The elevated prevalence of apolipoprotein E2 in patients with gout is associated with reduced renal excretion of urates

F. Cardona, F. J. Tinahones, E. Collantes, A. Escudero, E. García-Fuentes and F. J. Soriguer

Objective. Previous studies have demonstrated that the lower renal excretion of urates in patients with hyperuricaemia is inversely related to plasma very low-density lipoprotein (VLDL) levels, and the different genotypes of the apolipoprotein E gene are related to the plasma levels of lipids. The aim of this study was to determine the prevalence of apolipoprotein E in hyperuricaemic patients and to investigate whether the renal excretion of urates is conditioned by the apolipoprotein E genotype.

Method. The plasma levels of lipoproteins, cholesterol, triglycerides and uric acid, and the renal excretion of uric acid were studied in 68 patients with gout and in another control group of 50 healthy subjects. Both groups were genotyped for apolipoprotein E by means of an amplification technique and inverse hybridization.

Results. The prevalence of the E2 allele was greater in the patients than in the control group. The levels of cholesterol, triglycerides and uric acid were greater in the patients, whereas the levels of high-density lipoprotein were lower. The patients with the E2 allele had higher levels of triglycerides in VLDL and intermediate-density lipoproteins and a lower renal excretion of urates.

Conclusions. These results show that the reduced renal excretion of uric acid in patients with gout is mediated by high levels of VLDL and by the high prevalence of the E2 allele of apolipoprotein E.

KEY WORDS: Apolipoprotein E, Gout, Hyperuricaemia.
described by Utermann et al. [10] in 1977, since when Zannis et al. [11] determined that ApoE exhibits three isoforms (designated ApoE2, ApoE3 and ApoE4) encoded by the respective apoE alleles (apoE2, E3, E4). The phenotypes of apoE are known to be associated with lipid metabolism. The products of apoE2 and E4 are functionally and metabolically different from the common form apoE3, the isoprotein ApoE2 is deficiently bound to its receptor, resulting in decreased catabolism of the triglyceride-rich lipoproteins, whereas ApoE4 is associated with increased clearance of these lipoproteins [12]. Other studies have related ApoE4 with a hyper-response to the content of dietary fat in the concentrations of LDL cholesterol [13, 14]. Thus, it would appear important to determine the apoE genotypes as part of an investigation of the relationship between uric acid and lipids. The relationship between uric acid and lipids and between lipoproteins and renal excretion of uric acid could be partly explained by apoE genotypes.

In this study we determined the apoE genotypes in persons with gout. The aims were to evaluate the possible contribution of the apoE genotype to the lipid anomalies, especially hyperlipaemia, and to the renal excretion of urates.

Material and methods

Subjects

A total of 68 Caucasian patients with gout were studied. One week before the start of the study, allopurinol was withdrawn from those patients receiving treatment. Plasma levels of glucose, cholesterol, triglycerides, creatinine, HDL cholesterol, low-density lipoprotein (LDL) cholesterol and uric acid were measured. All patients who were found to have glycaemic figures indicative of type II diabetes after a basal glycemic or an oral glucose tolerance test were excluded. Their height and weight were also measured, from which the body mass index was calculated (weight in kilograms divided by the square of height in metres), as well as uric acid clearance and the excretory fraction of uric acid in 24-h urine samples and after a purine-free diet for 1 week.

The control group was composed of 50 healthy Caucasian persons, from the same geographical area as the patients, with cholesterol and triglyceride levels <2.28 mmol/l and no history of gout.

DNA analysis

DNA was isolated from venous blood by the salting-out method. The polymerase chain reaction was used for amplification of genomic DNA and apoE genotyping was made with the commercial kit Inno-Lipa ApoE (Innogenetics N.V. Belgium), which is based on the principle of inverse hybridization [15].

Laboratory analyses

The samples of venous blood were collected after a 12-h fast. The serum was separated by centrifugation at 2000 g for 15 min for the lipid and lipoprotein determinations. The VLDL was separated by ultracentrifugation at 55 000 rpm for 18 h at 7°C with a 45° rotor (Beckman TLA 100.3). After separation of the VLDL, the density of the infranatant was adjusted to 1.300 g/ml by the direct addition of potassium bromide and saccharose, and the other lipoproteins were separated by density gradient centrifugation at 45 000 rpm for 22 h at 7°C with a swinging-bucket rotor (Beckman SW 60 Ti). To visualize the position of the lipoprotein bands a small amount (25 ml) of saturated solution of Coomassie blue was added. The concentrations of cholesterol and triglycerides were determined in each isolated fraction by the methods of cholesterol oxidase-peroxidase and glycerol oxidase-peroxidase.

All the analytical parameters of uric acid, cholesterol, triglycerides and renal excretion urates were measured in an autoanalyzer with colorimetric assays (Ecoline 25, Diagnostica Merck).

