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Sex-specific effects of naturally occurring variants in the dopamine receptor D2 locus on insulin secretion and Type 2 diabetes susceptibility

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Abstract

Aims Modulation of dopamine receptor D2 (DRD2) activity affects insulin secretion in both rodents and isolated pancreatic β-cells. We hypothesized that single nucleotide polymorphisms in the *DRD2/ANKK1* locus may affect susceptibility to Type 2 diabetes in humans.

Methods Four potentially functional variants in the coding region of the *DRD2/ANKK1* locus (rs1079597, rs6275, rs6277, rs1800497) were genotyped and analysed for Type 2 diabetes susceptibility in up to 25 000 people (8148 with Type 2 diabetes and 17687 control subjects) from two large independent Dutch cohorts and one Danish cohort. In addition, 340 Dutch subjects underwent a 2-h hyperglycaemic clamp to investigate insulin secretion. Since sexual dimorphic associations related to *DRD2* polymorphisms have been previously reported, we also performed a gender-stratified analysis.

Results rs1800497 at the *DRD2/ANKK1* locus was associated with a significantly increased risk for Type 2 diabetes in women (odds ratio 1.14 (1.06–1.23); $P = 4.1*10^{-4}$) but not in men (odds ratio 1.00 (95% CI 0.93–1.07); $P = 0.92$) or the combined group. Although rs1800497 was not associated with insulin secretion, we did find another single nucleotide polymorphism in this locus, rs6275, to be associated with increased first-phase glucose-stimulated insulin secretion in women ($P = 5.5*10^{-4}$) but again not in men ($P = 0.34$).

Conclusion The present data identify *DRD2/ANKK1* as a potential sex-specific Type 2 diabetes susceptibility gene.

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Introduction

Pancreatic β-cell dysfunction is one of the major determinants in the pathogenesis of Type 2 diabetes [1]. In response to glucose, insulin is released by the β-cells in a biphasic

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What's new?

- The rs1800497 single nucleotide polymorphism at the *DRD2/ANKK1* locus was associated with a significantly increased risk for Type 2 diabetes in women but not in men.
- The rs6275 single nucleotide polymorphism in the *DRD2* gene is associated with increased first-phase glucose-stimulated insulin secretion in women only.
- Our data identify *DRD2/ANKK1* as a potential sex-specific Type 2 diabetes susceptibility gene.

fashion consisting of a rapid and transient first-phase followed by a sustained second phase [2]. The molecular events linking glucose metabolism to insulin secretion involve the so-called triggering and metabolic amplifying pathways which interact to achieve temporal control and amplitude modulation of insulin secretion [3]. An early sign of Type 2 diabetes is the loss of first-phase glucose-stimulated insulin secretion and reduction of second-phase release [4].

Dopamine is one of the major neurotransmitters in the brain and controls a variety of key functions, such as cognition, feeding behaviour, motor control and secretion of various endocrine hormones [5]. The dopaminergic system is also involved in both central and peripheral regulation of whole-body fuel and energy homeostasis [6]. Alterations of this central regulatory pathway have been reported in insulin-resistant rodent models [7,8] and patients with Type 2 diabetes [9]. Dopamine signalling is mediated by two different G-protein-coupled receptor subtypes belonging to D1-like (D1 and D5) and D2-like (D2, D3 and D4) families [5]. Interestingly, it has been shown that all of these receptors are expressed in pancreatic β-cells, suggesting that some of this glucose-stimulated insulin secretion could be involved in the regulation of insulin secretion [10]. Accordingly, dopamine, as well as various dopamine receptor D2 (DRD2) agonists such as quinpirole and bromocriptine, inhibits insulin secretion in both β-cell lines and isolated islets from rodents [10,11]. In addition, a time-release form of bromocriptine was recently approved for the treatment of Type 2 diabetes [12], suggesting that DRD2 might be involved in the regulation of key metabolic processes, although the exact mechanism of action remains unclear. Furthermore, siRNA-mediated knockdown of DRD2, but not of the other dopamine receptor subtypes, affects glucose-stimulated insulin secretion in insulin-secreting INS-1E cells [13]. Finally, it has been reported recently that whole-body knockdown of DRD2 in mice impairs insulin secretion and glucose homeostasis [14], supporting the concept that DRD2 might play a role in the regulation of glucose-stimulated insulin secretion.

