Lipoprotein Glomerulopathy: Molecular Characterization of Three Italian Patients and Literature Survey

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Abstract

Background: Lipoprotein glomerulopathy (LPG) is a rare kidney disease, mainly reported in Asian subjects, linked with rare APOE gene mutations resulting in a structurally abnormal Apolipoprotein E (ApoE), the plasma accumulation of lipoprotein remnants and the formation of lipid thrombi in glomerular capillaries. This study reports the molecular characterization of three unrelated Italian patients with long standing LPG.

Methods: We sequenced the APOE gene in the LPG patients and their family members and determined the haplotype co-segregating with the APOE mutation. We also sequenced other genes and looked at the distribution of common SNPs, known to be involved in lipoprotein metabolism. Finally we reviewed the LPG cases reported in the literature.

Results: The three patients showed the clinical, biochemical and histological features of LPG. They were found to be heterozygous for the APOE c.527 G>C, p.(Arg176Pro) mutation, previously reported in two Japanese subjects. Patients’ relatives, carrying the mutation, were free of LPG. The mutation co-segregated with the same haplotype in all carriers, suggesting a common ancestor. The search for additional genetic variants promoting plasma accumulation of lipoprotein remnants failed to show differences between APOE mutation carriers with or without LPG. The literature survey showed that 145 LPG cases had been clinically described and rare APOE mutations had been documented in 102. The analysis of 95 fully characterized LPG cases suggests that the phenotypic expression of the disease in APOE mutation carriers mainly depend on the plasma accumulation of lipoprotein remnants above a threshold value. Indeed, APOE mutation carriers without LPG had lower plasma lipids than carriers with LPG.

Conclusions: The p.(Arg176Pro) substitution in ApoE is associated with LPG in Caucasians. However, as in the case of other rare APOE mutations found in other populations, this mutation is necessary but not sufficient for the development of LPG.

Keywords: Lipoprotein glomerulopathy (LPG); APOE gene mutation; Dominant inheritance with incomplete penetrance; Glomerular lipoprotein thrombi; Hyperlipidemia

Introduction

Lipoprotein Glomerulopathy (LPG), first described by Saito et al. in 1989 [1], is a pathological condition characterized by proteinuria, edema and lipid accumulation in the glomerular capillaries, which frequently progresses to renal failure. The histological hallmark of LPG is the presence of laminated thrombi, consisting of lipid droplets within the lumina of dilated glomerular capillaries. Under the electron microscope these lipid deposits show a layered texture resembling fingerprints [2]. These thrombi contain apolipoprotein B (ApoB) and apolipoprotein E (ApoE), suggesting deposition of plasma lipoprotein particles containing these apolipoproteins [3]. The plasma lipid profile of patients with LPG is characterized by an increased plasma level of ApoE in most cases and a variable elevation of Very Low Density Lipoproteins (VLDL) and Intermediate Density Lipoproteins (IDL), resembling that reported in type III hyperlipidemia, a disorder commonly associated with homozygosity for the e2 allele of APOE gene encoding the ApoE2 isoform [2]. Occasionally intraglomerular lipoprotein thrombi have been reported in the classic type III hyperlipidemia but in these cases the histological features did not show the layered structure of the lipid deposits (which is the key feature of LPG) and included glomerulosclerosis with mesangial and interstitial foam cells accumulation [4,5]. In fact, in most LPG cases the ApoE phenotype, determined by isoelectric focusing polyacrylamide gel electrophoresis, is characterized by heterozygosity for E2 isoform (E1/E2, E2/E3, E2/E4 or E2/E5), associated with a variable genotype (ε2ε2, ε2ε3, ε3ε3, ε3ε4). In genetically characterized LPG patients the disease was found to be associated with heterozygosity for rare APOE gene mutations, leading to substitutions of a single amino acid residue, amino acid deletions or single amino acid duplication [6] (Supplementary information: Table S1 and references). The presence of these abnormal ApoE proteins has been considered as the main cause of the formation of lipid thrombi in the glomerular capillaries and the deposition of lipoproteins in the kidney. However, some family members of LPG patients found to carry the pathogenic ApoE mutation often did not develop LPG [5] (Supplementary information: Tables S1 and S2), suggesting that other factors are involved in the
development of kidney lesions. Within this context LPG may be regarded as a dominant disorder with incomplete penetrance [7].