Statistical analysis

Data are expressed as the mean ± standard deviation. Comparison between groups was made by Student’s t-test for independent samples. The apoE genotypes were examined by χ². A value of P < 0.05 was considered as statistically significant. The study was authorized by the Hospital Carlos Haya Ethics Committee.

Results

Clinical characteristics of the subjects

The clinical characteristics and the biochemical values of the two study groups (control and hyperuricaemic patients) are summarized in Table 1. The levels of cholesterol, triglycerides and plasma uric acid were greater in the hyperuricaemic patients than the controls (5.5 ± 1.04 vs 4.37 ± 0.62, 2.82 ± 2.53 vs 0.78 ± 0.31, and 458.15 ± 101.15 vs 309.4 ± 85, respectively) (P < 0.0001), whereas the plasma levels of HDL cholesterol were lower in the patients (0.92 ± 0.23 vs 1.23 ± 0.27) (P < 0.0001). The body mass index was higher in the patients (27.9 ± 8.7 vs 24.9 ± 3.7) (P < 0.0001).

Apolipoprotein E polymorphisms

The distribution of the apoE genotypes differed between the patients and the controls (P < 0.0001) (Fig. 1). The most common genotype in the patients was E3/2 (50%), followed by E3/3 (25%), E4/2 (14%) and E4/3 (11%), while the most common in the controls was E3/3 (58%), followed by E4/2 (22%), E4/3 (14%) and E3/2 (6%). The frequency of the E2 allele was greater in the patients (64%) than in the controls (28%) (P < 0.0001).

Table 2 shows the mean values in the control group of the different variables. There were no significant differences between persons with the E2 allele and those with any other allele of the apoE gene (E3, E4).

| TABLE I. Comparison of the means of the Student’s t-test of the quantitative variables between controls and hyperuricaemic patients |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | Controls (n=50)          | Hyperuricaemic patients (n=68) | P            |
| Age (yr)                 | 41.3 ± 9.4              | 49.1 ± 12.3               | 0.09         |
| Cholesterol (mmol/l)     | 4.37 ± 0.62             | 5.5 ± 1.04                | <0.0001      |
| Triglycerides (mmol/l)   | 0.78 ± 0.31             | 2.82 ± 2.53               | <0.0001      |
| HDL cholesterol (mmol/l) | 1.23 ± 0.27             | 0.92 ± 0.23               | <0.0001      |
| Plasma uric acid (mmol/l)| 309.4 ± 85              | 458.15 ± 101.15           | <0.0001      |
| LDL cholesterol (mmol/l) | 2.77 ± 0.63             | 2.27 ± 0.75               | <0.0001      |
| BMI (kg/m²)              | 24.9 ± 3.7              | 27.9 ± 8.7                | 0.011        |
Table 3. Comparison of the means of the Student’s t-tests of the quantitative variables in persons with and without the E2 allele in the control group.

<table>
<thead>
<tr>
<th></th>
<th>E3 or E4 allele (n = 36)</th>
<th>E2 allele (n = 14)</th>
<th>P</th>
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<tbody>
<tr>
<td>HDL cholesterol</td>
<td>4.95 ± 0.37</td>
<td>5.13 ± 0.26</td>
<td>0.19</td>
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<tr>
<td>Triglycerides</td>
<td>0.76 ± 0.32</td>
<td>0.80 ± 0.75</td>
<td>0.60</td>
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<tr>
<td>Uric acid clearance</td>
<td>90.0 ± 37.2</td>
<td>77.1 ± 38.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Uric acid clearance (ml/min)</td>
<td>6.3 ± 3.3</td>
<td>4.2 ± 2.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Excretory fraction</td>
<td>7.1 ± 1.9</td>
<td>5.5 ± 1.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the means of the Student’s t-tests of the quantitative variables in patients with and without the E2 allele in the patient group.

<table>
<thead>
<tr>
<th></th>
<th>No E2 allele (n = 20)</th>
<th>E2 allele (n = 33)</th>
<th>P</th>
</tr>
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<tr>
<td>HDL cholesterol</td>
<td>4.95 ± 0.37</td>
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<td>0.01</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.5 ± 7.4</td>
<td>27.6 ± 8.6</td>
<td>0.39</td>
</tr>
<tr>
<td>VLDL triglycerides (mmol/l)</td>
<td>0.87 ± 0.31</td>
<td>1.49 ± 1.5</td>
<td>0.037</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.51 ± 0.38</td>
<td>0.56 ± 0.34</td>
<td>0.61</td>
</tr>
<tr>
<td>LDL triglycerides (mmol/l)</td>
<td>0.51 ± 0.22</td>
<td>0.76 ± 0.92</td>
<td>0.14</td>
</tr>
<tr>
<td>IDL triglycerides (mmol/l)</td>
<td>0.19 ± 0.087</td>
<td>0.31 ± 0.29</td>
<td>0.028</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.12 ± 0.25</td>
<td>1.21 ± 0.36</td>
<td>0.99</td>
</tr>
<tr>
<td>IDL triglycerides (mmol/l)</td>
<td>0.29 ± 0.16</td>
<td>0.49 ± 0.67</td>
<td>0.12</td>
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Discussion

This study demonstrates that hyperuricaemic patients with gout have a high prevalence of apolipoprotein E2, which is accompanied by increased levels of VLDL and IDL triglycerides and a lower renal excretion of urates. These findings could partly explain the relationship between hyperuricaemia and hyperlipidaemia.