The above findings led us to hypothesize that gene variants in the *DRD2*/ankyrin repeat and kinase domain containing 1 (*ANKK1*) locus might affect glucose-stimulated insulin secretion and Type 2 diabetes susceptibility in humans. Although associations between *DRD2* gene polymorphisms and cognitive behaviours, such as smoking addiction, alcohol dependence and depression, have been intensively investigated [15], there are, to our knowledge, no data available on the relationship between *DRD2* variants, glucose-stimulated insulin secretion and Type 2 diabetes. The gene variants examined in the present study were in the coding regions of the *DRD2* gene or the nearby *ANKK1* gene (Taq1a variant, rs1800497) and were selected according to previous studies, which have linked them to phenotypes involving the dopamine system, such as tobacco and alcohol abuse [15]. For some of these single nucleotide polymorphisms (SNPs), *in vivo* or *in vitro* studies support a functional role in dopamine receptor signalling. For instance, the rs6277 SNP (Pro319Pro) in exon 7 was found to be associated with alterations of mRNA folding and stability, and decreased translation of the protein [16], whereas the rs1800497 SNP, although located in the *ANKK1* gene, has been associated with a reduced DRD2 receptor density [17]. Furthermore, the rs1079597 SNP in intron 6 affects splicing of *DRD2* mRNA, leading to a shift in equilibrium between long and short isoforms of the protein [18]. rs6275 was included as a proxy for rs6276, rs6279 (both in the 3'untranslated region of *DRD2*) and rs4938016 (*ANKK1*, G442R) (D'=1; $r^2 \geq 0.89$), which have been previously reported in the literature to be associated with various phenotypes, but await proof of functionality [19].

The main objectives of the present study were, therefore, to investigate the effects of these potentially functional SNPs in the coding region of the *DRD2/ANKK1* locus on Type 2 diabetes susceptibility. As sexual dimorphic associations related to *DRD2* polymorphisms have been reported previously [20–23], we also performed a gender-stratified analysis. Furthermore, we investigated their effect on first- and second-phase glucose-stimulated insulin secretion in a Dutch cohort subjected to a standardized hyperglycaemic clamp.

Material and methods

Association study

To test the effect of the *DRD2* gene on Type 2 diabetes susceptibility, data from one discovery and two replication cohorts were used. For the discovery cohort we used participants from the New Hoorn Study, a population-based study in the West-Friesland region of the Netherlands which aims to identify risk factors for Type 2 diabetes. Details have been described previously [24]. The definition of glucose tolerance status in the New Hoorn Study cohort was based on WHO criteria after a fasting oral glucose tolerance test. In addition, known diabetes was defined as the use of insulin or oral glucose-lowering agents and (self-)reported known diabetes. We randomly selected 2103 people (163 with Type 2 diabetes and 1940 with normal glucose tolerance, age 53 ± 7 years, BMI 25.6 ± 3.6 kg/m^2 (SD), 45% men) from

the New Hoorn Study. Furthermore, we randomly selected 3062 people with Type 2 diabetes from the West-Friesland Diabetes Care System (age 64 ± 11 years, BMI 30.1 ± 5.3 kg/m^2 (SD), 56% men). The West-Friesland Diabetes Care System provides diabetes care to people with Type 2 diabetes living in the same geographical region of the Netherlands [25]. In short, each patient visits the diabetes research centre annually for a physical examination and nutritional advice, and is invited for follow-up visits when necessary.

For the replication studies, we used two different cohorts, one from the Netherlands and one from Denmark. The people in the first replication cohort (1398 with Type 2 diabetes and 10761 with normal glucose tolerance) were participants in the Hoorn Study, the Leiden Longevity Study, the Rotterdam Study and the Netherlands Twin Register/the Netherlands Study of Depression and Anxiety cohorts in the Netherlands. The second replication cohort included a total of 3525 people with Type 2 diabetes and 4986 people with normal glucose tolerance from the Copenhagen area in Denmark. Clinical characteristics and details of criteria used to define Type 2 diabetes in the replication cohorts are provided in the online Supporting Information.