LPG cases have mostly been reported in Japanese and Chinese/Taiwanese subjects; only few cases of LPG have been described in Caucasians [8]. We have recently characterized, at the molecular level, an Italian case of LPG clinically described by others in 2009 [9] and identified a novel ApoE mutation [p.(Arg150Cys), designated ApoE Modena] forming ApoE homodimers [10].

Here we describe the molecular characterization of three other LPG cases identified in Italy, who were found to share the same rare APOE gene mutation, leading to a non-conservative amino acid substitution p.(Arg176Pro) [p.(Arg158Pro) in the mature protein].

### Subjects and Methods

#### Subjects: Proband LPG-1

At the time of the molecular diagnosis the patient (subject II.1, Kindred LPG-1 in Figure 1) was a 54 year-old male, who at the age of 45 had been admitted to the Nephrology Unit of the S. Chiara Hospital of Pisa (Tuscany Region) for proteinuria (3.7 g/day), moderate ankle edema, creatinine level 1.5 mg/dl, GFR 54 ml/min/1.73 m², arterial hypertension (175/95 mmHg) and a moderate mixed hyperlipidaemia (Table 1).

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### Table 1: Clinical and biochemical data of the subjects investigated

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (y)*, Sex</th>
<th>BMI (kg/m²)</th>
<th>TC (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>ApoAI (mg/dL)</th>
<th>ApoB (mg/dL)</th>
<th>ApoE (mg/dL)</th>
<th>APOE genotype</th>
<th>LPG</th>
<th>ApoE mutation</th>
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<tbody>
<tr>
<td>Kindred LPG-1</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I.1</td>
<td>86, M</td>
<td>26.0</td>
<td>5.34</td>
<td>3.31</td>
<td>1.78</td>
<td>1.03</td>
<td>155</td>
<td>100</td>
<td>4.00</td>
<td>ε2ε4</td>
<td>No</td>
<td>p.(Arg176Pro)</td>
</tr>
<tr>
<td>I.2</td>
<td>83, F</td>
<td>24.5</td>
<td>4.29</td>
<td>2.32</td>
<td>1.86</td>
<td>0.81</td>
<td>170</td>
<td>78</td>
<td>3.80</td>
<td>ε2ε3</td>
<td>No</td>
<td>p.(Arg176Pro)</td>
</tr>
<tr>
<td>II.1</td>
<td>54, M</td>
<td>27.0</td>
<td>5.84†</td>
<td>4.21†</td>
<td>0.90†</td>
<td>2.37†</td>
<td>98†</td>
<td>110†</td>
<td>8.00†</td>
<td>ε2ε4</td>
<td>LPG</td>
<td>p.(Arg176Pro)</td>
</tr>
<tr>
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<td>22, F</td>
<td>27.6</td>
<td>2.90</td>
<td>1.29</td>
<td>1.39</td>
<td>0.68</td>
<td>131</td>
<td>36</td>
<td>4.27</td>
<td>ε2ε2</td>
<td>No</td>
<td>p.(Arg176Pro)</td>
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<td>17, F</td>
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<td>2.75</td>
<td>1.12</td>
<td>1.34</td>
<td>0.71</td>
<td>129</td>
<td>29</td>
<td>4.82</td>
<td>ε2ε2</td>
<td>No</td>
<td>p.(Arg176Pro)</td>
</tr>
</tbody>
</table>

| Subjects: Kindred LPG-2 |               |             |             |                 |                |             |               |              |              |                |     |                |
| I.1     | 50, F         | 23.2        | 4.24        | 2.52           | 1.47           | 0.53        | 148          | 66           | 2.88         | ε3ε3            | No  | p.(Arg176Pro) |
| I.2     | 45, M         | 21.3        | 8.07†       | 5.68†          | 1.39†          | 2.65†       | 150†         | 155†         | 9.68†        | ε2ε3            | LPG | p.(Arg176Pro) |
| II.1    | 23, M         | 22.1        | 3.46        | 1.84           | 1.37           | 0.55        | 134          | 55           | 2.96         | ε2ε3            | No  | p.(Arg176Pro) |

