Glucocorticoid receptor gene polymorphisms are associated with reduced first-phase glucose-stimulated insulin secretion and disposition index in women, but not in men


1Diabetes Center, Department of Internal Medicine, VU University Medical Center, Amsterdam, 2Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, 3The EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, 4Department of Internal Medicine, Utrecht University Medical Center, Utrecht, The Netherlands, 5Department of Internal Medicine, Eberhard-Karls University of Tübingen, Tübingen, Germany, 6Biological Psychology, VU University, Amsterdam, 7Department of Internal Medicine, VU University Medical Center, Amsterdam, The Netherlands, 8Eli Lilly and Company, Indianapolis, IN, USA and 9Section Molecular Epidemiology, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

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Abstract

Aim Glucocorticoids are efficacious anti-inflammatory agents, but, in susceptible individuals, these drugs may induce glucose intolerance and diabetes by affecting β-cell function and insulin sensitivity. We assessed whether polymorphisms in the glucocorticoid receptor gene NR3C1 associate with measures of β-cell function and insulin sensitivity derived from hyperglycaemic clamps in subjects with normal or impaired glucose tolerance.

Methods A cross-sectional cohort study was conducted in four academic medical centres in the Netherlands and Germany. Four hundred and forty-nine volunteers (188 men; 261 women) were recruited with normal glucose tolerance (n = 261) and impaired glucose tolerance (n = 188). From 2-h hyperglycaemic clamps, first- and second-phase glucose-stimulated insulin secretion, as well as insulin sensitivity index and disposition index, were calculated. All participants were genotyped for the functional NR3C1 polymorphisms N363S (rs6195), Bcl1 (rs41423247), ER22/23EK (rs6189/6190), 9β A/G (rs6198) and ThtIII (rs10052957). Associations between these polymorphisms and β-cell function parameters were assessed.

Results In women, but not in men, the N363S polymorphism was associated with reduced disposition index (P = 1.06 × 10⁻⁴). Also in women, the ER22/23EK polymorphism was associated with reduced first-phase glucose-stimulated insulin secretion (P = 0.011) and disposition index (P = 0.003). The other single-nucleotide polymorphisms were not associated with β-cell function. Finally, none of the polymorphisms was related to insulin sensitivity.

Conclusion The N363S and ER22/23EK polymorphisms of the NR3C1 gene are negatively associated with parameters of β-cell function in women, but not in men.


Keywords β-cell function, glucocorticoid receptor, polymorphisms

Introduction

Excess glucocorticoid levels induce glucose intolerance [1,2] and are associated with incident diabetes [3]. In addition to glucocorticoid-induced insulin resistance [4], glucocorticoid-induced β-cell dysfunction is a hallmark of glucocorticoid-induced
adverse metabolic effects [2,5–7]. Glucocorticoids exert many effects by binding to its the cytosolic glucocorticoid receptor, following which the ligand-activated glucocorticoid receptor translocates to the nucleus where it regulates target gene transcriptional activity. Considerable variability exists in the sensitivity to glucocorticoids across individuals, a phenomenon that was linked to functional polymorphisms in the glucocorticoid receptor gene (NR3C1) [8–10]. As such, the NR3C1 variants ER22/23EK [two single-nucleotide polymorphisms (SNPs) that are in complete linkage disequilibrium] [11] and 9 A/G [12] may induce relative glucocorticoid resistance, whereas the NR3C1 gene variants Bcl C/G [13] and N363S [14] are associated with enhanced glucocorticoid sensitivity. The effects of the TthIII polymorphism are currently less clear [15]. Importantly, several of these SNPs have been linked to metabolic variables. In some studies, glucocorticoid resistance was correlated with insulin sensitivity, increased lean body mass and reduced waist circumference [11,16,17]. In contrast, glucocorticoid sensitivity may be associated with a less favourable metabolic profile [18,19]. Importantly, gender-specific effects have frequently been observed [8–10,17,19]; for example, the ER22/23EK variant was associated with beneficial body composition, muscle strength and metabolic profile in men, but not in women [16].

It is currently unknown whether these NR3C1 gene polymorphisms affect β-cell function. Interestingly, mice with specific over expression of the glucocorticoid receptor in the β-cell become diabetic because of β-cell failure [20]. We hypothesized that alterations in glucocorticoid sensitivity attributable to SNPs in the NR3C1 gene could relate to β-cell function. This hypothesis was addressed for the first time in the present study, where 449 subjects were genotyped for NR3C1 polymorphisms and β-cell function was measured by the gold-standard hyperglycaemic clamp.