Hyperglycaemic clamp

The present study includes 340 subjects originating from three independent studies from the Netherlands who all underwent a 2-h hyperglycaemic clamp procedure at 10 mmol/l glucose as previously described [26]. Briefly, after an initial priming infusion of glucose, blood glucose levels were measured with a glucose analyser and kept constant at 10 mmol/l during the whole clamp procedure [26]. Insulin levels were measured with immunoassays. Both glucose and insulin levels were measured at 2.5 min (first 10 min of the procedure) or 10–20-min intervals (subsequent 10–120 min of the procedure). First-phase glucose-stimulated insulin secretion was calculated as the sum of the insulin levels during the first 10 min of the clamp procedure. Second-phase glucose-stimulated insulin secretion was calculated as the mean insulin level for the last 40 min (80–120 min). The insulin sensitivity index was calculated by dividing the glucose infusion rate (M in μmol/min/kg) by the mean plasma insulin level during the last 40 min of the clamp procedure (insulin, pmol/l). The disposition index was computed as the product of first-phase insulin secretion and insulin sensitivity index. Details of the three studies have been described previously [26]. In short, 139 people participated in the New Hoorn Study (all with impaired glucose tolerance), 78 people (66 with normal glucose tolerance and 12 with impaired glucose tolerance) were participants in a study in the University Medical Center Utrecht and 123 people (116 with normal glucose tolerance and seven with impaired glucose tolerance) were recruited by the Netherlands Twin Register. The Netherlands Twin Register sample includes 66 monozygotic and 28 dizygotic twins, and 29 of their non-twin siblings recruited from 50 families. The clinical characteristics of the study participants are given in Table S1.

Informed consent was given by participants in all studies and the studies were conducted in accordance to the principles of the Helsinki declaration and were approved by the local medical ethics committees.

Genotyping

Four SNPs in the *DRD2* locus were genotyped: rs1079597 (in high linkage disequilibrium (r^2 = 1.0, Hapmap CEU, www.hapmap.org) with rs1076560), rs6275 (H313H), rs6277 (P319P) and rs1800497 (*ANKK1*, E713K). The latter SNP, also known as the Taq1A polymorphism, was previously mapped to the *DRD2* locus but, more recently, it appeared that this SNP is actually located in the *ANKK1* gene, 10 kb downstream of the *DRD2* gene. SNPs were genotyped using either Sequenom MassARRAY technology (rs6275 and rs1800497 in the cohort who underwent hyperglycaemic clamp; Sequenom Inc., San Diego, CA, USA), Taqman SNP genotyping assays (Life Technologies, Bleiswijk, The Netherlands) or KASPar chemistry (KBiosciences, Hoddeston, UK). Duplicate samples (~5%) revealed no genotyping errors, genotyping success rates were all > 99% and there were no deviations from Hardy–Weinberg equilibrium. Furthermore, we did not detect any genotype errors when results from different platforms were compared. The four SNPs studied in the present study are only in moderate linkage ($r^2 \leq 0.69$) with each other based on the Hapmap data for Europeans. Three of them (rs6275, rs6277 and rs1079597) are located within a single linkage disequilibrium block whereas rs1800497 is located in another block.

Statistics

Genotype and allele frequencies in healthy individuals and people with Type 2 diabetes were compared using chi-squared tests assuming additive models. Logistic regression was used to calculate odds ratios (ORs) with 95% CIs. Comprehensive META-ANALYSIS v2 software (www.meta-analysis.com) was used for meta-analysis of the case–control studies using a fixed-effects model. ANOVA was used to compare anthropometric variables. Linear regression analysis assuming additive models was used to compare measures of β-cell function between different genotypes, unless otherwise stated. All β-cell measures were log transformed before analysis for normalization. To take into account family relatedness (in the twin sample), empirical standard errors were used (using generalized estimating equations). The analyses of first- and second-phase glucose-stimulated insulin secretion were adjusted for age, sex, BMI, study centre, glucose tolerance status (normal glucose tolerance/impaired glucose tolerance) and insulin sensitivity index. For the analysis of insulin sensitivity index and disposition index,