| Subjects: Kindred LPG-3 |               |             |             |                 |                |             |               |              |              |                |     |                |
| I.1     | 58, M         | 27.0        | 5.69        | 3.85           | 1.16           | 1.58        | 135          | 95           | 4.30         | ε3ε3            | No  | p.(Arg176Pro) |
| I.2     | 56, F         | 25.9        | 4.57        | 2.25           | 2.17           | 0.33        | 168          | 58           | 3.40         | ε2ε3            | No  | p.(Arg176Pro) |
| II.1    | 24, M         | 27.8        | 9.21†       | 7.27†          | 1.32†          | 1.35†       | 148†         | 161†         | 5.62†        | ε2ε2            | LPG | p.(Arg176Pro) |

ND: not determined; *age of subjects at molecular diagnosis; † before therapy with HMG-CoA reductase inhibitors; ‡ pseudo ε2 allele; data of the probands are reported in bold characters.
Family history for kidney disease was negative. Kidney biopsy, performed at 45, revealed the typical histological lesions of LPG, characterized by enlargement of the glomeruli, dilatation of the capillaries obstructed by pale, amorphous, partially laminated or reticulated lipid deposits [11].

Proband LPG-2

At the time of the molecular diagnosis the patient (subject I.2, Kindred LPG-2 in Figure 1) was a 45 year-old male living in the Tuscany Region, who at the age of 42 had been referred to the Nephrology Unit for a nephrotic syndrome with heavy proteinuria (6.9 g/day), hypoalbuminemia (2.7 g/dl), creatinine level of 1.25 mg/dl, GFR of 67 ml/min/1.73 m², arterial hypertension (180/100 mmHg) and mixed hyperlipidaemia (Table 1). The light microscopy of kidney biopsy, performed at 42, was consistent with the diagnosis of LPG [11].

Proband LPG-3

This was a novel patient (subject II.1, Kindred LPG-3 in Figure 1) not previously clinically described. At the time of molecular diagnosis the patient, also living in the Tuscany Region, was a 23 year-old male, referred to the Nephrology Unit for heavy proteinuria (>15 g/day), hypoalbuminemia (1.9 g/dl), edema at lower limbs, creatinine level of 1.10 mg/dl, GFR of 94 ml/min/1.73 m², arterial hypertension (180/110) and isolated hypercholesterolemia (Table 1). The histological examination of the renal biopsy performed during hospitalisation revealed the typical lesions of LPG (Figure 2).

Informed consent for clinical and genetic studies was obtained from the index subjects and their family members. The study protocol was approved by the institutional human investigation committee of each participating institution.

Biochemical analyses

Plasma concentrations of total cholesterol (TC), LDL cholesterol (LDL-C), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by standard enzymatic methods. Plasma apolipoproteins AI, B and E concentrations were measured by nephelometry (Siemens AG Healthcare Diagnostics, München, Germany); reference range values: ApoAI 115-225 mg/dL, ApoB 60-150 mg/dl, ApoE 1-5 mg/dl.

APOE Genotyping and Resequencing

APOE genotype was obtained by PCR amplification of genomic DNA and Hha I digestion [12]. The APOE coding regions were amplified by PCR using the primers and conditions reported in Supplementary information (Tables S3) and sequenced by automatic sequencer CEQ2000 DNA Analysis System (Beckman Coulter, Fullerton, CA). The analysis of common SNPs or the sequences of other genes involved in lipoprotein metabolism are reported in Supplementary information (Methods, Results, Table S4).

The mutations were designated according to the Human Genome Variation Society, 2012 version (http://www.hgvs.org/mutnomen/recs-DNA.html). ApoE protein sequence variants were designated
according to http://www.hgvs.org/mutnomen/recs-prot.html: the numerical series of codons includes the sequence of the signal peptide (18 residues).

**APOE haplotype co-segregating with APOE mutation in carriers**

The haplotype co-segregating with the APOE mutation was assessed, as previously described [13], through the analysis of five informative biallelic SNPs at the APOE locus: -491 A/T (rs449647, MAF 0.175) and -219 G/T (rs405509, MAF 0.493) located in the transcriptional regulatory region [14]; 2440 G/A (rs769450, MAF 0.326) located in intron 2 (c.45+520G/A); rs429358 T/C (MAF 0.149) and rs7412 C/T (MAF 0.07) located in exon 4.

