Glucagon Receptor Gene Mutation (Gly40Ser) in Human Essential Hypertension

The PEGASE Study

Eva Brand, Lise Bankir, Pierre-François Plouin, Florent Soubrier

Abstract—A missense mutation (Gly40Ser) in exon 2 of the glucagon receptor gene (GCG-R) was shown to reduce ligand affinity and impair cAMP response. We conducted a case-control study with a sample of 741 French hypertensive patients with moderate to severe hypertension and 412 normotensive control subjects, who were genotyped for this biallelic variant by use of hybridization with allele-specific oligonucleotides. The Gly40Ser polymorphism was not significantly associated with hypertension in the whole study population, although the frequency of 40Ser carriers in hypertensive subjects was double that in normotensive subjects (3.1% in hypertensives versus 1.5%; \( P=0.087 \)). However, the separate analysis of both genders revealed that 40Ser allele carriers were significantly more frequent (\( P=0.035 \)) among male patients (17/429; 4.0%) than among normotensive male controls (2/242; 0.8%), whereas no significant difference was observed in female subjects (6/312 in hypertensives and 4/170 in normotensives). Further studies are required to interpret the significance of this association. (Hypertension. 1999;34:15-17.)

Key Words: linkage, genetic ▪ polymerase chain reaction ▪ oligonucleotide ▪ blood pressure ▪ genotype

Several findings have offered significant support that the glucagon receptor gene (GCG-R) represents a putative candidate for predisposition to human essential hypertension. This hypothesis is drawn from the potential role of glucagon in the regulation of electrolyte and water homeostasis. Glucagon has long been known to be natriuretic, but this effect required high doses of the hormone and, intriguingly, was not reproduced when glucagon was infused into the renal artery. The mechanism of the natriuretic effect of glucagon has been recently elucidated.\(^1\) It involves a prior action of glucagon on the liver, stimulating cAMP formation and cAMP release into the circulation. Although cAMP is usually an intracellular messenger, it is now clear that it also behaves as an extracellular, interorgan mediator between liver and kidney. In the kidney, cAMP potently reduces solute and water reabsorption in the pars recta of the proximal tubule. This results in a significant increase in water, sodium, phosphate, and urea excretion.\(^1\) Accordingly, solute and water excretion by the kidney are permanently modulated by the intensity of cAMP release by the liver.\(^2\)

The effects of glucagon are mediated through its binding to a specific receptor (GCG-R), a 480–amino acid protein, which belongs to the superfamily of G protein–coupled transmembrane receptors.\(^3\) The recently described Gly40Ser missense mutation in exon 2 of this receptor results in a lower affinity of the receptor for glucagon and a reduced cAMP response in transfected cells.\(^4\) Carriers of the mutation have a significantly lower increase in plasma glucose concentration in response to glucagon infusion, suggesting that the mutation also results in a blunted cAMP response in humans.\(^5\) It is tempting to speculate that a decrease in receptor activity in vivo might contribute to common essential hypertension by reducing the renal natriuretic effect of glucagon. A case-control study showed that the Gly40Ser mutation of the GCG-R was 5-fold more common in essential hypertensives with 2 hypertensive parents (\( n=130, 5\% \)) than in normotensives (\( n=90, 1\% \)) in the white population of Australia.\(^6\) All hypertensive carriers of the mutation except 1 were women and had a later onset of hypertension (52 ± 5 years; \( P<0.0001 \)) than hypertensives not bearing this mutation. A case-control study was performed in a Sardinian group of non–insulin-dependent diabetic patients and nondiabetic subjects.\(^5\) Results are difficult to compare because the group of nondiabetic subjects is small, and some subjects are family related.\(^5\) This mutation was not found in a group of 188 Japanese.\(^7\)

To estimate the importance of the potentially functional Gly40Ser mutation of the GCG-R, we performed a large case-control study in the French population using this variant.

