
and triglyceride levels were measured using enzyme assays
(Boehringer Mannheim, Mannheim, Germany). High
density lipoprotein (HDL) cholesterol was measured after
sodium phosphotungstate/magnesium chloride precipi-
tation (Boehringer Mannheim, Mannheim, Germany).
Glucose was measured using the standard glucose hexoki-
tation (Boehringer Mannheim, Mannheim, Germany).

Results

Table 1 shows the biological and clinical characteristics of
the subjects who were homozygous for the frequent allele.
Likewise, the polymorphism was also assessed in an Amplifluor®
system using the following primers: forward 5'-

APOA5 Ser19Trp polymorphism was assessed in an
Amplifluor® genotyping system using the following
probes: APOA5-F1: 5' GAAGGTGACCAAGTTCATGCCT-
CCTTCACACGGCTTTTC-3'; APOA5-F2: 5'-
GAAGGTCCAGTTCAACGGATTTCCCTCACCAGGGTTTG-3'
and APOA5-R: 5'-TGAAGTATGCCAGAGCCGCTTTT-3'. The sample, 1.6% was undetermined.

A 20 mL blood sample was drawn into a disodium EDTA
tube (after the subjects had fasted for at least 10 hours),
stored at room temperature and centrifuged within 4
hours. All measurements were performed in a central lab-
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and triglyceride levels were measured using enzyme assays
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density lipoprotein (HDL) cholesterol was measured after
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tation (Boehringer Mannheim, Mannheim, Germany).

Statistical analyses

General linear models (GLM) and chi-square tests were used
to compare the clinical and biological characteristics
of subjects with or without metabolic syndrome. Further-
more, GLM and Chi-square tests were used to compare
centers in terms of mean values and the distributions of
variables and genotypes, respectively. A GLM adjusted for
age, gender and center was used to compare genotype
groups in terms of metabolic syndrome criteria. Subjects
receiving a treatment that may have affected the level of
the variable were excluded from these analyses. Chi-
square tests and logistic regression analyses were used to
test the association between the various genotypes and
metabolic syndrome. For logistic regression analyses, car-
riers of at least one minor allele were compared with sub-
jects who were homozygous for the frequent allele
(dominant model), while adjusting for age, gender and
center. The threshold for statistical significance was set to
5%. Linkage disequilibrium between loci was tested using
a log-likelihood-ratio test [29]. Disequilibrium was
expressed in terms of normalized difference D' = D/Dmax
or D/Dmin [30]. Haplotype frequencies were estimated
using a stochastic version of the expectation-maximiza-
tion algorithm, as implemented in Thesias software
[31,32]. Differences in haplotype frequencies when com-
paring groups of subjects with or without metabolic syn-
drome were examined using a log-likelihood ratio statistic
test, which was computed from the haplotype frequency
log-likelihoods for each of the two groups estimated in
Thesias.

Results

Table 1 shows the biological and clinical characteristics of
the subjects with (n = 932) and without (n = 2206) me-
tabolic syndrome as defined by NCEP III. As expected, body
mass index, waist girth, triglyceridemia, cholesterolemia,
glycemia, systolic and diastolic blood pressure were
higher and HDL-cholesterol levels were lower in subjects
with metabolic syndrome than in subjects without meta-

tabolic syndrome (all p < 0.0001). Subjects with metabolic
syndrome were less physically active and had spent less
time in education than those without metabolic syn-
drome. The proportion of smokers was lower in the meta-
abolic syndrome group.

The APOA5 Ser19Trp, APOA5 -12,238T>C, APOA4
Thr347Ser, APOC3 -482C>T and APOC3 3238C>G geno-
types did not deviate from Hardy-Weinberg equilibrium.
Table 2 shows the linkage disequilibrium matrix in sub-
jects without metabolic syndrome. Overall, the D' varied
from 0.29 to 1. The APOA5 and APOA4 SNPs were in the
same haplotype block (\(|D'| \geq 0.85\)); however, due to differences in minor allele frequencies, the \(r^2\) values were almost nil. Within this block, both APOA5 -12,238T>C and APOA4 Thr347Ser SNPs were in negative disequilibrium with the APOA5 Ser19Trp SNP. Both APOC3 SNPs were within the same linkage disequilibrium block (\(D' = 0.91\)). Lastly, the APOA4 Thr347Ser SNP was in negative linkage disequilibrium with the APOC3 3238C>G SNP (\(D' = -0.92\)) and thus showed some overlap with the APOC3 haplotype block.