**Literature search**

A literature search was undertaken to identify LPG patients previously described (http://www.ncbi.nlm.nih.gov/pubmed) after the original report [1]. The survey of the reports published in English led to the identification of 145 patients with the clinical diagnosis of LPG (Supplementary information: Tables S2), including the three patients reported in the present study, individual laboratory data (proteinuria, plasma creatinine, GFR and plasma cholesterol and triglyceride levels) were available for 95 patients; the plasma levels of HDL-C and ApoE were available for 50 and 78 patients, respectively (Supplementary information: Tables S5).

**Statistical analyses**

Statistical analyses were performed using SPSS (PASW Statistics 18, Release Version 18.0, SPSS, Inc., 2009, Chicago, IL, www.spss.com). The correlations between variables were assessed by Pearson and Spearman tests. Differences between groups for continuous variables were assessed by Mann-Whitney test. Triglyceride levels were logarithmically transformed before analysis. Differences in the distribution of categorical variables were assessed by Fisher’s exact test.

**Results**

**Kindred LPG-1**

The proband, now aged 54, had been on treatment with dietary restriction, rosuvastatin 10 mg/day, n-3 fatty acids 1.7 g/day, aliskiren 150 mg/day, doxazosin 1 mg/day and nebivolol 5 mg/day from hospital discharge to present time. This treatment resulted in a satisfactory control of arterial hypertension (147/93 mmHg), a reduction of proteinuria to 0.3 g/day, creatinine level to 1.35 mg/dl and in a small increase of GFR (59 ml/min/1.73 m²). The plasma lipid profile was greatly improved (TC 3.05, LDL-C 1.12, TG 2.01, HDL-C 1.19 mmol/L) with respect to the basal levels detected at 45 and shown in Table 1.

The proband was a carrier of the ε2ε4 APOE genotype; he was also heterozygous for a rare mutation in exon 4 of APOE gene: c.527 G>C, p.(Arg176Pro) [p.(Arg158Pro) in the mature protein] (Table 1 and Figure 3).

**Figure 3:** Analysis of APOE gene in probands LPG-1, LPG-2 and LPG-3. Each panel shows the partial sequence of exon 4 in the three LPG patients carrying the c.527 G>C, p.(Arg176Pro) mutation.

This mutation abolishes the HhaI restriction site (GGC↓C), thus simulating the presence of an ε2 allele (pseudo ε2) after DNA digestion with this enzyme. The screening of the mutation among patient’s family members revealed that his mother (subject I.2 in Figure 1) and his two daughters (subjects III.1 and III.2 in Figure 1) were carriers of the mutation but had neither clinical nor laboratory signs of LPG (Table 1). The haplotype analysis revealed that the mutation co-segregated with rs449647 A, rs405509 G, rs769450 G, rs429358 T and rs7412 C SNPs at the APOE locus.

**Kindred LPG-2**

The proband, now aged 45, had been treated with atorvastatin 10 mg/day for two years. Following treatment, his plasma lipid profile (TC 6.07, LDL-C 3.77, TG 0.74, HDL-C 1.96 mmol/L, ApoAI 179, ApoB 89 mg/dl, ApoE 4.05 mg/dl) improved with respect to the pre-treatment profile (Table 1). However, in spite of this treatment and pharmacological control of arterial hypertension (140/80 mmHg) by telmisartan 80 mg/day and ramipril 10 mg/day, the kidney function showed a progressive deterioration (creatinine level 1.60 mg/dl, GFR 50 ml/min/1.73 m², with an increase of proteinuria (9.9 g/day). In view of these findings, LDL-apheresis treatment was started. The proband was a carrier of the ε2ε3 APOE genotype and heterozygous for the p. (Arg176Pro) mutation (Table 1 and Figure 3). The patient’s normolipidemic sister (subject I.1 in Figure 1) was found to be mutation negative, while his son (subject II.1 in Figure 1) was a carrier of the mutation, but had low plasma lipid levels and normal renal function (Table 1). The haplotype co-segregating with the mutation was the same as that found in the carriers of kindred LPG-1.
Kindred LPG-3

