Leptin receptor gene polymorphisms in patients with post-transplant diabetes mellitus

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Research Article

Keywords:

Posted Date: February 10th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2552558/v1

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Abstract

Post-transplant diabetes mellitus (PTDM) is a metabolic complication that often occurs after kidney transplantation. Factors that increase the risk of this complication are currently being researched, including polymorphisms in genes affecting carbohydrate-lipid metabolism. Leptin is a hormone that affects appetite and adipose tissue and plays an important role in regulating insulin secretion as well as glucose and lipid metabolism. The aim of this study was to examine the association between leptin receptor gene polymorphisms and the development of post-transplant diabetes mellitus. The study was carried out in a group of 201 patients who underwent kidney transplantation. The follow-up period was 12 months. PTDM was diagnosed in 35 patients. There were no statistically significant differences in the distribution of the LEPR rs1137100 and LEPR rs1805094 polymorphisms between patients with and without PTDM. Analysing the LEPR gene rs1137101 polymorphism, we observed in patients with PTDM an increased frequency of GG allele carriers (GG vs AA; OR 3.36; 95% CI (0.99–11.46), p = 0.044). Multivariate regression analysis confirmed that female sex, advanced age, increased BMI and a higher number of LEPR rs1137101 G alleles were independent risk factors for PTDM development. The risk of PTDM development was almost 3.5 times greater in LEPR rs1137101 G allele carriers than in AA homozygotes (GG + AG vs AA; OR 3.48; 95%CI (1.09–11.18), p = 0.035). The results suggest that patients after kidney transplantation with the LEPR gene rs1137101 G allele have an increased risk of post-transplant diabetes development.

Introduction

Kidney transplantation is currently a common treatment for end-stage renal failure, but this requires long-term immunosuppressive therapy, which is associated with numerous complications. Post-transplant diabetes mellitus (PTDM) is a metabolic complication that occurs frequently after kidney transplantation. This complication may develop in up to 30% of recipients [1]. The known risk factors include recipient age, increased body mass index and use of calcineurin inhibitors, especially tacrolimus [2]. Numerous studies have supported that calcineurin inhibitors are involved in the development of PTDM. Their diabetogenic effects appear to be due to multiple mechanisms, particularly their toxic effects on pancreatic β-cells and increased insulin resistance [3, 4]. Studies suggest that tacrolimus therapy is associated with a higher risk of PTDM than cyclosporine therapy [5, 6]. Post-transplant diabetes mellitus is a complication that increases the risk of cardiovascular complications and loss of the transplanted kidney [7]. Due to the similarity of PTDM to type 2 diabetes, the role of hormones that affect carbohydrate-lipid metabolism in the pathogenesis of PTDM is being investigated.

Leptin is a 16-kDa single-chain protein synthesised in human adipose tissue. This hormone regulates appetite and body fat and plays an important role in regulating insulin secretion as well as glucose and lipid metabolism [8]. Additionally, leptin affects bone metabolism and angiogenesis, as well as the immune system. Previous studies suggest that increased serum leptin levels are associated with metabolic syndrome and are a risk factor for the development of diabetes [9]. In addition to obesity and diabetes, hyperleptinemia is associated with hypertension and other cardiovascular diseases [3–7].
peripheral actions of leptin include enhancement of the inflammatory response, oxidative stress, atherogenesis, endothelial dysfunction and insulin resistance [8–10]. Recent studies have highlighted the clinical relevance of leptin in the development of diabetes. Polymorphisms of the leptin gene have been studied in patients with obesity, insulin resistance and type 2 diabetes, suggesting that they may be risk factors for these diseases [10].

Due to the numerous complications of PTDM and the reduced survival of the transplanted kidney in patients with PTDM, factors that increase the risk of developing PTDM are being sought. Previous studies have shown an association between some genetic polymorphisms and the risk of developing PTDM [11–13].

The aim of this study was to examine the association between leptin receptor gene polymorphisms and the development of post-transplant diabetes mellitus in patients treated with tacrolimus.

**Materials And Methods**

**Patients**

The study was carried out in a group of 201 patients who underwent kidney transplantation at the Department of Nephrology, Transplantology and Internal Medicine of the Pomeranian Medical University in Szczecin. The follow-up period was 12 months. PTDM was diagnosed in 35 patients with haemoglobin A1c consistently above 6.5% or fasting glucose ≥ 7.0 mmol/l or who required treatment with oral hypoglycaemic agents or insulin for more than three months after transplantation [14]. Patients who were diagnosed with diabetes mellitus (as a cause of renal disease or comorbidities) before transplantation and patients with graft failure or death within one month after transplantation were excluded.

Standard immunosuppression consisted of the calcineurin inhibitor tacrolimus, mycophenolate mofetil and steroids. Tacrolimus administration was started at 0.1 mg/kg, and doses were adjusted to maintain serum concentrations between 10 and 12 ng/ml in the first month after transplantation and between 8 and 10 ng/ml thereafter. Mycophenolate mofetil was administered at doses of 2 g per day and prednisolone at doses of 10–20 mg per day. The study was approved by the local ethics committee, and written informed consent was obtained from all participants.