Table 3 shows the metabolic syndrome-related anthropometric, biological and clinical parameters in subjects without metabolic syndrome and as a function of the 5 polymorphisms. Multivariate analyses (adjusted for age, gender and center) revealed that mean triglyceride levels were significantly higher in APOA5 Trp19 (\(p < 0.0001\); p

Table 1: Anthropometric, clinical and biological characteristics of subjects with or without metabolic syndrome

|                         | Without metabolic syndrome | With metabolic syndrome | p       |
|-------------------------|----------------------------|-------------------------|---------|
| n                       | 2206                       | 932                     | <0.0001 |
| age (y)                 | 49.4 ± 8.4                 | 54.2 ± 8.1              |         |
| men                     | 1044 (47.3)                | 553 (59.3)              | <0.0001 |
| BMI (kg/m²)             | 24.8 ± 3.6                 | 29.9 ± 4.7              | <0.0001 |
| Waist (cm)              | 85.3 ± 11.3                | 101.5 ± 11.6            | <0.0001 |
| Cholesterol (mmol/l)    | 5.8 ± 1.0                  | 6.1 ± 1.1               | <0.0001 |
| Triglycerides (mmol/l)  | 1.00 ± 0.52                | 2.20 ± 2.32             | <0.0001 |
| HDL (mmol/l)            | 1.58 ± 0.43                | 1.19 ± 0.34             | <0.0001 |
| Glucose (mmol/l)        | 5.20 ± 0.76                | 5.95 ± 1.30             | <0.0001 |
| Diabetes (%)            | 2.4                        | 19.5                    | <0.0001 |
| SBP (mm Hg)             | 125.7 ± 15.8               | 140.6 ± 16.9            | <0.0001 |
| DBP (mm Hg)             | 79.0 ± 10.1                | 87.9 ± 11.2             | <0.0001 |

| Smoking                 |                            |                         | 0.032   |
| never                   | 1069 (48.5)                | 432 (46.4)              |         |
| former                  | 629 (28.5)                 | 308 (33.0)              |         |
| current                 | 508 (23.0)                 | 192 (20.6)              |         |

| Physical activity       |                            |                         | <0.0001 |
| none                    | 449 (20.4)                 | 244 (26.3)              |         |
| light                   | 1058 (48.0)                | 490 (52.7)              |         |
| intense                 | 697 (31.6)                 | 195 (21.0)              |         |

| Alcohol intake          |                            |                         | 0.0013  |
| Q1                      | 760 (34.5)                 | 303 (32.5)              |         |
| Q2                      | 574 (26.0)                 | 201 (21.6)              |         |
| Q3                      | 535 (24.3)                 | 241 (25.9)              |         |
| Q4                      | 337 (15.2)                 | 187 (20.0)              |         |

| Educational level       |                            |                         | <0.0001 |
| low                     | 433 (19.6)                 | 312 (33.5)              |         |
| intermediate            | 952 (43.2)                 | 410 (44.0)              |         |
| high                    | 821 (37.2)                 | 210 (22.5)              |         |

Values are means± SD or n (%).
BMI : body mass index ; HDL : high density lipoprotein ; SBP/DBP : systolic and diastolic blood pressure ; alcohol intake is in quartiles.