insulin sensitivity index was removed from the covariates. An *a priori* power calculation showed that we had at least 80% power ($\alpha = 0.05$) to detect a difference in first- or second-phase insulin secretion of ~15–20% (depending on allele frequency). Data in the discovery phase of the study were corrected for eight tests (four SNPs and two genders) and $P \leq 6.3*10^{-3}$ (0.05/8) was regarded significant. For the replication phase and the sex interaction, P values between 0.05 and $6.3*10^{-3}$ were regarded as nominally significant.

For all statistical analyses SPSS version 20.0 software (SPSS, Chicago, Il, USA) was used, unless otherwise stated.

Results

All genotyped SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$) and minor allele frequencies were similar to previously reported allele frequencies in Caucasian populations.

We first tested whether the variants affected Type 2 diabetes susceptibility. In our discovery case–control cohort we compared allele and genotype frequencies between 3225 cases and 1940 controls. In the cohort as a whole we did not observe significant associations (data not shown). As sexual dimorphic associations related to *DRD2* polymorphisms have been previously reported [20–23], we tested for a genotype × sex interaction on diabetes risk and found that it was nominally significant for rs1800497 ($P = 0.02$). We next repeated the analysis for men and women separately and identified a borderline significant association between rs1800497 and Type 2 diabetes in women (OR 1.22, 95% CI 1.06–1.40; $P = 7.0*10^{-3}$) but not in men (OR 0.94, 95% CI 0.81–1.09; $P = 0.40$; Table 1). In the next step, we expanded our study with two replication panels comprising a Dutch (replication 1, $n = 12159$) and a Danish cohort (replication 2, $n = 8511$; Table 1). In the Dutch replication cohort, the minor allele (T) of rs1800497 was nominally associated with higher risk of Type 2 diabetes in women (OR 1.14, 95% CI 1.00–1.30; $P = 0.046$) but not in men. Results from the Danish replication cohort did not reach statistical significance; however, the direction and size of the effect in women was similar to that in the Dutch populations (OR 1.10, 95% CI 0.98–1.23), $P = 0.12$) without any effect in men (Table 1).

After a test for homogeneity, which did not reveal significant heterogeneity ($P \geq 0.5$), we performed a meta-analysis with fixed effects of all available data which led to a summary OR for rs1800497 of 1.14 (95% CI 1.06–1.23); $P = 4.1*10^{-4}$) in women but no significant effect in men (OR 1.00, 95% CI 0.93–1.07; $P = 0.92$; Table 1). Notably, we also found a similar trend towards significance for rs1079597 which is in moderate linkage disequilibrium with rs1800497 ($r^2 = 0.69$; $P = 0.048$) in women (Table 1). None of the other SNPs were associated with Type 2 diabetes risk after meta-analysis of the discovery and replication cohorts. We also explored genome-wide association study