The proband, now aged 24, was treated with a combination of ramipril 10 mg/day, olmesartan 20 mg/day, amlodipine 5 mg/day, potassium canrenate 50 mg/day and furosemide 25 mg every two days with satisfactory control of arterial hypertension (135/80 mmHg) and with atorvastatin 10mg/day with a poor improvement of the lipid profile (TC 7.21, LDL-C 5.17, TG 0.93, HDL-C 1.60 mmol/L, ApoA1 161, ApoB 136, ApoE 4.9 mg/dl). In view of the persistent high level of proteinuria (12.5±1.8 g/day; mean of 10 determinations during the last 4 months) and the worsening of renal function (creatinine 1.40 mg/dl, GFR 66 ml/min/1.73 m²), LDL-spheresis treatment was started. The sequencing of APOE gene revealed that the patient was heterozygous for the p.(Arg176Gln) (Table 3). The proband’s mother (subject 2 in Figure 1) was also a carrier of this mutation but she was normolipemic (Table 1) and had neither clinical nor laboratory signs of LPG. In the patient, as well as in his mother, APOE genotype was ε2ε3 (pseudo-ε2 allele). The haplotype co-segregating with the mutation was the same as that found in carriers of LPG-1 and LPG-2 kindred.

Analysis of the Phenotypic Expression of LPG: Survey of the Literature

The survey of the clinical data of 95 fully characterized LPG patients (94 previously reported in the literature, including the clinically described probands LPG-1 and LPG-2, and proband LPG-3 reported here for the first time) (Supplementary information: Tables S5) showed a large inter-individual variability in terms of plasma lipid profile and kidney involvement. A mixed hyperlipidemia (TC>5.17 and TG>1.70 mmol/L) was reported in 67.4% of patients while high levels of plasma ApoE (>10 mg/dl) was reported in 66.6%. Some patients had isolated hypercholesterolemia (8.4%) or isolated hypertriglyceridemia (14.7%) and some had normal plasma lipids (9.5%). The analysis of the correlations between pairs of variables showed that plasma ApoE concentration was highly correlated with TC concentration (P<0.01) and Log-TG concentration (P<0.03), suggesting an accumulation in plasma of cholesterol- and triglyceride-rich lipoprotein remnant. The parameters of renal function were significantly correlated with age, plasma TC and non-HDL-C concentrations; more specifically proteinuria and serum creatinine showed that plasma ApoE concentration was highly correlated with age, plasma TC and non-HDL-C (P<0.01 and P<0.002, respectively) and to non-HDL-C (P<0.002 and P<0.03, respectively); GFR was inversely related to age (P<0.001) and to TC levels (P<0.007). No significant correlations were found between the parameters of renal function and ApoE levels. The comparison of the parameters of renal function according to the first and the third tertile of TC distribution (adjusted for age and gender) showed significant differences: proteinuria 3.66 ± 2.84 vs 6.24 ± 4.39 g/d (P<0.01), plasma creatinine 1.06 ± 0.38 vs 1.51 ± 0.60 mg/dl (P<0.001), GFR 80.6 ± 23.6 vs 57.0 ± 22.9 ml/min/1.73m² (P=0.004).

APOE Mutation Carriers with and without LPG

To gain insight into the development of LPG in the APOE mutation carriers we selected 6 families from the literature and the 3 families reported in the present paper, whose complete plasma lipid profile and renal laboratory parameters were available for the LPG patients (group 1), as well as for their relatives without LPG (group 2) (Supplementary information: Tables S6). The two groups did not significantly differ in terms of age and gender distribution; however, the subjects of group 2 (APOE mutation carriers without LPG) had significantly lower levels of TC, LDL-C, non-HDL-C and TG and marginally significant lower levels of ApoE with respect to the subjects of group 1 (LPG patients).

To ascertain whether variants in some lipid related genes were involved in the development of LPG in carriers of p.(Arg176Gln) of our kindreds, we resequenced some genes of the intra-vascular lipolytic cascade and looked at the distribution of some SNPs known to affect the plasma lipids (Supplementary information: Tables S4). These analyses failed to identify genetic factors discriminating mutation carriers with and without LPG.