Table 2: \(D'\) and \(r^2\) values for linkage disequilibrium among polymorphisms in subjects without metabolic syndrome

|                  | APOA5 Ser19Trp | APOA5 -12,238 T>C | APOA4 Thr347Ser | APOC3 -482 C>T | APOC3 3238 C>G |
|------------------|----------------|-------------------|----------------|----------------|----------------|
| APOA5 Ser19Trp   | -              | -0.85             | -1             | -0.73          | -0.41          |
| APOA5 -12,238 T>C| 0.02           | -                 | 0.95           | 0.29           | -0.58          |
| APOA4 Thr347Ser  | 0.02           | 0.40              | -              | 0.45           | -0.92          |
| APOC3 -482 C>T   | 0.01           | 0.06              | 0.13           | -              | 0.91           |
| APOC3 3238 C>G   | 0.00           | 0.02              | 0.02           | 0.23           | -              |

\(D'\) upper right corner ; \(r^2\) lower left corner
< 0.0001 in a dominant model), APOC3 -482T (p = 0.04 in a dominant model) and APOC3 3238G (p < 0.0001; p < 0.0001 in a dominant model) allele carriers. Conversely, mean triglycerides were lower in APOA5 -12,238C (p < 0.01; p = 0.003 in a dominant model), APOA4 Ser347 (p = 0.04 in a dominant model) allele carriers than in non-carriers. The mean HDL-cholesterol value was significantly higher in APOA5 -12,238C (p = 0.04; p = 0.02 in a dominant model) allele carriers than in non-carriers. Systolic and diastolic blood pressures were higher in APOA5 Trp19 carriers (p = 0.03 in a dominant model) and systolic blood pressure was lower in APOA4 Ser347 carriers (p = 0.03 in a dominant model). There were no other statistically significant associations for any of the SNPs. Further adjustment for physical activity, alcohol consumption, smoking habits and educational level yielded similar results (data not shown). These associations were similarly observed in all three centers (i.e. there was no significant interaction with geographical area).

In order to assess the contribution of the genetic variability to plasma triglyceride levels, haplotype analyses were performed in subjects without metabolic syndrome using the 5 SNPs in the following order: APOA5 (Ser(C)19Trp(G), -12,238T>C), APOA4 (Thr(A)347Ser(T)) and APOC3 (-482C>T, 3238C>G). Eight haplotypes

### Table 3: Mean levels of metabolic syndrome-related anthropometric, biological and clinical parameters in subjects without metabolic syndrome according to the 5 polymorphisms

| APOA5 Ser19Trp (p) | Ser19Ser | Ser19Trp (p) | Trp19Trp (p) | p | p^a | p^b |
|--------------------|---------|-------------|-------------|-----|-----|-----|
| Waist (cm)          | 85 ± 11 | 84 ± 11     | 81 ± 8      | 0.49 | 0.23 | 0.77 |
| TG (mmol/l)         | 1.26 ± 1.1 | 1.59 ± 2.4 | 2.46 ± 2.3 | <0.0001 c | <0.0001 c | 0.07 c |
| HDL (mmol/l)        | 1.48 ± 0.4 | 1.49 ± 0.5 | 1.2 ± 0.5 | 0.56 | 0.78 | 0.59 |
| Glucose (mmol/l)    | 5.4 ± 1 | 5.4 ± 0.8 | 5.5 ± 0.4 | 0.72 | 0.43 | 0.83 |
| SBP (mm Hg)         | 129 ± 17 | 131 ± 18 | 135 ± 13 | 0.081 | 0.03 | 0.47 |
| DBP (mm Hg)         | 81 ± 11 | 82 ± 10 | 84 ± 10 | 0.082 | 0.03 | 0.39 |

| APOA5 -12,238 T>C (p) | TT | TC | CC |
|-----------------------|----|----|----|
| Waist (cm)            | 85 ± 11 | 85 ± 11 | 85 ± 11 | 0.49 | 0.23 | 0.15 |
| TG (mmol/l)           | 1.31 ± 1.0 | 1.29 ± 1.4 | 1.32 ± 1.9 | 0.01 c | 0.003 c | 0.08 c |
| HDL (mmol/l)          | 1.47 ± 0.4 | 1.49 ± 0.4 | 1.52 ± 0.5 | 0.04 | 0.02 | 0.10 |
| Glucose (mmol/l)      | 5.4 ± 1 | 5.4 ± 1.1 | 5.3 ± 0.8 | 0.31 | 0.52 | 0.15 |
| SBP (mm Hg)           | 129 ± 17 | 129 ± 18 | 129 ± 17 | 0.24 | 0.11 | 0.30 |
| DBP (mm Hg)           | 81 ± 11 | 81 ± 11 | 81 ± 10 | 0.79 | 0.54 | 0.49 |