Research design and methods

Cohorts

Four hundred and forty-nine Caucasian subjects were recruited from three independent studies from the Netherlands and one from Germany [21–25]. Characteristics and inclusion criteria of the separate cohorts are provided in the Supporting Information (Tables S1 and S2).

Hyperglycaemic clamp procedure

All participants underwent a hyperglycaemic clamp at 10 mmol/L glucose for at least 2 h as described previously [21,22,24,25]. First-phase glucose-stimulated insulin secretion was computed as the sum of the insulin levels during the first 10 min of the clamp. Second-phase glucose-stimulated insulin secretion was determined as the mean insulin level during the last 40 min of the clamp (80–120 min). The insulin sensitivity index was defined as the glucose infusion rate (M, μmol min⁻¹ kg⁻¹) divided by the plasma insulin concentration (I, pmol/l) during the last 40 min of the clamp (M/I, μmol min⁻¹ kg⁻¹ pmol⁻¹ l⁻¹), which was shown to correlate well with insulin sensitivity measured by the hyperinsulinaemic-euglycaemic clamp [26]. Insulin secretion adjusted for insulin sensitivity was expressed as the disposition index, calculated by multiplying first-phase glucose-stimulated insulin secretion and insulin sensitivity index [27].

Genotyping

Based on the available literature, five SNPs were genotyped: TthIII (rs10052957), ER22/23EK (rs6189/6190), N363S (rs6195), Bcl site (rs41423247) and 9 A/G (rs6198), using the Sequenom platform (Sequenom, San Diego, CA, USA). The genotyping success rate was above 98% for all SNPs (see also Supporting Information, Tables S1 and S2).

Statistics

The effect of the SNPs on β-cell function was examined with linear regression assuming an additive model. To take into account the family relatedness, empirical standard errors were used (using the generalized estimating equations). For the monozygotic twins we computed the mean of the β-cell measures and included only these mean measures in the analysis. The data from the non-identical twins were both used. The analyses of both first- and second-phase glucose-stimulated insulin secretion were adjusted for age, BMI, study centre, glucose tolerance status (normal/impaired glucose tolerance) and insulin sensitivity index. For the analysis of the insulin sensitivity and disposition indices, the insulin sensitivity index was removed from the covariates. All outcomes were log transformed prior to analysis. Because NR3C1 polymorphisms have previously been shown to display gender-specific effects, male and female participants were analysed separately [8–10]. All data are given as estimated mean (95% CI). After Bonferroni correction for multiple testing, results were regarded to be significant at a level of P < 0.012 (four tests). For all statistical analyses, SPSS version 18.0 for Mac OS X (SPSS, Chicago, IL, USA) was used.

Results

Subject characteristics

In total, 449 participants were recruited from four study centres (see also Supporting Information, Tables S1 and S2).

Genotypes and haplotypes

The observed genotype and haplotype frequencies are shown in Fig. 1 and were similar to those previously reported [8–20].
Associations with β-cell function

The N363S variant was associated with reduced disposition index \( (P = 1.06 \times 10^{-5}) \) and showed a trend towards reduced first-phase insulin secretion in women (Table 1). Also, in women only, the ER22/23EK polymorphism was associated with reduced first-phase glucose-stimulated insulin secretion \( (P = 0.011) \) and disposition index \( (P = 0.003) \). No other associations were observed between NR3C1 SNPs and measures of β-cell function, neither in men, nor in women. Similar results were obtained for the associations between NR3C1 haplotypes and β-cell function parameters (see Supporting Information, Table S2). The results of the analyses were not different when subjects with normal glucose tolerance and those with impaired glucose tolerance were analysed separately (data not shown).

Associations with insulin sensitivity

None of the NR3C1 SNPs or haplotypes was significantly associated with insulin sensitivity (Table 1 and Supporting Information, Table S2).