**Methods**

All samples were genotyped in duplicate using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Carlsbad, California, USA) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). To discriminate the LEPR rs1137100, rs1137101 and rs1805094 alleles, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C____2412786_10, C____7497299_10 and C__15966471_20, respectively), including appropriate primers and fluorescently
labelled (FAM and VIC) MGB™ probes to detect the alleles. Genotypes were assigned using all of the data from the study simultaneously.

**Statistical analysis**

The consistency of the genotype distribution with Hardy-Weinberg equilibrium (HWE) was assessed with the use of Fisher the exact test. The frequencies of genotypes and alleles were compared with chi-square and Fisher's exact tests. Multivariate Cox proportional hazards models were used to assess the risk of PTDM in one year of follow-up after renal transplantation. P < 0.05 was considered statistically significant.

**Results**

As shown in table 1, there were no statistically significant differences in the distribution of the *LEPR* rs1137100 and *LEPR* rs1805094 polymorphisms between patients with and without PTDM. *Analysing the LEPR* gene rs1137101 polymorphism, we observed in patients with PTDM an increased frequency of GG allele carriers (GG vs AA, OR 3.36; 95% CI 0.99–11.46; p = 0.04).

In multivariate regression analysis we examined the PTDM risk during 1-year follow up. This analysis confirmed in additive model that female sex, advanced age, increased BMI and the number of LEPR rs1137101 G alleles were independent risk factors of PTDM development (Table 2).

The risk of PTDM development in dominant model was almost 3.5 times greater in *LEPR* rs1137101 G allele carriers than in AA homozygotes (GG+AG vs AA, OR 3.48; 95% CI 1.09–11.18) (Table 3).

**Table 1. Distribution of LEPR genotypes and alleles in PTDM and no PTDM groups.**
<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>No PTDM</th>
<th>PTDM</th>
<th>$p^*$</th>
<th>OR (95% CI)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><strong>LEPR rs1137100</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>88</td>
<td>53.01%</td>
<td>14</td>
<td>40.00%</td>
<td>0.30</td>
</tr>
<tr>
<td>AG</td>
<td>62</td>
<td>37.35%</td>
<td>18</td>
<td>51.43%</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>16</td>
<td>9.64%</td>
<td>3</td>
<td>8.57%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>238</td>
<td>71.69%</td>
<td>46</td>
<td>65.71%</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>94</td>
<td>28.31%</td>
<td>24</td>
<td>34.29%</td>
<td></td>
</tr>
<tr>
<td><strong>LEPR rs1137101</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>44</td>
<td>26.51%</td>
<td>4</td>
<td>11.43%</td>
<td>0.13</td>
</tr>
<tr>
<td>AG</td>
<td>86</td>
<td>51.81%</td>
<td>20</td>
<td>57.14%</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>36</td>
<td>21.69%</td>
<td>11</td>
<td>31.43%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>174</td>
<td>52.41%</td>
<td>28</td>
<td>40.00%</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>158</td>
<td>47.59%</td>
<td>42</td>
<td>60.00%</td>
<td></td>
</tr>
<tr>
<td><strong>LEPR rs1805094</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Multivariate analysis of PTDM hazard during 1 year follow up (additive model).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male vs female)</td>
<td>0.43 (0.19-0.98)</td>
<td>0.044</td>
</tr>
<tr>
<td>Recipient’s age (years)</td>
<td>1.04 (1.00-1.07)</td>
<td>0.031</td>
</tr>
<tr>
<td>Recipient’s BMI (kg/m²)</td>
<td>1.17 (1.02-1.34)</td>
<td>0.025</td>
</tr>
<tr>
<td>LEPR rs1137101 (number of G alleles) (additive model)</td>
<td>1.91 (1.05-3.47)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Table 3. Multivariate analysis of PTDM hazard during 1 year follow up (dominant model).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male vs female)</td>
<td>0.43 (0.19-0.98)</td>
<td>0.043</td>
</tr>
<tr>
<td>Recipient’s age (years)</td>
<td>1.04 (1.00-1.07)</td>
<td>0.029</td>
</tr>
<tr>
<td>Recipient’s BMI (kg/m²)</td>
<td>1.17 (1.02-1.34)</td>
<td>0.022</td>
</tr>
<tr>
<td>LEPR rs1137101 (GG+AG vs AA) (dominant model)</td>
<td>3.48 (1.09-11.18)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Discussion
Post-transplant diabetes mellitus (PTDM) is a common complication after kidney transplantation leading to a number of metabolic and cardiovascular complications as well as dysfunction and loss of the transplanted kidney. PTDM occurs as early as the first months after transplantation. Accordingly, most cases of PTDM in our study were diagnosed in the first few months. We assessed the association between leptin receptor gene polymorphisms and the development of PTDM in one year of follow-up after renal transplantation. We found that this complication occurs more frequently in carriers of the \textit{LEPR} rs1805094 G allele. This relationship was confirmed by multivariate regression analysis, in which the number of G alleles was an independent risk factor for PTDM in addition to known PTDM risk factors such as recipient age, female sex and increased BMI. Furthermore, the risk of developing PTDM was almost 3.5 times higher in carriers of the G allele.

