

## EXTENDED REPORT

# Contribution of polymorphisms in the apolipoprotein AI-CIII-AIV cluster to hyperlipidaemia in patients with gout

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**Background:** Studies have shown that hyperuricaemia is independently related to the insulin resistance syndrome and that polymorphisms of the apolipoprotein AI-CIII-AIV cluster are also related to insulin resistance.

**Objective:** To study the prevalence of polymorphisms of the apolipoprotein AI-CIII-AIV cluster in persons with gout and to determine whether these polymorphisms contribute to the pathophysiology of gout or to altered lipid concentrations.

**Methods:** Plasma cholesterol, triglycerides, uric acid, VLDL, LDL, IDL, and HDL triglycerides, cholesterol, and the renal excretion of uric acid were measured in 68 patients with gout and 165 healthy subjects. Polymorphisms were studied by amplification and RFLP in all subjects, using XmnI and MspI in the apolipoprotein AI gene and SstI in the apolipoprotein CIII gene.

**Results:** The A allele at position –75 bp in the apolipoprotein AI gene was more common in patients with gout than in controls ( $p=0.01$ ). Levels of cholesterol, triglycerides, uric acid, basal glycaemia, and HDL cholesterol were higher in the patients ( $p<0.001$ ). In the patients there was also an interaction between mutations at the two polymorphic loci studied in the apolipoprotein AI gene ( $p=0.04$ ). An absence of the mutation at position –75 bp of the apolipoprotein AI gene resulted in increased plasma triglyceride levels.

**Conclusions:** Gouty patients have an altered allelic distribution in the apolipoprotein AI-CIII-AIV cluster, which could lead to changes in levels of lipoproteins. This is not caused by a single mutation but rather by a combination of different mutations.

Hyperuricaemia appears to be an independent risk factor for coronary heart disease.<sup>1–3</sup> Although the reason for this is not well known, evidence suggests that hyperuricaemia forms part of the metabolic syndrome. A high concentration of uric acid is closely related to hyperinsulinaemia, glucose intolerance, hypertension, dyslipidaemia, very high levels of triglycerides, low levels of high density lipoprotein (HDL) cholesterol, and obesity (especially abdominal adiposity).<sup>4–12</sup> Regarding the association that has already been demonstrated between hyperuricaemia and hyperlipidaemia, we have previously shown the existence of two groups of hyperuricaemic patients—those in whom the hyperuricaemia is associated with hyperlipidaemia, who could be considered to have the metabolic syndrome, and those having no association with any metabolic disorder.<sup>13</sup> We have also reported that hyperuricaemic-hypertriglyceridaemic patients have high levels of very low density lipoprotein (VLDL) components, a reduced fractionated excretion of uric acid, and an increased ratio of CIII to CII apolipoproteins. Apolipoproteins therefore play an important role in the pathophysiology of the changes seen in hyperuricaemia.<sup>14</sup>

Apolipoproteins AI-CIII-AIV are lipid binding proteins that are involved in the transport of lipids in plasma. Defects or variations in these apolipoproteins are also associated with altered plasma concentrations of lipids and lipoproteins. The relation between variations in the apolipoprotein AI-CIII-AIV gene cluster and plasma lipids has long been recognised. Most studies have concentrated on the association between plasma triglycerides and variations in the gene cluster.<sup>15</sup> Upregulation of apolipoprotein CIII in transgenic mice induces hypertriglyceridaemia because this apoprotein is involved in VLDL clearance.<sup>16</sup> The apoprotein CIII gene is closely linked to the apolipoprotein AI and AIV genes on