Table 1 *DRD2/ANKK1* polymorphisms and Type 2 diabetes susceptibility

SNP	Sex	Alleles	Discovery			Replication 1			Replication 2			Meta-analysis	
			MAF	OR (95% CI)	*P*	MAF	OR (95% CI)	*P*	MAF	OR (95% CI)	*P*	OR (95% CI)	*P*
rs1079597	**Men**	**G/A**	0.14	0.91 (0.77–1.07)	*0.26*	0.14	0.87 (0.73–1.04)	*0.12*	0.15	0.99 (0.88–1.11)	*0.81*	0.94 (0.86–1.02)	*0.14*
	Women		0.14	1.15 (0.98–1.35)	*0.10*	0.15	1.03 (0.89–1.20)	*0.68*	0.15	1.09 (0.96–1.24)	*0.17*	1.09 (1.00–1.18)	*0.048*
rs6275	**Men**	**C/T**	0.31	1.00 (0.88–1.14)	*0.97*	0.31	1.15 (1.01–1.30)	*0.035*	0.31	0.94 (0.86–1.03)	*0.17*	1.00 (0.94–1.07)	*0.90*
	Women		0.30	1.02 (0.90–1.15)	*0.79*	0.30	1.02 (0.91–1.15)	*0.74*	0.31	1.04 (0.94–1.14)	*0.49*	1.03 (0.96–1.09)	*0.44*
rs6277	**Men**	**C/T**	0.45	0.97 (0.87–1.09)	*0.64*	0.45	1.02 (0.90–1.14)	*0.80*	0.46	0.94 (0.86–1.02)	*0.12*	0.96 (0.91–1.02)	*0.23*
	Women		0.45	1.08 (0.97–1.21)	*0.18*	0.46	0.99 (0.89–1.10)	*0.82*	0.47	1.09 (0.99–1.19)	*0.073*	1.06 (0.99–1.12)	*0.081*
rs1800487	**Men**	**C/T**	0.20	0.94 (0.81–1.09)	*0.40*	0.19	1.05 (0.89–1.24)	*0.55*	0.19	1.01 (0.91-1.12)	*0.88*	1.00 (0.93–1.07)	*0.92*
	Women		0.20	1.22 (1.06–1.40)	***7.0*10^-3***	0.19	1.14 (1.00–1.30)	*0.046*	0.20	1.10 (0.98–1.23)	*0.12*	1.14 (1.06–1.23)	***4.1*10^-4***

SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio.
The major allele at each SNP is depicted in bold. MAF in discovery cohort. Data are presented as allelic ORs for the minor allele.

data from the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium (9580 cases and 53810 controls) and, as evidenced in the present study, they did not show a significant association between our *DRD2* SNPs and Type 2 diabetes susceptibility in a joint analysis of men and women (rs1800497 [OR 1.03, 95% CI 0.99–1.08; $P = 0.14$]; rs1079597 [OR 1.02, 95% CI 0.97–1.07; $P = 0.41$]; rs6275 [OR 1.02, 95% CI 0.98–1.06, $P = 0.32$]); however, sex-stratified analysis were unfortunately not publicly available (www.diagram-consortium.org).

We next analysed whether the SNPs studied were associated with measures of β-cell function assessed using the 'gold standard' hyperglycaemic clamp technique. After correction for multiple hypothesis testing, none of the SNPs were associated with β-cell function (Table S3). Given the sex-specific association with Type 2 diabetes susceptibility, we repeated the analysis in men and women separately and found gender-specific differences in insulin response (Table 2). Although rs1800497 was not associated with glucose-stimulated insulin secretion, we did find a significant association between rs6275 and first-phase glucose-stimulated insulin secretion in women ($P = 5.5*10^{-4}$) but, again, not in men ($P = 0.34$, Table 2). Furthermore there were no significant effects on insulin sensitivity or the disposition index (Table 2).

Discussion

In the present study we report that genetic variation in the coding region of the *DRD2* locus is associated with increased susceptibility to Type 2 diabetes in women but not in men, which might be mediated via alterations in glucose-stimulated insulin secretion.

Numerous SNPs within both coding and non-coding regions of the human *DRD2* gene have been described [15]. The gene variants examined in the present study were in the coding regions of the *DRD2* gene or the nearby *ANKK1* gene (Taq1a variant, rs1800497) and were selected according to previous studies which have linked them to phenotypes involving the dopamine system, such as tobacco and alcohol abuse [15]. For most of these SNPs, *in vivo* or *in vitro* studies support a functional role in dopamine receptor signalling [16–18].

Our most important finding was that, after sex stratification, the rs1800497 variant was associated with a higher risk of developing Type 2 diabetes only in women. One of the possible mechanisms whereby this SNP might increase risk of Type 2 diabetes could be impairment of DRD2 signal transduction in pancreatic β cells. Rubi *et al.* [10] have shown that dopamine receptors, including DRD2, are widely expressed in human pancreatic β cells and that both dopamine and selective DRD2 agonists inhibited glucose-stimulated insulin secretion [10]; however, our genetic study in people without diabetes who underwent a hyperglycaemic clamp procedure did not reveal significant associations between this SNP and β-cell function; this might be attributable to the relatively low *a priori* power. Furthermore, we and others have also shown previously that genetic effects in people without diabetes may also be overridden by other factors upon development of Type 2 diabetes [27,28]. This might also explain why women carriers of the rs6275 variant do not have an altered risk of Type 2 diabetes.