Previous studies indicated that leptin has a diabetogenic effect by decreasing insulin secretion and increasing insulin resistance. Studies have confirmed the inhibitory effect of leptin on insulin secretion in pancreatic \(\beta\)-cells. By inhibiting JAK/STAT signalling, leptin blocks the transcription of the proinsulin gene in pancreatic \(\beta\)-cells [15]. In addition, leptin blocks the phosphorylation of glucose transporter 2 (GLUT2), resulting in reduced glucose transport into cells and thus reduced intracellular adenosine triphosphate (ATP) concentrations [16]. This is the reason for the increased insulin resistance. In addition, leptin activates ATP-sensitive potassium channels and causes \(\beta\)-cell hyperpolarisation, thereby reducing intracellular calcium concentrations. Leptin also reduces insulin secretion induced by cyclic adenosine monophosphate, glucagon-like peptide 1 and protein kinase C [17].

Dedinska et al. have shown that leptin levels are significantly increased in patients after kidney transplantation. Additionally, leptin levels are positively correlated with the level of triacylglycerols, post-transplant diabetes mellitus (PTDM) development and acute rejection [18].

One of the factors that increase the risk of PTDM is the immunosuppressive pharmacotherapy used, particularly calcineurin inhibitors. The calcineurin inhibitors cyclosporine and tacrolimus also show significant diabetogenic effects, with tacrolimus having a stronger effect than cyclosporine. Cyclosporine significantly reduces both insulin synthesis and insulin secretion by pancreatic \(\beta\)-cells, due to increased polyamines that regulate \(\beta\)-cell growth and function [19]. The diabetogenic effect of tacrolimus is mainly due to its toxic effect on \(\beta\)-cells, which leads to reduced insulin secretion [20, 21]. The drug causes damage to \(\beta\)-cells, swelling of their cytoplasm, vacuolisation and increased apoptosis. Tacrolimus has also been shown to significantly reduce transcription of insulin and insulin receptor genes and inhibit insulin secretion by \(\beta\)-cells [22, 23]. Tacrolimus can also reduce glucose uptake through increased endocytosis of the GLUT4 transporter from the cell surface [24].

Factors influencing the risk of PTDM are currently being investigated. To date, associations between PTDM and genes connected with an increased risk of type II diabetes and affecting the synthesis of tacrolimus and cyclosporine metabolising enzymes have been analysed [25–28]. Many of the genes associated with type II diabetes have also been linked to increased risk of PTDM. Previous studies have suggested that the development of PTDM may be related not only to determinants of tacrolimus and
cyclosporine metabolism but also to factors affecting pancreatic β-cell function as well as carbohydrate and lipid metabolism [29]. Type II diabetes is characterised by impaired pancreatic β-cell function and increased insulin resistance. In PTDM, both insulin secretion and peripheral insulin action appear to be impaired. However, impaired insulin secretion appears to be the predominant mechanism leading to the development of PTDM [5, 30].

Leptin gene polymorphisms were studied in patients with diabetes in different populations. The results are variable and depend on the population studied. In most cases, these polymorphisms appear to be predisposing factors for diabetes or diabetic complications [31–35]. The association of leptin and leptin receptor gene polymorphisms with PTDM has not been widely investigated to date. In our previous study, we demonstrated the association of the LEP rs2167270 gene polymorphism with the occurrence of PTDM [11]. Mota-Zamorano et al. have shown an association between rs1137101 of the LEPR gene and PTDM [36]. The results of our study confirm these findings in our population and suggest that the rs1137101 polymorphism of the LEPR gene is associated with the risk of PTDM, and patients with the G allele have an increased risk of developing PTDM. Our results suggest that the leptin receptor gene polymorphism rs1137101 may be one of the factors that increase the risk of developing PTDM. Due to the multifactorial pathogenesis of this complication, this polymorphism must be taken into account together with other known risk factors for the development of PTDM such as recipient's sex, age and BMI. Consideration of multiple risk factors can help to identify patients at increased risk of developing this complication following renal transplantation and to implement appropriate early prophylaxis.

**Conclusion**

Patients with the LEPR gene rs1137101 G allele have an increased risk of post-transplant diabetes mellitus development after kidney transplantation.

**Declarations**

**Ethical Approval:** The study was approved by the local ethics committee (BN-001/59/04) and is in accordance with the Declaration of Helsinki.

**Consent to Participate:** Informed consent was obtained from all subjects involved in the study.

**Consent to Publish:** Not applicable

**Competing interests:** The authors declare no conflict of interest.

**Authors’ contributions:**

VD-investigation, manuscript preparation, conceptualization, KS-statistical analysis, MKN-investigation, patients collection, DM-investigation, AP-conceptualization, manuscript preparation
Funding: The project is financed from the program of the Minister of Science and Higher Education under the name “Regional Initiative of Excellence” in 2019–2022 project number 002/RID/2018-19.

Availability of data and materials: The data that support the findings of this study are available upon reasonable request from the corresponding author.

References