chromosome 11, forming a cluster. Study of polymorphisms in this cluster has shown that the S2 allele of the polymorphism is related to hypertriglyceridaemia and an increased risk for coronary heart disease.<sup>17, 18</sup> López-Miranda *et al* found that carriers of the S2 allele had reduced concentrations of LDL cholesterol when given a diet rich in monounsaturated fats.<sup>19</sup> Furthermore, the change of a G for an A at position –75 base pairs (bp) in the promoter region of the apolipoprotein AI gene, digested by the restriction enzyme MspI, induces increased transcription of this gene and increased serum levels of apolipoprotein AI.<sup>20</sup> Individuals with this mutation also have higher levels of HDL cholesterol. Although not yet demonstrated, it would appear that this can be explained by linkage disequilibrium between the A allele and another functional mutation in the gene. There is another mutation in the 5' region near the apolipoprotein AI gene –2500 bp upstream from the restriction start site, which is the marker for combined family hyperlipidaemia or hypertriglyceridaemia.<sup>21–23</sup> These data show that the cluster of apolipoprotein AI-CIII-AIV genes is involved in hypertriglyceridaemia, and may also be involved in predisposition to atherosclerosis.

We studied the prevalence of polymorphisms of the apolipoprotein AI-CIII-AIV cluster in persons with gout and determined whether these polymorphisms contribute to the pathophysiology of hyperuricaemia or to altered lipid levels in these individuals.

## METHODS

### Subjects

The study was undertaken in 68 men with gout attending the rheumatology service at the Hospital Reina Sofía in Córdoba, Spain. None was receiving lipid lowering or urate lowering

**Table 1** Biological variables in controls and patients with gout

	Controls	Hyperuricaemic subjects	p Value
n	165	68	
Age (years)	49.0 (10.0)	50.0 (10.8)	0.59
Blood glucose (mmol/l)	5.72 (1.37)	6.01 (1.07)	0.18
Cholesterol (mmol/l)	4.49 (0.45)	5.48 (1.04)	0.001*
Triglyceride (mmol/l)	1.01 (0.45)	2.79 (2.51)	0.001*
HDL cholesterol (mmol/l)	1.08 (0.24)	1.22 (0.32)	0.01*
Uric acid (mmol/l)	0.31 (0.07)	0.46 (0.10)	0.001
LDL cholesterol (mmol/l)	2.94 (0.43)	3.11 (0.88)	0.17
BMI (kg/m <sup>2</sup> )	27.3 (3.8)	30.2 (3.8)	0.001*

Values are mean (SD).

\*p<0.001 after adjustment for age.

BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein.

treatment. Height and weight were measured and the body mass index (BMI) calculated.

Patients with secondary hyperuricaemia (renal failure or use of diuretics) were excluded from the study.

A control group was composed of 165 healthy persons with no history of gout and with cholesterol and triglyceride concentrations lower than 5.2 and 2.3 mmol/l, respectively, selected from a local cross sectional study<sup>24</sup> and adjusted for age.

## Procedures

### DNA analysis

DNA was isolated from venous blood by the "salting out" method of Miller *et al*,<sup>25</sup> modified by Queipo-Ortuño *et al*,<sup>26</sup> and amplified by polymerase chain reaction in a thermocycler (mastercycler Eppendorf). Primers for the XmnI locus (at position -2500 bp upstream of the transcription start site of the apolipoproteins AI) were those described by Shoulders *et al*,<sup>27</sup> while those of Jeenah *et al* were used for the -75 bp locus of the apolipoprotein AI gene (determined by MspI),<sup>28</sup> and those of Dammerman *et al* for the SstI locus of the apolipoprotein CIII gene.<sup>29</sup>

### Laboratory analysis

Venous blood samples, collected after a 12 hour fast, were ultracentrifuged at 2000×g for 15 minutes for measurement of lipids and lipoproteins. Plasma levels of glucose, cholesterol, triglycerides, creatinine, HDL cholesterol, low density lipoprotein (LDL) cholesterol, and uric acid were measured in an autoanalyser using calorimetric assays (Ecoline 25, Diagnostica Merck). Calculations were made of uric acid clearance and the 24 hour uric acid urinary excretory fraction.