Another explanation for the association between rs1800497 and Type 2 diabetes but lack of an association with insulin secretion might be that the association is driven by either peripheral or central nervous system-driven effects, independently of modulation of insulin secretion. Indeed, central DRD2 activation by the synthetic dopamine agonist bromocriptine decreases hepatic glucose production and improves insulin sensitivity [29], suggesting that lack of functional DRD2 in specific hypothalamic area(s) would impair dopamine-mediated regulation of hepatic glucose production and whole-body metabolic homeostasis. Interestingly, although its exact molecular mechanism of action remains unknown to date, a timed-release form of bromocriptine has been recently approved by the U.S. Food and Drug Administration for the treatment of Type 2 diabetes [12]. Taken together, this indicates that central and/or peripheral DRD2 constitutes a potential drug target for treating metabolic disorders associated with Type 2 diabetes. It is worth mentioning that, wherever the drug acts, *DRD2* polymorphisms might therefore affect an individual patient's response to bromocriptine treatment. Future research examining *DRD2* polymorphisms in relation to sex-specific treatment success would then be interesting.

Notably, as mentioned above, rs1800497 was found to belong to the *DRD2* neighbouring gene *ANKK1*, coding for a serine/threonine kinase with unclear function [30]. It is therefore possible that alteration of the signal transduction mediated by this kinase might play a role in the pathophysiology of Type 2 diabetes. Further studies on the sex-specific function(s) of *ANKK1*, especially in metabolic tissues, are required for clarifying this point. Finally, it has been recently reported that rs1800497 is also associated with hypersensitivity to reward and increased risk for binge eating disorders [31], suggesting that this polymorphism might also foster overeating and promote the development of obesity and Type 2 diabetes.

In the present study, β-cell function and Type 2 diabetes susceptibility are both affected by *DRD2* variants in a sex-specific manner, suggesting that the effects of this gene are under the influence of as yet unknown sex-specific factors. Although not common, other traits also show sexual dimorphism in relation to genetic susceptibility [32,33]. Nonetheless, it remains to be elucidated how sex-specific factors affect the influence of *DRD2/ANKK1* gene polymorphisms on insulin secretion and Type 2 diabetes susceptibility.

Although our well-controlled hyperglycaemic clamp study is by far the largest available of its kind, it is still relatively small and thus underpowered to detect small effects on β-cell function while the chance of false-positive findings is also

Table 2 Insulin response in women and men according to *DRD2* rs1079597, rs6275, rs6277 and rs1800497 genotypes