| APOA4 Thr347Ser (p) | Thr347Thr | Thr347Ser (p) | Ser347Ser (p) | p | p^a | p^b |
|---------------------|------------|---------------|---------------|-----|-----|-----|
| Waist (cm)          | 85 ± 11 | 86 ± 11 | 86 ± 11 | 0.64 | 0.71 | 0.39 |
| TG (mmol/l)         | 1.31 ± 1.0 | 1.30 ± 1.8 | 1.22 ± 0.8 | 0.11 c | 0.04 c | 0.69 c |
| HDL (mmol/l)        | 1.48 ± 0.4 | 1.49 ± 0.4 | 1.55 ± 0.5 | 0.24 | 0.24 | 0.09 |
| Glucose (mmol/l)    | 5.4 ± 1.1 | 5.4 ± 0.9 | 5.2 ± 0.7 | 0.44 | 0.63 | 0.20 |
| SBP (mm Hg)         | 130 ± 18 | 129 ± 17 | 127 ± 16 | 0.09 | 0.03 | 0.82 |
| DBP (mm Hg)         | 81 ± 11 | 81 ± 11 | 81 ± 10 | 0.92 | 0.83 | 0.84 |

| APOC3 -482 C>T (p) | CC | CT | TT |
|--------------------|----|----|----|
| Waist (cm)         | 85 ± 11 | 85 ± 11 | 85 ± 11 | 0.75 | 0.78 | 0.49 |
| TG (mmol/l)        | 1.37 ± 1.1 | 1.34 ± 1.4 | 1.33 ± 1 | 0.09 c | 0.04 c | 0.07 c |
| HDL (mmol/l)       | 1.47 ± 0.4 | 1.49 ± 0.4 | 1.51 ± 0.4 | 0.68 | 0.54 | 0.66 |
| Glucose (mmol/l)   | 5.4 ± 1 | 5.4 ± 1 | 5.4 ± 0.8 | 0.93 | 0.78 | 0.87 |
| SBP (mm Hg)        | 129 ± 17 | 129 ± 18 | 128 ± 17 | 0.47 | 0.45 | 0.28 |
| DBP (mm Hg)        | 81 ± 10 | 81 ± 11 | 81 ± 11 | 0.93 | 0.72 | 0.85 |

| APOC3 3238 C>G (p) | CC | CG | GG |
|--------------------|----|----|----|
| Waist (cm)         | 85 ± 11 | 86 ± 11 | 84 ± 15 | 0.25 | 0.1 | 0.66 |
| TG (mmol/l)        | 1.27 ± 1.4 | 1.44 ± 1.2 | 1.56 ± 1.2 | <0.0001 c | <0.0001 c | 0.05 c |
| HDL (mmol/l)       | 1.49 ± 0.4 | 1.47 ± 0.4 | 1.43 ± 0.4 | 0.15 | 0.6 | 0.29 |
| Glucose (mmol/l)   | 5.4 ± 1 | 5.4 ± 0.8 | 5.5 ± 0.6 | 0.55 | 0.35 | 0.79 |
| SBP (mm Hg)        | 129 ± 17 | 129 ± 17 | 134 ± 21 | 0.19 | 0.52 | 0.28 |
| DBP (mm Hg)        | 81 ± 11 | 81 ± 11 | 84 ± 12 | 0.12 | 0.37 | 0.21 |

