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

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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), *Bcl*I (rs41423247), ER22/23EK (rs6189/6190), 9β A/G (rs6198) and *ThtIII*I (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 only 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.

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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

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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 9β A/G [12] may induce relative glucocorticoid resistance, whereas the *NR3C1* gene variants *Bcl*I C/G [13] and N363S [14] are associated with enhanced glucocorticoid sensitivity. The effects of the *TthIII*I polymorphism are currently less clear [15]. Importantly, several of these SNPs have been linked to metabolic variables. In some studies, glucocorticoid resistance was associated 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 second hour 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*I (rs10052957), ER22/23EK (rs6189/6190), N363S (rs6195), *Bcl*I site (rs41423247) and 9β A/G (rs6198), using the Sequenom platform (Sequenom, San Diego, CA, USA). The genotyping success rate was above 98% for all SNPs and samples measured in duplicate (~5%) were in complete concordance. The SNPs did not deviate from Hardy–Weinberg equilibrium (Haploview program; MIT, Harvard Broad Institute, Cambridge, MA, USA). Individual haplotypes were constructed using SNPHAP (http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt).

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].

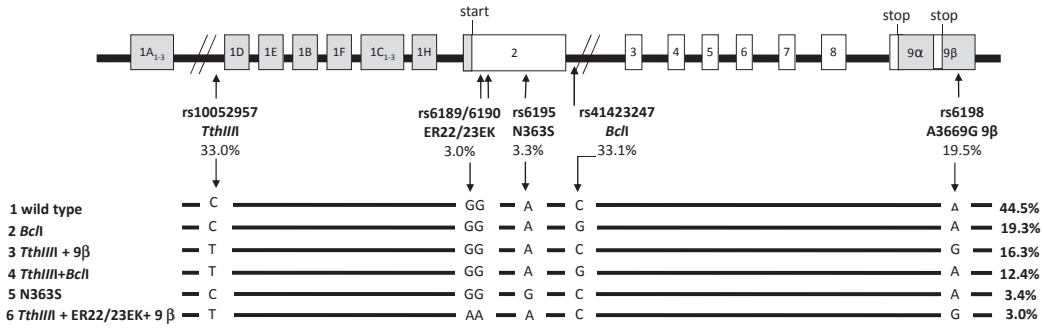

FIGURE 1 Schematic overview of the glucocorticoid receptor gene, *NR3C1*, polymorphisms and haplotypes. The location of each single-nucleotide polymorphism (SNP) in the *NR3C1* gene is indicated by a black arrow. Minor allele frequencies of the SNPs are indicated below the SNP name. Haplotype alleles are indicated with black lines containing the nucleic acid for each SNP. Haplotype allele frequencies are displayed to the left of the haplotype allele and haplotype alleles are numbered in order of decreasing frequency.

Associations with β-cell function

The N363S variant was associated with reduced disposition index ($P = 1.06\ 10^{-4}$) 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, respectively).

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 *in vitro*, and relative glucocorticoid resistance *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 HbA_{1c} 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 *in vitro* and *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