Lipoproteins were separated from serum by ultracentrifugation as follows: first, the VLDL fraction was separated from the serum by flotation at 125 000×g for 18 hours at 7°C in a 45° rotor (Beckman TLA 100.3) After separation of the VLDL, the density of the infranatant was adjusted to 1.30 g/ml by direct addition of potassium bromide and saccharose, separating the rest of the lipoproteins by density gradient centrifugation at 208 000×g for 22 hours at 7°C in a swinging bucket rotor (Beckman SW 40Ti). To visualise the position of the lipoprotein bands a small amount (25 µl) of saturated Coomassie blue was added. The concentrations of cholesterol and triglycerides were measured in each fraction as above.

The amount of HDL cholesterol was also measured by the alternative method of precipitation with phosphotungstic acid in patients and controls. The LDL cholesterol was calculated from the Friedewald equation.

## Statistical analysis

Data are expressed as mean (SD). Comparison between groups was made using Student's *t* test for independent variables or the Mann-Whitney U test, depending on the normality of the distribution. Differences in the genotype distribution of the apolipoprotein AI-CIII-AIV cluster were studied by  $\chi^2$  tests. Statistical analyses were carried out with SPSS 6.0 for Windows and probability (p) values <0.05 were considered significant.

## RESULTS

The clinical and laboratory data for the patients and controls are summarised in table 1. Plasma concentrations of cholesterol, triglycerides, and uric acid were higher in patients with gout than in the controls: (mean (SD) 5.48 (1.04) v 4.49 (0.45); 2.79 (2.51) v 1.01 (0.45); and 0.46 (0.10) v 0.31 (0.07) mmol/l, respectively; p<0.001. The patients also had higher plasma HDL cholesterol levels (1.22 (0.32) v 1.08 (0.24) mmol/l; p<0.001) and a higher BMI (30.2 (3.8) v 27.3 (3.7) kg/m<sup>2</sup>; p<0.001) than the controls (table 1).

### Frequency distribution of the polymorphisms of the apolipoprotein AI-CIII-AIV cluster

The frequency of the A allele—that is, the presence of the mutation in both homozygotes and heterozygotes—was higher in patients than in controls (p=0.01). The distribution of the different genotypes of this polymorphism is shown in table 2. The unusual alleles in homozygotes located -2500 bp upstream of the transcription start site of the apolipoprotein AI gene (determined by XmnI) and of exon 4 of the apolipoprotein CIII gene (determined by SstI) were also more frequent in patients than controls, though the difference was not significant.

### Influence of the polymorphisms of the apolipoprotein AI-CIII-AIV cluster on biological variables

The effect of these polymorphisms on the study variables differed. The presence of the X2 allele in the controls was not

**Table 2** Genotype distribution (%) of the polymorphisms of the cluster in controls and patients with gout

Apo AI	Controls	Patients	Apo AI	Controls	Patients	Apo CIII	Controls	Patients
n	165	64	n	165	64	n	165	64
GG	66.1	51.6	X1X1	79.2	70.3	S1S1	78.8	86.7
GA	27.3	29.7	X1X2	18.3	25.0	S1S2	20.7	11.7
AA	6.7	18.8*	X2X2	2.5	4.7	S2S2	0.5	1.7

\*p=0.01.

**Table 3** Biological variables in patients with gout according to the presence or absence of the mutated allele (determined by XmnI) of the polymorphism of the apolipoprotein AI gene