Methods

An association study was performed by comparing allele and genotype frequencies for the biallelic polymorphism of the GCG-R (Gly40Ser) in a group of hypertensive patients and in normotensive control subjects, as described below. Clinical characteristics of the 2 groups are listed in Table 1.
TABLE 1. Clinical Parameters of Hypertensive Subjects and Normotensive Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age, y</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>BMI, kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT (all)</td>
<td>741</td>
<td>48.1 (9.0)</td>
<td>175.0 (19.3)</td>
<td>109.8 (6.8)</td>
<td>28.0 (5.1)</td>
</tr>
<tr>
<td>HT men</td>
<td>429</td>
<td>47.8 (9.0)</td>
<td>173.2 (19.0)</td>
<td>110.1 (7.3)</td>
<td>28.3 (4.5)</td>
</tr>
<tr>
<td>HT women</td>
<td>312</td>
<td>48.5 (9.1)</td>
<td>177.3 (19.4)</td>
<td>109.4 (6.1)</td>
<td>27.6 (5.8)</td>
</tr>
<tr>
<td>NT (all)</td>
<td>412</td>
<td>46.1 (6.7)</td>
<td>123.3 (13.8)</td>
<td>76.3 (9.8)</td>
<td>24.3 (3.3)</td>
</tr>
<tr>
<td>NT men</td>
<td>242</td>
<td>47.1 (6.5)</td>
<td>127.0 (13.1)</td>
<td>78.5 (10.0)</td>
<td>25.0 (2.9)</td>
</tr>
<tr>
<td>NT women</td>
<td>170</td>
<td>44.7 (6.8)</td>
<td>117.8 (13.0)</td>
<td>72.9 (8.7)</td>
<td>23.3 (3.7)</td>
</tr>
</tbody>
</table>

Values are mean (SD). SBP and DBP indicate systolic and diastolic blood pressure; BMI, body mass index; HT, hypertensive subjects; and NT, normotensive subjects.

Study Population

Hypertensive Index Cases (PEGASE Study)
The Projet d’Etude des Gènes de l’Hypertension Artérielle Sèvère à modérée Essentielle (PEGASE) was designed to identify genes for susceptibility to moderate and severe hypertension. A total of 741 hypertensive cases were previously recruited in the PEGASE Study6 from 15 regions of France, according to the following criteria: white European origin with both parents born in France and 4 grandparents born in Europe, aged <60 years, diastolic blood pressure ≥105 mm Hg without antihypertensive treatment or ≥110 mm Hg with treatment, creatinine level <120 μmol/L, age >40 years, and absence of proteinuria. Patients with secondary hypertension were excluded. Blood pressure was measured 3 times with a mercury sphygmomanometer and following the World Health Organization/International Society of Hypertension recommendations, with subjects in a sitting position and after 15 minutes of rest. The mean values of the 3 measurements were used in analyses.

Normotensive Control Subjects
Normotensive unrelated control subjects (n=412), collected in preventive medicine centers in Paris and Nancy, had systolic blood pressure ≤145 mm Hg, diastolic blood pressure ≤90 mm Hg, and no history of antihypertensive treatment or of chronic disease. All individuals were of white European origin.

Genotyping Gly40Ser Polymorphism of the GCG-R Gene
One set of oligonucleotides was used to cover a part of exon 2 and to generate a polymerase chain reaction product of 196 bp in length (Table 2). The amplification was performed with 100 ng of DNA in a total volume of 50 μL containing 10 mmol/L Tris-HCl (pH 9), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.1% Triton X-100, 0.2 mg/mL BSA, 200 μmol/L dNTPs, 25 pmol of each primer, and 0.2 U Taq polymerase (Perkin Elmer). The samples were denatured at 94°C for 5 minutes, followed by 35 amplification cycles at 94°C for 45 seconds, 53°C for 1 minute, and 72°C for 1 minute and 1 cycle at 72°C for 10 minutes.

Genotyping of the whole study population was performed with the use of allele-specific oligonucleotides. One fifth of the polymerase chain reaction product was denatured in 150 μL of 0.5 mol/L NaOH and 1.5 mol/L NaCl and dotted onto nylon membranes (N+., ICM). Membranes were then neutralized in 2× SSC and cross-linked with UV light. Each membrane was hybridized in 7% polyethylene glycol/10% sodium dodecyl sulfate at 49°C (Gly40 probe) and 47°C (Ser40 probe) for 4 hours, with 100 pmol of either of the 2 oligonucleotides (Table 2), end-labeled with [γ-^32P]ATP. The membranes were washed twice at room temperature in 0.5× SSC for 5 minutes followed by 5 minutes in 0.1× SSC at 51°C (Gly40) and 49°C (Ser40), followed by autoradiography.