p values adjusted on age, gender and center; p^a and p^b values for a dominant and recessive models, respectively; analyses on log-transformed data; BMI: body mass index; TG: triglycerides; HDL: high density lipoprotein; SBP/DBP: systolic and diastolic blood pressure; Exclusion of subjects treated with fibrates, oral antidiabetic or insulin, BP lowering therapy.
had an estimated frequency of above 1% (accounting for more than 97% of the existing haplotypes) and were used in the analyses (Table 4). The overall association between haplotypes and triglyceride levels was highly significant (p < 4 x 10^{-8}). By reference to the most common haplotype (CTACC; estimated frequency: 0.45), two haplotypes were significantly associated with raised plasma triglycerides. The GTACC haplotype (estimated frequency: 0.05) that carried the APOA5 Trp19 allele on the common background was associated with a 30% increase in triglyceride levels (p < 10^{-6}). The other haplotype (CTATG) carried both the APOC3 -3238G and 33% greater risk of metabolic syndrome (p < 0.005). The APOA4 Thr347Ser (p = 0.034) SNPs. The adjusted odds ratio (OR) and 95% confidence interval (CI) for metabolic syndrome in carriers of at least one minor allele (compared with subjects homozygous for the frequent allele) are presented in Table 6. The APOA5 Trp19 allele conferred an increased risk of metabolic syndrome (p = 0.03). In contrast, the APOA5 -12,238C (p = 0.01) and the APOA4 Ser347 (p = 0.04) alleles were associated with a lower risk of metabolic syndrome. Adjustment for plasma triglycerides but not for other metabolic syndrome criteria (i.e. waist girth, HDL, glycemia and systolic blood pressure) abolished these associations. Lastly, there was no significant association between the APOC3 -482C>T and 3238G>C polymorphisms and metabolic syndrome. Using the IDF criteria for metabolic syndrome, the ORs [95%CI] for metabolic syndrome were similar in magnitude (data not shown).

Haplotype analyses using the 5 SNPs were performed to assess the relationship with metabolic syndrome. The overall association between the haplotypes and metabolic syndrome was statistically significant (p = 0.011). With respect to the most common haplotype (CTACC), 2 haplotypes were significantly associated with metabolic syndrome. The GTACC haplotype (carrying the APOA5 Trp19 allele on the common background) was associated with a 33% greater risk of metabolic syndrome (p < 0.005). The other haplotype (CCTTC, carrying the APOA5 -12,238G, the APOA4 Ser347 and the APOC3 -482C>T alleles) was associated with a 26% reduction in metabolic syndrome risk (p = 0.03). Since the APOC3 and APOA5 SNPs are in different haplotype blocks, the association with metabolic syndrome was tested for the two blocks independently. Using only the SNPs of APO5 (Ser19Trp, -12,238T>C) and APOA4 Thr347Ser, we found 4 possible haplotypes with a frequency of above 1%. When compared with the common haplotype (CTA; estimated frequency: 0.58), the GT (estimated frequency 0.05) and the CG (estimated frequency 0.19) haplotypes were respectively associated with a 33% greater risk (p < 0.02) and a 17% lower risk (p = 0.034) of metabolic syndrome. Both associations disappeared after adjustment for triglyceride levels. In contrast,

Table 5 shows the genotype distribution of the 5 SNPs in subjects with or without metabolic syndrome. The two groups of subjects differed in terms of the genotype distribution of APOA5 -12,238T>C (p = 0.026) and APOA4 Thr347Ser (p = 0.034) SNPs. The adjusted odds ratio (OR) and 95% confidence interval (CI) for metabolic syndrome in carriers of at least one minor allele (compared with subjects homozygous for the frequent allele) are presented in Table 6. The APOA5 Trp19 allele conferred an increased risk of metabolic syndrome (p = 0.03). In contrast, the APOA5 -12,238C (p = 0.01) and the APOA4 Ser347 (p = 0.04) alleles were associated with a lower risk of metabolic syndrome. Adjustment for plasma triglycerides but not for other metabolic syndrome criteria (i.e. waist girth, HDL, glycemia and systolic blood pressure) abolished these associations. Lastly, there was no significant association between the APOC3 -482C>T and 3238G>C polymorphisms and metabolic syndrome. Using the IDF criteria for metabolic syndrome, the ORs [95%CI] for metabolic syndrome were similar in magnitude (data not shown).