Variable	Hyperuricaemic patients	
	X1X1	X1X2/X2X2
n	44	19
Blood glucose (mmol/l)	6.01 (1.22)	5.88 (0.70)
HDL cholesterol (mmol/l)*	0.89 (0.22)	0.92 (0.24)
Uric acid (mmol/l)	0.45 (0.10)	0.48 (0.10)
LDL cholesterol (mmol/l)**	2.25 (0.60)	2.26 (1.04)
Cholesterol (mmol/l)	5.36 (0.98)	5.67 (1.17)
Triglyceride (mmol/l)	2.31 (1.68)	3.82 (3.76)†
BMI (kg/m <sup>2</sup> )	28.0 (8.5)	27.6 (8.0)
VLDL cholesterol (mmol/l)	0.50 (0.33)	0.68 (0.40)
VLDL triglyceride (mmol/l)	1.05 (0.71)	1.77 (1.96)†
LDL cholesterol (mmol/l)	3.07 (0.78)	3.31 (1.16)
LDL triglyceride (mmol/l)	0.61 (0.63)	0.81 (0.98)
IDL cholesterol (mmol/l)	0.39 (0.28)	0.40 (0.27)
IDL triglyceride (mmol/l)	0.25 (0.21)	0.33 (0.32)
HDL cholesterol (mmol/l)	1.23 (0.33)	1.13 (0.31)
HDL triglyceride (mmol/l)	0.38 (0.47)	0.53 (0.69)

†p<0.05, after adjustment for age.  
 \*HDL cholesterol was measured by the alternative method of precipitation with phosphotungstic acid.  
 \*\*LDL cholesterol was calculated from the Friedewald equation.  
 BMI, body mass index; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein.

related to any increase in the variables studied, whereas its presence in the patients was related to an increased plasma concentration of triglycerides (2.31 (1.68) v 3.82 (3.76) mmol/l; p = 0.03) (table 3). The other polymorphisms studied in the apolipoprotein AI-CIII-AIV cluster were not related significantly to any differences in the distribution of the study variables (data not shown).

The content of VLDL triglycerides was greater in those patients with the X2 allele than in non-carriers, adjusted for age and BMI (1.77 (1.96) v 1.05 (0.71) mmol/l; p = 0.05) (table 3). The levels of IDL and LDL triglycerides were also higher in carriers of the mutated allele, though the difference was not statistically significant. There were no significant differences in any of the other lipoprotein fractions studied (table 3), or in the distribution of the biological variables in either of the other two polymorphisms of the apolipoprotein AI and CIII cluster (data not shown).

The combined distribution of the polymorphisms of the apolipoprotein AI-CIII-AIV cluster differed between the patients and the controls; 63.5% of the patients had some mutation at the two polymorphic sites of the apolipoprotein AI gene studied, compared with 41.7% of the controls (p = 0.007) (table 4). The presence of at least one unusual allele was related to significant differences in the lipid content of the lipoprotein fractions (table 5). VLDL cholesterol was higher in patients with at least one mutation in any

**Table 4** Differences in the genotype distribution (%) of the polymorphisms of the cluster classified as the presence or absence of some mutation in the two polymorphisms of the apolipoprotein AI studied by  $\chi^2$  test in controls and patients with gout

	No mutation	At least one mutation
Controls (n = 139)	58.3	41.7
Patients (n = 52)	36.5	63.5*

\*p = 0.007 v controls.

of the polymorphisms of the cluster than in patients with no mutation (p = 0.044). The triglyceride content was greater in carriers of some mutation in the cluster than in persons with no mutation (p = 0.014). The IDL cholesterol was higher in carriers of some mutation than in non-carriers (p = 0.021). HDL cholesterol was also higher in carriers, but the difference was not significant.

**DISCUSSION**

Hyperuricaemia is a risk factor for coronary heart disease.<sup>30</sup> The relation between hyperuricaemia-hyperlipidaemia and renal excretion of urates is known—individuals with hyperuricaemia-hyperlipidaemia have reduced renal excretion of urates compared with those who have only isolated hyperuricaemia; and the latter have also been reported to have low renal excretion of urates.<sup>31 32</sup> In patients with hyperuricaemia and increased VLDL there is a close relation between the VLDL levels and renal excretion of urates.<sup>32 33</sup> This relation appears to be mediated through the high prevalence of the E2 isoform of apolipoprotein E.<sup>34</sup>