Discussion

In four cohorts of subjects with normal glucose tolerance and those with impaired glucose tolerance, we found that the NR3C1 SNPs N363S and ER22/23EK were associated with reduced β-cell function parameters in women. Both first-phase glucose-stimulated insulin secretion and disposition index, which denotes the adaptation of β-cells to prevailing insulin sensitivity, were influenced. As expected, the corresponding NR3C1 haplotypes containing these polymorphisms provided identical results. The N363S SNP enhances glucocorticoid sensitivity by increasing gene transcription [14]. Indeed, in various studies, a link was established between the N363S SNP and characteristics of a Cushingoid phenotype, including increased BMI and waist circumference, dyslipidaemia and augmented fasting insulin levels, indicating reduced insulin sensitivity [10,19]. The ER22/23EK SNP, however, demonstrated reduced glucocorticoid receptor activation \( \text{in vitro} \), and relative glucocorticoid resistance \( \text{in vivo} \) [11,16]. As such, in men, the ER22/23EK was associated with a beneficial metabolic phenotype, including increased muscle mass and strength, lower LDL cholesterol and insulin levels [11,16]. In contrast, female carriers of the ER22/23EK SNP were at increased risk to develop cardiovascular disease [28]. In another cohort, carriers of the ER22/23EK had higher HbA1c levels as compared with non-carriers [29], thus raising doubt on the hypothesis that this SNP may induce a more favourable metabolic profile, especially in women.

More recently, impaired glucose-stimulated insulin secretion was shown to be another hallmark of glucocorticoid-induced adverse metabolic effects both \( \text{in vitro} \) and \( \text{in vivo} \) in humans, where several measures of β-cell function were impaired [2,5–7]. Furthermore, mice over expressing the glucocorticoid receptor specifically in β-cells developed diabetes through β-cell failure [20]. Our present data support the concept that glucocorticoids impair β-cell function.

Interestingly, the associations between SNPs in the NR3C1 gene and β-cell function parameters were only observed in women, not in men. As outlined above, gender-specific effects of NR3C1 gene variants have been observed in various studies for various anthropometric and metabolic variables [8–10,16,17,19]. Additionally, gender-related hormonal factors are known to affect β-cell function [30]. As such, pre-menopausal women and women receiving oestrogen replacement therapy have reduced prevalence of diabetes, which has been attributed to the β-cell protective effects of oestrogens [30]. Furthermore, the male sex hormone testosterone may also affect β-cell function [31]. The effects of NR3C1 polymorphisms on β-cell function may therefore interact differently with sex hormones.

An important limitation of the present study is the relatively small number of participants that were included, although this cohort is the largest to undergo a hyperglycaemic clamp in the context of genetic analysis currently available in the literature. We cannot rule out the possibility to have missed subtle effects of other NR3C1 polymorphisms on β-cell function variables.
Table 1: Insulin response according to NR3C1 SNP in women and men