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Table 4: Haplotype frequencies and delta values of mean triglycerides in subjects without metabolic syndrome

| Haplotype | Frequency | ΔTG | p   |
|-----------|-----------|-----|-----|
| CTACC     | 0.454     | -   |     |
| CCACC     | 0.130     | 0.037 | 0.13 |
| CCTTC     | 0.018     | 0.003 | 0.93 |
| CTATG     | 0.079     | 0.165 | <10^{-4} |
| CCTCC     | 0.075     | -0.004 | 0.92 |
| GTACC     | 0.050     | 0.165 | <10^{-4} |
| CTATC     | 0.043     | -0.021 | 0.71 |
| CCATC     | 0.022     | -0.013 | 0.83 |

Only haplotypes with a frequency above 1% are represented. Order of the SNPs: APOA5 (Ser(C)19Trp(G), 12,238T>C), APOA4 (Thr(A)347Ser(T)) and APOC3 (-482C>T, 3238G>C). p values adjusted for age, gender and center.

Table 5: Genotype distributions in subjects with or without metabolic syndrome (MS)

| SNP       | Ser19Ser | Ser19Trp | Trp19Trp | p   |
|-----------|----------|----------|----------|-----|
| Without MS| 1960 (88.8) | 241 (10.9) | 5 (0.2) | 0.10 |
| With MS   | 807 (86.6) | 120 (12.9) | 5 (0.5) |     |
| APOA5 -12,238 T>C | TT | CT | CC |     |
| Without MS| 912 (41.3) | 995 (45.1) | 299 (13.5) | 0.026 |
| With MS   | 413 (44.3) | 424 (45.5) | 95 (10.2) |     |
| APOA4 Thr347Ser | Thr347Thr | Thr347Ser | Ser347Ser |     |
| Without MS| 1412 (64.0) | 702 (31.8) | 92 (4.2) | 0.034 |
| With MS   | 627 (67.3) | 282 (30.3) | 23 (2.5) |     |
| APOC3 -482 C>T | CC | CT | TT |     |
| Without MS| 1148 (52.0) | 894 (40.5) | 164 (7.4) | 0.26 |
| With MS   | 508 (54.5) | 349 (37.4) | 75 (8.0) |     |
| APOC3 3238 C>G | CC | CG | GG |     |
| Without MS| 1799 (81.5) | 388 (17.6) | 19 (0.86) | 0.71 |
| With MS   | 750 (80.5) | 172 (18.4) | 10 (1.1) |     |

Values are number (%).

Table 6: Odds ratios for metabolic syndrome in carriers of the minor allele

| SNP       | OR      | [95% CI] | p   |
|-----------|---------|----------|-----|
| APOA5 Ser19Trp | 1.30 | [1.03–1.66] | 0.03 |
| APOA5 -12,238 T>C | 0.81 | [0.69–0.95] | 0.01 |
| APOA4 Thr347Ser | 0.84 | [0.70–0.99] | 0.04 |
| APOC3 -482 C>T | 0.89 | [0.76–1.06] | 0.17 |
| APOC3 3238 C>G | 1.03 | [0.84–1.26] | 0.76 |

The reference groups were homozygous subjects for the frequent allele. Odds ratios and p values adjusted for age, gender and center.
haplotype analyses with the two APOC3 SNPs did not reveal any significant association with metabolic syndrome.

Discussion

The goal of the present study was to assess the contribution of SNPs in the APOA5/A4/C3/A1 cluster to the risk of metabolic syndrome. Our results showed that the APOA5 Trp19 allele was associated with an increased risk of metabolic syndrome and that this association could possibly be explained by an increase in plasma triglyceride levels. Furthermore, the APOA5 -12,238C and APOA4 Ser347 alleles were associated with a lower risk of metabolic syndrome – possibly due to their negative linkage disequilibrium with the APOA5 Trp19 allele. In contrast, the APOC3 -482C>T and 3238C>G SNPs did not appear to be related to metabolic syndrome in this French sample.

All 5 SNPs of the cluster tested in this study were associated with triglyceride levels. Compared with the most common haplotype, haplotypes that carried either the APOA5 Trp19 allele only or both the APOC3 -482T and APOC3 3238G alleles were found to be independently associated with elevated triglycerides. These results are consistent with earlier studies in different population samples that reported similar linkage disequilibrium patterns and associations with triglycerides (driven by the APOA5 Trp19 and APOC3 -482T alleles [20]). In addition to elevated triglyceride levels, the APOA5 Trp19 and APOA4 Thr347 alleles were associated with higher blood pressure. A direct effect of APOA5 or APOA4 on blood pressure regulation is unlikely. In contrast, there is experimental evidence to suggest that chronic hypertriglyceridemia leads to endothelium dysfunction, which is associated with an impaired response to vasodilator stimulation [33] and a subsequent decrease in nitric oxide availability [34] – phenomena which may result in increased blood pressure.