Recent studies have related polymorphisms of the cluster to variations in plasma lipid levels. Variations in these genes contribute to combined family hyperlipidaemia, modifying plasma concentrations of cholesterol and triglycerides, as well as those of apolipoprotein B and apolipoprotein CIII.<sup>35</sup>

Patients with gout have a greater prevalence of mutated genotypes at polymorphic sites of the apolipoprotein AI gene; in our study, 18.8% had AA at position -75 bp from the transcription start site v 6.7% in controls (p = 0.01). This high prevalence may be responsible for the high levels of HDL cholesterol seen in patients with gout, as has been described for other disease groups.<sup>36-39</sup>

We also found a greater frequency of the mutated allele at position -2500 bp in the apolipoprotein AI gene, although the difference was not significant. We showed that 63.5% of persons with gout have some mutation at the two polymorphic sites of the apolipoprotein AI gene, compared with 36.7% who do not have these alleles (p = 0.007). These patients with a mutated allele at position -2500 bp upstream of the apolipoprotein AI gene had higher plasma and VLDL triglyceride levels than those without the mutation (p = 0.05), corrected for BMI (patients with the mutation had a lower BMI than those without the mutation). This allele has been associated with hypertriglyceridaemia in patients with combined family hyperlipidaemia.<sup>40 41</sup> In our

**Table 5** Means of the biological variables in patients with gout according to the presence or absence of some mutation in the apolipoprotein AI-CIII-AIV cluster

Variable	No mutation	At least one mutation
N	19	33
BMI (kg/m <sup>2</sup> )	29.9 (8.0)	27.5 (9.0)
Uric acid (mmol/l)	0.48 (0.12)	0.46 (0.09)
Cholesterol (mmol/l)	5.33 (0.80)	5.65 (1.03)
Triglyceride (mmol/l)	2.80 (2.50)	2.95 (2.94)
Blood glucose (mmol/l)	6.22 (1.53)	5.94 (0.78)
Uric acid clearance (ml/min)	5.0 (2.9)	4.9 (2.7)
Excretory fraction	5.5 (1.7)	6.1 (1.9)
VLDL cholesterol (mmol/l)	0.24 (0.20)	0.48 (0.36)*
VLDL triglyceride (mmol/l)	0.28 (0.17)	0.45 (0.28)†
LDL cholesterol (mmol/l)	2.32 (0.69)	2.30 (0.85)
LDL triglyceride (mmol/l)	0.20 (0.07)	0.21 (0.09)
IDL cholesterol (mmol/l)	0.18 (0.08)	0.30 (0.25)‡
IDL triglyceride (mmol/l)	0.07 (0.02)	0.09 (0.06)
HDL cholesterol (mmol/l)	0.84 (0.15)	0.96 (0.24)
HDL triglyceride (mmol/l)	0.13 (0.06)	0.12 (0.05)

\*p = 0.044; †p = 0.014; ‡p = 0.021.  
 BMI, body mass index; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein.

group of patients there seemed to be a synergic effect of the two polymorphic sites in the influence on plasma triglycerides. Patients with gout who had some mutation at the three polymorphic sites studied had higher contents of VLDL, intermediate density lipoprotein (IDL), HDL cholesterol, and VLDL triglycerides. Thus their metabolism of lipoproteins seems to be altered, thereby contributing to the expression of hypertriglyceridaemia. The lipoprotein phenotype characteristic of the insulin resistance syndrome was more prevalent in the gouty subjects with a mutation in the cluster despite their BMI being no greater than in subjects without the mutation.

We conclude that the high prevalence of mutations in the apolipoprotein AI-CIII-AIV cluster in patients with gout partly explains their lipoprotein phenotype and that, jointly, these mutations exert more influence on the polymorphic sites of the apolipoprotein AI gene than the apolipoprotein CIII gene.

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