Statistical Analysis
Data were analyzed with SAS statistical software (SAS Institute Inc.). Hardy-Weinberg equilibrium was tested by a χ² test with 1 df. Genotype frequencies were compared between cases and control subjects by a χ² test.

Results
Table 3 displays the results of the association study. The frequency of the Gly40Ser mutation did not exhibit significant deviation from Hardy-Weinberg expectations in the case or control groups. In the whole population, the mutation was twice as frequent among hypertensives than among controls. However, this difference did not reach statistical significance (P=0.087). When genders were analyzed separately, a significant difference in genotype frequency was found between cases and controls in men (P=0.035) but not in women (Table 3).

Seventeen of 429 male hypertensives exhibited the mutation on at least 1 allele versus only 2 of 242 male normotensives (4.0% versus 0.8%).

Discussion
This is the largest case-control study ever performed with white hypertensives for the Gly40Ser mutation of the GCG-R. The Gly40Ser mutation is relatively rare (allele frequency <1% in normotensive controls of this study) and, as a consequence, the informational value of this marker is low for an association study. This low informational value is compensated by the large number of severe essential hypertensives who were included in this study and by the fact that the Gly40Ser mutation is a potentially functional variant. Although we did not find a significant difference in the whole group, the frequency of the 40Ser allele carriers was 2-fold higher in hypertensives than in normotensives. Further analysis of both genders separately revealed that the 40Ser allele was significantly more frequent in male hypertensives than in male controls. In contrast, Chambers and Morris found a difference for the Gly40Ser mutation frequency, which was restricted to women. In the absence of consistency between these results, either these associations are
TABLE 3. Comparison of Genotype Distribution of the GCG-R Gly40Ser Polymorphism in Controls and Hypertensive Cases

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlyGly</td>
<td>741</td>
<td>429</td>
<td>312</td>
<td>412</td>
<td>242</td>
<td>170</td>
</tr>
<tr>
<td>GlySer</td>
<td>718</td>
<td>96.9</td>
<td>412</td>
<td>96.0</td>
<td>306</td>
<td>98.1</td>
</tr>
<tr>
<td>SerSer</td>
<td>22</td>
<td>3.0</td>
<td>16</td>
<td>3.7</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

χ² = 2.9, 5.5, 0.10
P = 0.087, 0.035, 0.9

χ² comparisons are made between hypertensives and corresponding normotensive control groups for genotype frequencies.

both spurious or they can result from differences in the type of patients collected. Indeed, the significant association of the Gly40Ser polymorphism in hypertensive women was restricted to a group of patients with family history of hypertension in both parents, an inclusion criterion that was not used in our group of patients and that could result in a reduction of men with the deleterious genotype in the hypertensive population by a higher death rate. Such a hypothesis was proposed by Morris et al to explain variations in angiotensin I–converting enzyme genotypes in older hypertensive patients. In view of our large sample of men, the association we observe in this gender could be considered biologically supported. The association that was found only in men could be due to an associated factor, such as the higher activity of the vasopressin system observed in this gender. It is well established, in rats and in different human ethnic groups, that vasopressin secretion is significantly higher in males than in females, and that both the antidiuretic and the vasoconstrictive responses to vasopressin are more intense in males than in females. In addition to its well-known effect on water permeability, vasopressin is also known to stimulate sodium reabsorption in the renal collecting duct. Several observations suggest that low urinary flow rate (due to high endogenous vasopressin) reduces the ability of the kidney to excrete sodium by enhancing the amount of sodium reabsorbed in the late part of the nephron. Obviously, the conjunction of a too strong sodium reabsorption in both the proximal and distal parts of the nephron (due to Gly40Ser mutation and to intense vasopressin actions, respectively) would favor the appearance of hypertension in those subjects who tend to produce strongly hypertonic urine. This interpretation, however, still needs confirmation because no information is available concerning urinary flow rate, osmolality, and vasopressin status in the PEGASE study.

In conclusion, this study reveals that the Gly40Ser variant of the GCG-R is present in ≈1.5% of French normotensive subjects. It exhibits a significant association with hypertension in men (with a 5-fold higher frequency in hypertensives than in normotensives) but not in women. These results should encourage additional studies designed to clarify the responsibility of this variant in hypertension, alone or in interaction with other risk factors.

Acknowledgments
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References
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