Discussion

Lipoprotein glomerulopathy (LPG) is a rare kidney disease reported so far mainly in Japanese and Chinese/Taiwanese subjects; it is exceedingly rare in Caucasians [8]. The key biochemical feature of LPG is the presence of rare mutations of APOE gene, resulting in structurally abnormal apolipoprotein E [5,6]. Here we describe the clinical and molecular features of three apparently unrelated patients, living in the same geographical area of Central Italy (Tuscany Region), who were found to share the same rare APOE mutation.

ApoE (mature protein 299 residues) is the ligand for the plasma clearance of triglyceride- and cholesterol-rich lipoproteins (chylomicron remnants, VLDL and IDL). It binds to LDL receptor, LDL-receptor-related protein (LRP), VLDL receptor, Megalin, ApoER-2 and heparan sulfate proteoglycans. The N-terminal domain (1-191 amino acid residues) of ApoE contains four amphipathic bundle α-helices. The LDL-receptor binding region (136-150 amino acid residues and the arginine residue at position 172) contains a cluster of basic amino acids, which interact with acidic residues of the ligand-binding domain of the members of the LDL receptor family. The Arg172 is in the hinge region connecting the N-terminal domain with the C-terminal domain (~225-299 residues). This latter domain contains the lipid-binding region (244-272 residues). It has been suggested that the lipid-binding to the C-terminal domain induces a conformational change in the N-terminal domain, which facilitates the binding to the LDL receptor family [15,16].

ApoE consists of three isoforms (E2, E3, and E4). The ApoE3 (Cys112, Arg158) and the ApoE4 (Arg112, Arg158) isoforms bind to LDL receptors with similarly high affinity. The ApoE2 (Cys112, Cys158) isoform has a much weaker LDL receptor binding activity (about 1-2% of that of E3 isoform) [17], since the Cys for Arg substitution at position 158 eliminates the salt bridge between Arg158 and Asp154 and induces the formation of a new salt bridge between Arg150 and Asp154, which alters the conformation of the binding domain and disrupts the binding to the receptors [15,16].

Up to now sixteen different mutations of APOE gene have been identified world-wide in patients with LPG (11 missense, 4 amino acid deletions, 1 amino acid duplication). Most of these mutations are located in, or close to, the LDL receptor-binding domain. Among the missense mutations, four are proline for arginine substitutions (at position 145, 147, 150 and 158 of the mature protein); the deletions involve the region encompassing the amino acid residues 141-146 (141-143, 142-144 and 144-146 in the central region of the binding domain) or the region encompassing the amino acid residues 156-173 (which includes the Arg172 residue involved in the binding to LDL receptor); one amino acid duplication, recently reported, involves the residue Asp151 (Supplementary information: Tables S1 and references).
The patients LPG-1, LPG-2 and LPG-3 (index cases in Figure 1), who resulted to be unrelated at least up to three generations back, were heterozygous carriers of the c.527 G→C, p.(Arg176Pro) mutation, [p. (Arg158Pro) in the mature protein]. This mutation, found for the first time in Caucasians, had been previously reported independently in two Japanese patients with LPG and named ApoE Osaka [18] and ApoE2 Kurashiki [19], respectively. The substitution of proline for arginine, also found in ApoE Sendai p.(Arg145Pro), in ApoE Chicago p.(Arg147Pro) and in ApoE Guangzhou p.(Arg150Pro), deeply affects the structural and conformational integrity of ApoE protein, since the introduction of proline in a highly helical region acts as “helix-breaker” [20]. The effects of three of these mutations (Arg145Pro, Arg147Pro and Arg158Pro) on the structure and conformation of the respective mutant ApoE proteins, all encoded by an ε3 allele, had been extensively investigated using biophysical techniques [21]. These analyses showed that these amino acid substitutions are responsible for multiple striking alterations: i) unfolding of the entire N-terminal region of the protein not restricted to the 4 helix; ii) decrease of the thermodynamic stability of the protein at physiological temperature; iii) increase of the hydrophobic area exposure; iv) disruption of lipoprotein assembly producing malformed particles, which result from the lack in maintaining the monomeric form of ApoE when bound to lipoproteins [22] with the induction of protein and lipoprotein aggregates prone to deposit in the glomerular capillaries and v) increased sensitivity to proteolysis, which may be relevant in sites of local kidney inflammation [21]. In the case of the Pro for Arg substitution at position 158, an additional ApoE2-like structural perturbation may be the abolition of the salt bridge between Arg158 and Asp154 and the formation of a new salt bridge between Arg150 and Asp154, which contributes to alter the conformation of the LDL receptor-binding region, substantially reducing the affinity of this domain for the LDL receptor [15,21].

