Investigation of P213S SELL gene polymorphism in type 2 diabetes mellitus and related end stage renal disease. A case-control study

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Abstract
SELL (L-selectin) is a candidate gene for several complex diseases including diabetes mellitus and renal failure. Our aim was to investigate the involvement of P213S SELL gene polymorphism (rs2229569) in type 2 diabetes mellitus (T2DM) and related end stage renal disease (ESRD). Type 2 diabetes mellitus patients without ESRD (n=250) or with ESRD (n=90), ESRD patients without diabetes (n=119) and sex and age matched healthy subjects (n=459) were analyzed in this study. DNA samples from all these subjects were genotyped for the P213S polymorphism by PCR–RFLP technique. Statistical analysis indicated that SELL P213S genotypes and alleles were similar distributed in the patients and control groups (OR SS=0.37, CI 95%: 0.131>0.372>1.06, \( p = 0.05 \), Yate’s correction \( p = 0.09 \), for T2DM patients without ESRD, OR SS=2.04, CI 95%: 0.365>2.047>1.465, \( p = 0.4 \), Yate’s correction \( p = 0.67 \), for T2DM patients with ESRD and OR SS=1, CI95%: 0.198>1>5.057, \( p = 1 \), Yate’s correction \( p = 0.7 \), for non-diabetic with ESRD patients). Also, no significant differences were noticed when we compared the ESRD subjects with diabetes vs. non-diabetic ones (OR=1.798, CI 95%: 0.392>1.798>8.245, \( p = 0.44 \), Yate’s correction \( p = 0.7 \)). No statistically significant results were found in order to sustain the hypothesis of association between SELL gene P213S polymorphism, type 2 diabetes mellitus and end stage renal disease.

Keywords: T2DM, ESRD, SELL, P213S genetic polymorphism.

Introduction

In recent years, we have been gathering evidence that in T2DM there is a cytokine-associated acute-phase reaction, but the involvement of SELL in susceptibility for DM is still debated [7].

Renal disease is a microvascular complication, which occurs in roughly one-third of diabetic patients [10]. The role of inflammation in the pathogenesis of renal disease [11] is supported by identification of leukocyte infiltration in the renal biopsy and prone benefic effects of agents responsible for infiltration or immune cells activation block [12–14].

SELL may be involved in T2DM and related renal disease not only through the inflammatory pathways, but also by interfering with intrauterine development. Normal expression of SELL is crucial to normal placenta implant and development [15, 16] and to intrauterine development [17, 18]. One of the SELL gene polymorphism (P213S or rs2229569) is associated with low birth weight [19] reported as a risk factor for type 2 diabetes [20–22], congenital oligonephronia and renal disease [23, 24].

Aim
The objective of this study was to investigate the involvement of P213S SELL gene polymorphism in type 2 diabetes mellitus (T2DM) and related end stage renal disease (ESRD).
Patients and Methods

Subjects

Type 2 diabetes mellitus patients without ESRD (n=250) or with ESRD (n=90), ESRD patients without diabetes (n=119) and matched healthy subjects (n=459) have been selected from the Emergency County Hospital of Craiova and “Nicolea Paulesc” Institute, Bucharest. The local ethic comity approved the research conducted in accordance with WMA Declaration of Helsinki (2008).

Diabetes was diagnosed based on World Health Organization guidelines and the patients were selected if they have at least two years of diabetes.

Healthy clinical controls have had normal à jeun glycemia (<110 mg/dL) and normal albumin excretion rate and declared no heredo-collateral antecedents for diabetes or renal diseases. They were selected to be similar as sex, age and residence place with the patients.

DNA genotyping

Blood samples collected on EDTA tubes were used to extract genomic DNA with AxyPrepTM Blood Genomic DNA Miniprep Kit. The P213S SELL gene polymorphism was detected by PCR using the following primers Fwd 5’ tggacctgttgcagcttg 3’ and Rev 5’ cttgacaggttggttctg 3’. Each amplification reaction (10 µL) contained 50 mM KCl, 10 mM tris-HCl pH 8.4, 1.5 mM MgCl2, 0.05 µL of each primer, 200 uM dNTP, 0.7 µL DNA and 1 U Taq polymerase. The PCR program (Corbett Research thermocycler) has an initial denaturation at 95°C for 2 minutes, 30 cycles at 94°C for one minute, 60°C for one minute and 72°C for one minute, and a final step at 72°C for 5 minutes. The genotypes were determined by digestion of each amplicon (5 µL) with 5U of Hph1 (Fermentas, Vilnius, Lithuania) followed by PAGE (8%). The genotypes have been established after ethidium bromide staining.

Statistical analyses

The distribution of genotypes and allele frequencies were compared using chi-square test while the disease risk was estimated by odds ratio (OR) calculated with 2×2 contingency table (SISA) [25]. A value of p<0.05 was considered significant.

Results

Clinical parameters of the subjects were recorded before P213S polymorphism genotyping. The mean age of each group was included in the range 50–57 years; diabetes duration was about 9.8 years for T2DM without ESRD patients and respectively 13.1 years for T2DM with ESRD subjects. Cardiovascular diseases were detected for 50.8% of the diabetic patients without ESRD, 81.1% of diabetic patients with ESRD and 31.9% of ESRD non-diabetic subjects. The genotypes distribution was counted by direct numbering. Chi-square values calculated for each of the analyzed groups were between 0.02 and 0.99 with p-values >0.05, in accordance with Hardy–Weinberg law. The equilibrium condition was also respected when the genotypes were distributed according to the gender, as it can be noticed in Table 1.