	Women					Men				
Genotype	*n*	First-phase glucose-stimulated insulin secretion (pmol/l)§	Second-phase glucose-stimulated insulin secretion (pmol/l)§	Insulin sensitivity index (μmol min^{-1} kg^{-1} $pmol^{-1}$ l^{-1})¶	Disposition index (μmol min^{-1} kg^{-1})¶	*n*	First-phase glucose-stimulated insulin secretion (pmol/l)§	Second-phase glucose-stimulated insulin secretion (pmol/l)§	Insulin sensitivity index (μmol min^{-1} kg^{-1} $pmol^{-1}$ l^{-1})¶	Disposition index (μmol min^{-1} kg^{-1})¶
*Bcl*I (rs41423247)										
CC	110	713 (656–766)	244 (225–264)	0.14 (0.12–0.15)	100 (91–109)	74	730 (643–828	262 (239–288)	0.15 (0.13–0.17)	107 (94–123)
CG	98	773 (712–839)	248 (229–269)	0.14 (0.13–0.16)	109 (100–118)	82	665 (594–745)	236 (217–257)	0.16 (0.14–0.18)	103 (91–117)
GG	30	837 (688–1017)	270 (233–312)	0.15 (0.12–0.19)	116 (95–143)	13	710 (518–972)	258 (195–341)	0.12 (0.08–0.17)	70 (70–119)
β (SE)		0.035 (0.020)	0.018 (0.017)	0.018 (0.024)	0.035 (0.021)		−0.021 (0.029)	−0.023 (0.023)	−0.013 (0.031)	−0.027 (0.028)
P‡		0.084	0.292	0.448	0.100		0.462	0.314	0.683	0.349
9β (rs6198)										
AA	156	733 (685–784)	244 (229–260)	0.15 (0.13–0.16)	104 (98–112)	111	687 (622–759)	250 (231–271)	0.15 (0.13–0.17)	102 (92–114)
AG	76	804 (723–893)	256 (233–281)	0.13 (0.11–0.15)	108 (96–121)	51	679 (577–798)	244 (216–275)	0.15 (0.13–0.18)	103 (87–122)
GG	8	758 (503–1144)	313 (210–468)	0.11 (0.07–0.17)	91 (59–122)	7	930 (722–1197)	240 (207–280)	0.17 (0.11–0.27)	142 (94–216)
β (SE)		0.028 (0.026)	0.033 (0.024)	−0.051 (0.030)	−0.003 (0.028)		0.022 (0.030)	−0.010 (0.020)	0.011 (0.034)	0.030 (0.034)
P		0.280	0.174	0.085	0.911		0.457	0.628	0.753	0.386
*Tth*III (rs10052957)										
CC	117	740 (686–798)	244 (226–264)	0.14 (0.13–0.16)	104 (97–113)	71	698 (619–787)	217 (239–286)	0.15 (0.13–0.17)	102 (94–123)
CT	99	787 (719–862)	260 (240–283)	0.14 (0.12–0.16)	109 (98–120)	77	686 (607–776)	243 (219–268)	0.15 (0.13–0.17)	103 (91–117)
TT	23	715 (569–897)	228 (193–271)	0.14 (0.10–0.16)	97 (78–121)	19	719 (553–934)	218 (184–259)	0.16 (0.12–0.22)	106 (70–119)
β (SE)		0.005 (0.021)	0.001 (0.018)	−0.018 (0.024)	−0.004 (0.022)		0.002 (0.027)	−0.037 (0.019)	0.011 (0.030)	0.007 (0.028)
P		0.808	0.938	0.466	0.865		0.949	0.055	0.707	0.798
N363S (rs6195)*										
AA	222	766 (722–812)	248 (235–262)	0.14 (0.13–0.15)	108 (101–115)	161	696 (637–760)	249 (232–267)	0.15 (0.13–0.17)	103 (94–114)
AG	17	613 (515–731)	263 (225–307)	0.14 (0.09–0.14)	74 (62–88)	8	658 (553–783)	229 (191–274)	0.20 (0.14–0.29)	118 (91–154)
β (SE)		−0.096 (0.041)	0.026 (0.036)	−0.111 (0.055)	**−0.165 (0.043)**		−0.024 (0.039)	−0.037 (0.041)	0.130 (0.085)	0.058 (0.058)
P		0.020	0.476	0.043	**0.0001**		0.534	0.375	0.125	0.327
ER22/23EK (rs6189/rs6190)†										
GG/GG	231	764 (722–808)	248 (235–262)	0.14 (0.13–0.15)	106 (100–113)	155	690 (632–754)	249 (232–266)	0.15 (0.14–0.17)	104 (94–114)
AA/GG	9	518 (386–694)	294 (205–420)	0.10 (0.08–0.14)	68 (51–90)	14	751 (595–947)	240 (210–274)	0.14 (0.10–0.18)	106 (78–144)
β (SE)		**−0.169 (0.066)**	0.073 (0.080)	−0.131 (0.064)	**−0.197 (0.065)**		0.037 (0.052)	−0.015 (0.030)	−0.047 (0.068)	0.011 (0.069)
P		**0.011**	0.360	0.040	**0.003**		0.485	0.618	0.491	0.874

Significant findings are shown in bold.

Data are estimated means (95% CI), the β (SE) is the β (SE) 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 and BMI.