The APOA5 Trp19 allele increased the odds ratio for metabolic syndrome, whereas the APOA5 -12,238C and APOA4 Ser347 alleles decreased it. Other studies have reported associations between APOA5 SNPs and metabolic syndrome [8-12]. Our results extend this observation to a different European population sample. The reduced risk of metabolic syndrome observed in carriers of APOA5 -12,238C and APOA4 Ser347 alleles merely reflected the negative linkage disequilibrium existing between these alleles and the APOA5 Trp19 allele. Several lines of evidence suggest that the association with metabolic syndrome is mediated by triglycerides. Firstly, the APOA5 Trp19 allele is associated strongly and independently with triglyceride levels but far less so with blood pressure. Secondly, adjustment for triglycerides (but not for other metabolic syndrome-related criteria) abolished the associations between APOA5 or APOA4 SNPs and the risk of metabolic syndrome. Overall, this suggests that APOA5 and APOA4 genetic variability affected susceptibility to metabolic syndrome.

In contrast, the APOC3 -482C>T and APOC3 3238C>G SNPs did not significantly affect the risk of metabolic syndrome in this sample of French origin, despite significant association with triglyceride level. These SNPs do not belong to the same haplotype as the APOA5 Ser19Trp SNP and have an independent effect on plasma triglycerides, which may explain the difference vis-à-vis APOA5. Furthermore and in contrast to the APOA5 Ser19Trp SNP, the APOC3 SNPs were not associated with another component of metabolic syndrome (such as blood pressure) which could explain this difference. Lastly, the prevalence of hypertriglyceridemia was higher in carriers of the APOA5 Trp19 allele (35%) than in carriers of the APOC3 -482T (23%) or APOC3 3238G (22%) alleles and thus increased the probability of fulfilling the definition of metabolic syndrome.

This study has both strengths and limitations. It was performed on a representative sample of the French population and therefore avoided potential selection bias for patients and controls. There were more than 900 subjects with metabolic syndrome, which yields enough statistical power to detect an OR of 1.3 for an allele frequency of 10% at α = 0.05 and β = 90%. Despite this large number of subjects with metabolic syndrome, the statistical power of the study was still not sufficient to account for possible type 1 error due to multiple testing. Therefore, corrections for multiple testing were not applied in the present analysis. In our sample, subjects without metabolic syndrome were, on average, 5 years younger than those with metabolic syndrome. Since metabolic syndrome prevalence increases with age, this may possibly confound (underestimate) the odds ratio associated with the SNPs. However, all our analyses were adjusted for age, in order to account for a possible impact of the latter parameter on the association. Since only a few SNPs per gene were studied, we cannot completely rule out the possibility that other SNPs in different gene loci or specific haplotypes can also contribute to genetic susceptibility to metabolic syndrome. Indeed, other studies have reported associations between APOA5 SNPs and metabolic syndrome [8-12]. Lastly, we used the NCEP working definition of metabolic syndrome; however, additional analyses using the IDF consensus definition generated very similar conclusions and suggested that choice of the definition of metabolic syndrome does not affect the relationship between metabolic syndrome and the SNPs studied here.

Conclusion

Over the past decades, genetic variability in the APOA5/A4/C3/A1 cluster has been associated (to varying extents) with variability in plasma lipid, apolipoprotein and lipo-
protein levels and with an increased risk of cardiovascular disease. The results of the present study further suggest that the association of SNPs in this cluster with metabolic syndrome may be explained by the propensity of plasma triglycerides to increase in APOA5 Trp19 allele carriers.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
DC, AW, PD, JBR, DA, AB, JF and PA participated in the design and coordination of the study. JD and AM carried out the molecular genetic studies, performed the statistical analyses, wrote the paper. All authors read and approved the final manuscript.

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