Table 1 – P213S genotypes and alleles in patients and control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>T2DM without ESRD</th>
<th>Healthy control (1)</th>
<th>T2DM with ESRD</th>
<th>Healthy control (2)</th>
<th>Non-diabetic with ESRD</th>
<th>Healthy control (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>W</td>
<td>Total</td>
<td>M</td>
<td>W</td>
<td>Total</td>
</tr>
<tr>
<td><strong>P213S genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>80</td>
<td>90</td>
<td>170</td>
<td>76</td>
<td>84</td>
<td>160</td>
</tr>
<tr>
<td>PS</td>
<td>37</td>
<td>38</td>
<td>75</td>
<td>37</td>
<td>40</td>
<td>77</td>
</tr>
<tr>
<td>SS</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td><strong>Allelic frequencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>F(P)</td>
<td>0.62</td>
<td>0.84</td>
<td>0.83</td>
<td>0.79</td>
<td>0.80</td>
<td>0.79</td>
</tr>
<tr>
<td>F(S)</td>
<td>0.18</td>
<td>0.16</td>
<td>0.17</td>
<td>0.21</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Hardy–Weinberg equilibrium</strong></td>
<td>Chi²</td>
<td>0.28</td>
<td>0.81</td>
<td>0.99</td>
<td>0.74</td>
<td>0.19</td>
</tr>
</tbody>
</table>

M – Men, W – Women.

Similar distribution of alleles’ frequencies has been observed in each of the three case/control studies. No statistical significant p values were noticed when we calculated the disease risk. Values of ORSS were statistical significant p values were noticed when we observed in each of the three case/control studies. No significant differences were noticed when we compared the three healthy subjects groups between them (p=0.94) or the ESRD subjects with diabetes versus non-diabetic ones (p=0.44). Also, no significant p-values were obtained when PP genotype distributions were compared in T2DM patients without ESRD (OR=1.05, p=0.87), non-diabetic ESRD patients (OR=1.12, p=0.67) and theirs controls.

Discussion

T2DM and related renal disease are major health problems worldwide. The onset of DM and its evolution to ESRD is modulated by different genetic and environmental factors. The combination of these factors may explain the familial aggregation, ethnic variation of prevalence and the clinical heterogeneity of diabetes [26, 27]. The precise pathophysiological roles for these factors are still under debate.

Several genes from both arms of chromosome 1 were evaluated for involvement in T2DM (e.g. NOTCH2-1p13-p11; APOA2, INSRR, PKLR, LMNA-1q21-q23). In the close proximity of these clusters was mapped SELL gene (1q23-q25) that may also interfere
with risk for T2DM and related renal disease. The P213S polymorphism (a C/T transition in exon 6) of the gene represents a good candidate in order to assess this risk. This genetic variant determines a Proline 213 Serine exchange in the protein domain 1 and thus seems to be responsible for the interaction between leucocytes and endothelium. A trend of association between this polymorphism and advanced stage of diabetic renal disease was previously reported by our research team [28] for type 1 diabetes mellitus patients, but additional studies are needed in order to clarify this finding.

Previous studies have investigated the role of P213S polymorphism in T2DM or renal disease. A research performed on 116 T2DM Chinese patients and 126 healthy subjects reported P-allele as a risk factor (OR=1.462, \( p<0.05 \)) for T2DM [29]. Also, an independent Chinese study investigated 64 type 2 diabetes mellitus patients without diabetic nephropathy, 55 patients with diabetic nephropathy, 112 healthy subjects and assessed that PP-genotype is a genetic risk factor for the development of renal disease in T2DM patients [30]. This finding confirmed the results previously obtained by Kamiuchi K et al. [31] who reported SELL 213PP genotype as a risk factor for diabetic renal disease in T2DM Japanese patients. The presence of 213P allele, in homozygous or heterozygous condition, seems to influence progression of diabetic renal disease rather than its onset [31]. The results were obtained after the investigation of 102 T2DM patients with diabetic nephropathy, 90 patients without diabetic nephropathy and 200 healthy controls.

The results of our present study, performed on Romanian Caucasians subjects, are not in agreement with the data reported for Asiatic T2DM patients. We did not obtain any \( p \)-value that achieves the statistical significance limit of 0.05. As a proving that our results are not due to a bias, healthy control groups' comparison did not reveal significant differences. Also, the frequencies of the rare allele in healthy Romanians (0.18; 0.19; 0.21) are in the range of values reported for other European populations (ex. frequency of T-allele is (0.18; 0.19; 0.21) are in the range of values reported for Asian T2DM patients.

Statistically significant results were found in order to compare did not reveal significant differences. Also, our results are not due to a bias, healthy control groups' statistical significance limit of 0.05. As a proving that We did not obtain any with the data reported for Asiatic T2DM patients.

We did not obtain any

With the data reported for Asiatic T2DM patients.

\[ \text{References} \]


\[ \text{Acknowledgments} \]

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