Gout and hyperuricaemia are related to several different conditions, including increased consumption of alcohol, obesity, diabetes, hyperlipidaemia, hypertension, renal disease, ischaemic heart disease and the use of diuretics [16–26]. Some of these form part of the metabolic syndrome [27–30].

The relationship between hyperuricaemia and hyperlipidaemia is known. Darlington and Scott [31] found a significant increase in triglycerides, phospholipids and non-esterified fatty acids in hyperuricaemic subjects compared with a control group. BerKowitz [32] and Frank [33] found high levels of triglycerides in 52–82% of patients with gout. In our study, too, the patients had higher levels of triglycerides than the controls, so that 44% of the patients were hyperlipaemic.

Different hypotheses have been proposed to explain this association. These include the action of exogenous factors which together result in hyperuricaemia and hyperlipidaemia. Such factors would include obesity, an increased intake of alcohol, the type of food or some type of genetic factor.

Other authors, however, have found that hypertriglyceridaemia persists in patients with gout after applying a correction factor for body weight. Many studies have demonstrated the existence of hypertriglyceridaemia in patients with gout in the absence of external factors [3, 34]. Moreover, numerous lipoprotein alterations have been found in hyperuricaemic patients, with the most common finding being increased levels of VLDL [35].

The relationship between hyperuricaemia/hyperlipaemia and renal excretion of urates is also known. Thus, hyperuricaemic/hyperlipaemic patients have a reduced renal excretion of urates compared with patients with just hyperuricaemia [2]. Moreover, in patients with hyperuricaemia there is a close relationship between renal excretion of urates and levels of VLDL [36]. Our group of patients with gout had a high rate of hypertriglyceridaemia and a high prevalence of the E2 allele compared with control subjects from the same area. In the patients with gout who had the E2 allele there were lower levels of renal excretion of urates and an important increase in triglyceride levels, both in VLDL and IDL.

Tinaheones et al. [35] demonstrated that hyperuricaemic hyperlipaemic patients exhibit a considerable reduction in the concentration of triglycerides and VLDL after a low calorie diet, concomitant with an increase in

triglycerides (0.31 ± 0.29 vs 0.19 ± 0.0087, respectively; P = 0.028).

The E4 allele was not associated with any differences in the biochemical parameters studied (data not shown).
the renal excretion of uric acid, which reverted with an increase in the VLDL after ceasing the hypocaloric diet, confirming the close relation between VLDL and renal excretion of urates.

The role of E2 in the clearance of VLDL is known, ApoE2 has been seen to have a reduced capacity to bind to the remnants receptor (ApoB/E), which would result in a decreased clearance rate and thus lead to differences in lipoprotein levels according to the apoE genotype [37–39]. Sijbrands et al. [40] showed that patients with combined familiar hyperlipaemia, heterozygous for the E2 allele, have significantly higher mean levels of VLDL cholesterol and VLDL triglycerides compared with persons without E2. Rats deficient in ApoE have high levels of ApoAII and this in turn produces an increase in VLDL, resulting in increased levels of triglycerides, cholesterol, ApoB, IDL and LDL and a reduced level of HDL, leading to an increased susceptibility to atherosclerosis. It would thus seem logical to suppose that ApoE plays an important role in the regulation of the levels of these lipoproteins, especially the VLDL [41]. The ApoCIII also seems to be involved, since high concentrations of this apoprotein displace ApoE from the remnants receptor (ApoB-E), which would result in a decreased clearance rate and thus lead to differences in the metabolism of urates.

The prevalence of E2 in the metabolic syndrome is known. Its frequency in the Framingham study was 7.8% (n = 1916) [43] and in combined familiar hyperlipaemia it was 25% compared with 6% in hypercholesterolaemic persons [44, 45]. We found that the prevalence of E2 in patients with hyperuricaemia was greater than in the controls, and probably greater than in the metabolic syndrome. The most prevalent apoE genotype in our hyperuricaemic patients was E3/2, followed by E3/3, E4/2 and E4/3 compared with E3/3, E4/2, E4/3 and E3/2 in the control group.

We conclude that the relationship between hyperuricaemia/hyperlipaemia and renal excretion of urates could be mediated by the high prevalence of the apoE2 allele. Reduced renal excretion of urates has been shown to be accompanied by high levels of VLDL [35] and the E2 isoform of ApoE could contribute to this reduction, since it has already been shown to contribute to high levels of VLDL.

Acknowledgements

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References