One hundred and forty-six LPG cases (including 145 previously reported and the new patient LPG-3 described in the present study) have been till now clinically identified. A mutation of APOE gene in heterozygous form was documented in 104 patients and in homozygous form in one (Supplementary information: Tables S1 and S2).

The survey of clinical and biochemical data in 95 fully characterized LPG patients (Supplementary information: Tables S3), and the comparison of APOE mutation carriers with and without LPG (reported in Chinese subjects with p.(Arg25Cys) mutation [23] and in carriers of other ApoE mutations) (Supplementary information: Tables S6) suggests that plasma accumulation of ApoE-containing remnant lipoproteins above a threshold value plays a key role in the development of LPG. This concept is reinforced by the observation that LPG develops in the kidneys transplanted to LPG patients [24-27], supporting the idea that a pre-renal circulating factor (i.e. plasma lipoprotein concentrations carrying mutant ApoE) plays a major role in the development of kidney lesions (even though the mechanisms leading to the formation of lipoprotein thrombi in glomerular capillaries are barely understood).

On the other hand, the finding of 64 probands’ family members (59 previously reported and 5 in the present study) (Supplementary information: Tables S1 and S2), who were mutation carriers but did not develop LPG, and the significant difference in lipoprotein profile between subjects with or without LPG and carrying the same ApoE mutations (ref. 23 and Supplementary information: Table S6) support the concept that the presence of an abnormal ApoE is necessary but not sufficient for the development of LPG. Other factors, possibly involved in a more pronounced lipoprotein remnant accumulation and consequently in the clinical expression of the disease, might be: i) additional allelic variants in exons, introns or regulatory regions of APOE which in cis or in trans may induce different levels of expression of the mutant vs the wild-type allele (a higher expression of the mutant APOE allele would translate into a higher plasma level of ApoE-containing lipoproteins which are prone to aggregate in kidney capillaries); ii) a rare mutation or a common polymorphism in another gene required for the full phenotypic expression of the disease; and iii) epigenetic mechanisms involved in the regulation of the mutant gene expression [7]. In this context we have resequenced some genes involved in the lipolytic pathway and looked at some SNPs potentially interfering with the lipoprotein remnants clearance from plasma (Supplementary information: Tables S4) but failed to identify additional genetic factors which could discriminate subjects with LPG from those without.

An additional mechanism in the development of LPG was suggested by experiments in mice showing that an impairment of macrophage activity resulting from Fcy receptor deficiency, which in turn suppress the activity of LDL receptor and CD36 scavenger receptor, may have effect in the formation of glomerular lipoprotein thrombi in association with ApoE mutations [6,28,29]. Whether this mechanism is involved in the pathogenesis of LPG in humans remains to be established.

The presence of the same ApoE mutation in three unrelated patients with LPG living in the same geographical area strongly suggests the presence of an ancestral familial relationship among these individuals (a founder gene effect for LPG in the Tuscany region of Italy?). This hypothesis is also supported by the fact that these patients share the same APOE haplotype linked to the mutation. Interestingly, the first Caucasian subject with the clinical diagnosis of LPG reported in literature in 1995 was an Italian living in France who came from Tuscany [30]. We wonder whether he too was a carrier of the p. (Arg158Pro) mutation.

Conclusion

In conclusion, we have genetically characterized three unrelated Italian patients with histologically proven LPG, a disease exceedingly rare in Caucasians. They were found to be heterozygous for the rare ApoE mutation p.(Arg158Pro), which was previously described in two Japanese subjects with LPG. Some patients’ family members were found to carry the ApoE mutation in the absence of either clinical or laboratory signs of kidney lesions, a situation also reported for other ApoE mutations associated with LPG. The p.(Arg158Pro) mutation co-segregated with the same APOE gene haplotype in all carriers suggesting a common ancestor. The analysis of some lipid-related genes failed to reveal the presence of a different distribution of common genomic variants between ApoE mutation carriers with or without LPG.

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References