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>First-phase glucose-stimulated insulin secretion (pmol/l) §</th>
<th>Second-phase glucose-stimulated insulin secretion (pmol/l) §</th>
<th>Insulin sensitivity index (µmol min⁻¹ kg⁻¹ pmol⁻¹ l⁻¹) §</th>
<th>Disposition index (µmol min⁻¹ kg⁻¹) §</th>
<th>n</th>
<th>First-phase glucose-stimulated insulin secretion (pmol/l) §</th>
<th>Second-phase glucose-stimulated insulin secretion (pmol/l) §</th>
<th>Insulin sensitivity index (µmol min⁻¹ kg⁻¹ pmol⁻¹ l⁻¹) §</th>
<th>Disposition index (µmol min⁻¹ kg⁻¹) §</th>
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<td>9β(rs6198)</td>
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<td>AA</td>
<td>156</td>
<td>733 (685–784)</td>
<td>244 (229–260)</td>
<td>0.15 (0.13–0.16)</td>
<td>104 (98–112)</td>
<td>111</td>
<td>687 (622–759)</td>
<td>250 (231–271)</td>
<td>0.15 (0.13–0.17)</td>
<td>102 (92–114)</td>
</tr>
<tr>
<td>AG</td>
<td>76</td>
<td>804 (723–893)</td>
<td>256 (233–281)</td>
<td>0.13 (0.11–0.15)</td>
<td>108 (96–121)</td>
<td>51</td>
<td>679 (577–798)</td>
<td>244 (216–275)</td>
<td>0.15 (0.13–0.18)</td>
<td>103 (87–122)</td>
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<tr>
<td>GG</td>
<td>8</td>
<td>758 (903–1144)</td>
<td>313 (210–468)</td>
<td>0.11 (0.07–0.17)</td>
<td>91 (59–122)</td>
<td>7</td>
<td>930 (722–1197)</td>
<td>240 (207–280)</td>
<td>0.17 (0.11–0.27)</td>
<td>142 (94–216)</td>
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<td>β (rs11052957)</td>
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<td>C677T</td>
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<tr>
<td>CC</td>
<td>117</td>
<td>740 (686–798)</td>
<td>244 (226–264)</td>
<td>0.14 (0.13–0.16)</td>
<td>104 (97–113)</td>
<td>71</td>
<td>698 (619–787)</td>
<td>217 (239–286)</td>
<td>0.15 (0.13–0.17)</td>
<td>102 (94–123)</td>
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<tr>
<td>CT</td>
<td>99</td>
<td>787 (719–862)</td>
<td>260 (240–283)</td>
<td>0.14 (0.12–0.16)</td>
<td>109 (98–120)</td>
<td>77</td>
<td>686 (607–776)</td>
<td>243 (219–268)</td>
<td>0.15 (0.13–0.17)</td>
<td>103 (91–117)</td>
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<td>TT</td>
<td>23</td>
<td>715 (569–897)</td>
<td>228 (193–271)</td>
<td>0.14 (0.10–0.16)</td>
<td>97 (78–121)</td>
<td>19</td>
<td>719 (553–934)</td>
<td>218 (184–259)</td>
<td>0.16 (0.12–0.22)</td>
<td>106 (70–119)</td>
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<tr>
<td>363N363S (rs6195)*</td>
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<tr>
<td>AA</td>
<td>222</td>
<td>766 (722–812)</td>
<td>248 (235–262)</td>
<td>0.14 (0.13–0.15)</td>
<td>108 (101–115)</td>
<td>161</td>
<td>696 (637–760)</td>
<td>249 (232–267)</td>
<td>0.15 (0.13–0.17)</td>
<td>103 (94–114)</td>
</tr>
<tr>
<td>AG</td>
<td>17</td>
<td>613 (515–731)</td>
<td>263 (225–307)</td>
<td>0.14 (0.09–0.14)</td>
<td>74 (62–88)</td>
<td>8</td>
<td>658 (553–783)</td>
<td>229 (191–274)</td>
<td>0.20 (0.14–0.29)</td>
<td>118 (91–154)</td>
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<td>ER22/23EK (rs6189/rs6190)</td>
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<td>GG/GG</td>
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<tr>
<td>AA/GG</td>
<td>9</td>
<td>518 (386–694)</td>
<td>294 (205–420)</td>
<td>0.10 (0.08–0.14)</td>
<td>68 (51–90)</td>
<td>14</td>
<td>751 (595–947)</td>
<td>240 (210–274)</td>
<td>0.14 (0.10–0.18)</td>
<td>106 (78–144)</td>
</tr>
<tr>
<td>β (rs2012 The Authors. © 2012 Diabetes UK)</td>
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</table>

Significant findings are shown in bold. Data are estimated means (95% CI). The β (st) is the β (st) of the log-transformed variable.

*Only one participant was homozygous for the N363S variant; the subject was added to the heterozygous group.

†For the ER22/23EK SNP, non-carriers are depicted as GG/GG. Heterozygotes are AA/GG. No homozygotes were identified in this cohort. All variables were log transformed before analysis.

‡P-values were computed for additive models using linear generalized estimating equations, which takes into account the family relatedness when computing the standard errors.

§First- and second-phase glucose-stimulated insulin secretion were adjusted for study centre, family relatedness, glucose tolerance status, age, BMI and insulin sensitivity index.

●Insulin sensitivity index and disposition index were adjusted for study centre, family relatedness, glucose tolerance status, age, BMI and insulin sensitivity index.
Another limitation is the fact that the SNPs did not tag the whole NR3C1 gene, therefore we cannot exclude that the associations found were caused by another untested SNP, although, given the extensive literature on the function of the SNPs, this seems unlikely. We fully subscribe to the need for replication of these data, although such replication is non-trivial because the hyperglycaemic clamp methodology is demanding for both researchers and participants.

In conclusion, this is the first report to show that the N363S and ER22/23EK NR3C1 gene variants are associated with reduced first-phase glucose-stimulated insulin secretion and disposition index in women, but not in men. This may point to a differential effect of genetically determined variation in glucocorticoid receptor activity in women as compared with men in the adaptation of (first-phase) insulin secretion to insulin sensitivity.

Competing interests
RJH is an employee of Eli Lilly & Company. The other authors have nothing to declare.

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References
NR3C1 polymorphisms and β-cell function • D. H. van Raalte et al.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Pairwise linkage disequilibrium values between the SNP quantified using $r^2$ in the Haploview program.

Table S1. Characteristics of the cohort.

Table S2. Major inclusion criteria of the study cohort.

Table S3. Insulin response according to NR3C1 haplotype in women and men.

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