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

1 Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 2002; **96**: 23–43.
2 van Raalte DH, Ouwens DM, Diamant M. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? *Eur J Clin Invest* 2009; **39**: 81–93.
3 Gulliford MC, Charlton J, Latinovic R. Risk of diabetes associated with prescribed glucocorticoids in a large population. *Diabetes Care* 2006; **29**: 2728–2729.
4 McMahon M, Gerich J, Rizza R. Effects of glucocorticoids on carbohydrate metabolism. *Diabetes Metab Rev* 1988; **4**: 17–30.
5 Linssen MM, van Raalte DH, Toonen EJ, Alkema W, van der Zon GC, Dokter WH *et al.* Prednisolone-induced beta-cell dysfunction is associated with impaired endoplasmic reticulum homeostasis in INS-1E cells. *Cell Signal* 2011; **23**: 1708–1715.
6 van Raalte DH, Nofrate V, Bunck MC, van Iersel T, Elassaiss Schaap J, Nassander UK *et al.* Acute and 2-week exposure to prednisolone impair different aspects of beta-cell function in healthy men. *Eur J Endocrinol* 2010; **162**: 729–735.
7 van Raalte DH, van Genugten RE, Linssen MM, Ouwens DM, Diamant M. Glucagon-like peptide-1 receptor agonist treatment prevents glucocorticoid-induced glucose intolerance and islet-cell dysfunction in humans. *Diabetes Care* 2011; **34**: 412–417.
8 Derijk RH. Single nucleotide polymorphisms related to HPA axis reactivity. *Neuroimmunomodulation* 2009; **16**: 340–352.
9 van Rossum EF, Lamberts SW. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. *Recent Prog Horm Res* 2004; **59**: 333–357.
10 Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF. Clinical features associated with glucocorticoid receptor polymorphisms. An overview. *Ann N Y Acad Sci* 2009; **1179**: 179–198.
11 van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO *et al.* A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids *in vivo*, is associated with low insulin and cholesterol levels. *Diabetes* 2002; **51**: 3128–3134.
12 Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J *et al.* A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol* 2001; **28**: 2383–2388.
13 van Rossum EF, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W *et al.* Identification of the *Bcl*I polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids *in vivo* and body mass index. *Clin Endocrinol (Oxf)* 2003; **59**: 585–592.
14 Russcher H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH *et al.* Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression. *J Clin Endocrinol Metab* 2005; **90**: 5804–5810.
15 van Rossum EF, Roks PH, de Jong FH, Brinkmann AO, Pols HA, Koper JW *et al.* Characterization of a promoter polymorphism in the glucocorticoid receptor gene and its relationship to three other polymorphisms. *Clin Endocrinol (Oxf)* 2004; **61**: 573–581.
16 van Rossum EF, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, Kemper HC *et al.* The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. *J Clin Endocrinol Metab* 2004; **89**: 4004–4009.
17 Syed AA, Irving JA, Redfern CP, Hall AG, Unwin NC, White M *et al.* Association of glucocorticoid receptor polymorphism A3669G in exon 9β with reduced central adiposity in women. *Obesity (Silver Spring)* 2006; **14**: 759–764.
18 Rosmond R, Chagnon YC, Holm G, Chagnon M, Perusse L, Lindell K *et al.* A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. *Obes Res* 2000; **8**: 211–218.
19 Roussel R, Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G. The N363S polymorphism in the glucocorticoid receptor gene is associated with overweight in subjects with type 2 diabetes mellitus. *Clin Endocrinol (Oxf)* 2003; **59**: 237–241.
20 Davani B, Portwood N, Bryzgalova G, Reimer MK, Heiden T, Ostenson CG *et al.* Aged transgenic mice with increased glucocorticoid sensitivity in pancreatic beta-cells develop diabetes. *Diabetes* 2004; **53**: S51–S59.
21 Fritsche A, Madaus A, Renn W, Tschritter O, Teigeler A, Weisser M *et al.* The prevalent Gly1057Asp polymorphism in the insulin receptor substrate-2 gene is not associated with impaired insulin secretion. *J Clin Endocrinol Metab* 2001; **86**: 4822–4825.
22 Ruige JB, Dekker JM, Nijpels G, Popp-Snijders C, Stehouwer CD, Kostense PJ *et al.* Hyperproinsulinaemia in impaired glucose tolerance is associated with a delayed insulin response to glucose. *Diabetologia* 1999; **42**: 177–180.
23 Simonis-Bik AM, Eekhoff EM, Diamant M, Boomsma DI, Heine RJ, Dekker JM *et al.* The heritability of HbA1c and fasting blood glucose in different measurement settings. *Twin Res Hum Genet* 2008; **11**: 597–602.
24 van Haeften TW, Dubbeldam S, Zonderland ML, Erkelens DW. Insulin secretion in normal glucose-tolerant relatives of type 2 diabetic subjects. Assessments using hyperglycemic glucose clamps and oral glucose tolerance tests. *Diabetes Care* 1998; **21**: 278–282.

25 van Haeften TW, Pimenta W, Mitrakou A, Korytkowski M, Jenssen T, Yki-Jarvinen H *et al.* Disturbances in beta-cell function in impaired fasting glycemia. *Diabetes* 2002; **51**: S265–S270.
26 Mitrakou A, Vuorinen-Markkola H, Raptis G, Toft I, Mokan M, Strumph P *et al.* Simultaneous assessment of insulin secretion and insulin sensitivity using a hyperglycemia clamp. *J Clin Endocrinol Metab* 1992; **75**: 379–382.
27 Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW *et al.* Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 1993; **42**: 1663–1672.
28 Koeijvoets KC, van Rossum EF, Dallinga-Thie GM, Steyerberg EW, Defesche JC, Kastelein JJ *et al.* A functional polymorphism in the glucocorticoid receptor gene and its relation to cardiovascular disease risk in familial hypercholesterolemia. *J Clin Endocrinol Metab* 2006; **91**: 4131–4136.
29 Kuningas M, Mooijaart SP, Slagboom PE, Westendorp RG, van Heemst D. Genetic variants in the glucocorticoid receptor gene (NR3C1) and cardiovascular disease risk. The Leiden 85-plus Study. *Biogerontology* 2006; **7**: 231–238.
30 Liu S, Mauvais-Jarvis F. Minireview: Estrogenic protection of beta-cell failure in metabolic diseases. *Endocrinology* 2010; **151**: 859–864.
31 Morimoto S, Fernandez-Mejia C, Romero-Navarro G, Morales-Peza N, Diaz-Sanchez V. Testosterone effect on insulin content, messenger ribonucleic acid levels, promoter activity, and secretion in the rat. *Endocrinology* 2001; **142**: 1442–1447.

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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