		Estimated mean (95% CI) first-phase insulin response, pmol/l		Estimated mean (95% CI) second-phase insulin response, pmol/l		Estimated mean (95% CI) insulin sensitivity index, μmol/min/kg/pmol/l		Estimated mean (95% CI) disposition index score, μmol/min/kg	
SNP	Women/Men, *n*	Women	Men	Women	Men	Women	Men	Women	Men-
Rs1079597									
G/G	142/100	766 (717–819)	636 (573–706)	247 (232–264)	230 (208–255)	0.148 (0.136–0.162)	0.163 (0.143–0.186)	115 (107–123)	106 (94–119)
G/A	50/40	743 (652–846)	732 (643–833)	263 (238–291)	257 (227–292)	0.151 (0.128–0.179)	0.164 (0.132–0.203)	111 (95–129)	120 (104–139)
A/A	4/4	802 (420–1532)	982 (758–1272)	474 (273–824)	240 (162–356)	0.101 (0.047–0.217)	0.227 (0.166–0.312)	91 (52–161)	182 (156–213)
P		*0.83*	*0.010*	*0.041*	*0.21*	*0.66*	*0.52*	*0.45*	*0.010*
Rs6275									
C/C	94/65	698 (639–762)	705 (627–792)	253 (232–275)	253 (226–283)	0.164 (0.147–0.182)	0.158 (0.133–0.187)	109 (99–120)	111 (97–126)
C/T	91/65	792 (727–864)	623 (538–721)	252 (232–274)	225 (197–255)	0.134 (0.119–0.150)	0.180 (0.153–0.212)	113 (102–125)	112 (96–130)
T/T	11/13	1081 (862–1356)	655 (485–884)	286 (230–354)	215 (165–282)	0.150 (0.116–0.193)	0.133 (0.102–0.172)	161 (125–207)	98 (67–143)
P		$5.5*10^{-4}$	*0.34*	*0.56*	*0.13*	*0.037*	*0.94*	*0.052*	*0.66*
Rs6277									
C/C	57/35	671 (601–729)	621 (536–720)	232 (209–257)	232 (200–270)	0.168 (0.147–0.192)	0.170 (0.141–0.205)	106 (95–119)	104 (88–123)
C/T	102/76	796 (704–813)	692 (610–785)	259 (240–279)	252 (227–280)	0.145 (0.132–0.159)	0.159 (0.135–0.188)	119 (108–130)	112 (98–128)
T/T	36/32	821 (684–871)	679 (558–826)	279 (242–322)	214 (180–253)	0.131 (0.105–0.163)	0.175 (0.145–0.213)	113 (93–137)	120 (96–150)
P		*0.021*	*0.51*	*0.032*	*0.46*	*0.040*	*0.82*	*0.47*	*0.32*
Rs1800497									
C/C	123/89	759 (706–815)	646 (575–727)	249 (232–267)	231 (207–257)	0.151 (0.139–0.164)	0.165 (0.144–0.190)	116 (107–125)	108 (95–122)
C/T	67/50	756 (679–840)	681 (609–762)	255 (233–279)	252 (223–284)	0.145 (0.124–0.169)	0.157 (0.131–0.189)	110 (97–125)	110 (97–125)
T/T	6/4	877 (555–1385)	984 (751–1290)	416 (277–623)	240 (163–352)	0.124 (0.060–0.257)	0.228 (0.171–0.304)	102 (63–167)	183 (152–219)
P		*0.74*	*0.14*	*0.09*	*0.27*	*0.50*	*0.88*	*0.42*	*0.21*

SNP, single nucleotide polymorphism. 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, sex, BMI and insulin sensitivity index. Insulin sensitivity and disposition indices were adjusted for study centre, family relatedness, glucose tolerance status, age, sex and BMI.

enhanced. Furthermore, due to its uniqueness it is, to our best knowledge, impossible to find comparable, sufficiently sized replication cohorts. Finally, we have only tested four known variants in the *DRD2/ANKK1* locus and much larger studies would therefore be needed for replication and thorough investigation of the whole *DRD2* locus to identify causal variants and to establish its effects on β-cell function and Type 2 diabetes susceptibility in men and women.

In conclusion, our main finding is the demonstration that a naturally occurring genetic variation at the *DRD2/ANKK1* locus (rs1800497) is associated with Type 2 diabetes susceptibility in a sex-specific manner.

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Competing interests

None declared.

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Supporting Information

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

Data S1. Description and clinical characteristics of the replication cohorts.
Table S1. Clinical characteristics of the three hyperglycemic clamp study samples
Table S2. Number of cases and controls genotyped for each SNP in the T2D discovery and replication cohorts
Table S3. Insulin response according to *DRD2* rs1079597, rs6275, rs6277, rs1800497 genotypes
Figure S1. Flow scheme describing the study design. Two different study designs were used: one to examine glucose-stimulated insulin secretion (GSIS) and the second for investigating Type 2 diabetes susceptibility. The genetic association study for GSIS includes Dutch subjects from the Hoorn and Utrecht studies, and from the Netherlands Twin Register (NTR). The discovery genetic association study for Type 2 diabetes risk consists in a meta-analysis based on a discovery and two replication cohorts. The discovery cohort includes Dutch subjects from the New Hoorn Study (NHS) and the Rotterdam study. The replication cohort 1 includes Dutch subjects from the Leiden Longevity Study (LLS), the NTR/Netherlands Study of Depression and Anxiety (NESDA) cohort, and from the Hoorn and Rotterdam studies. The replication cohort 2 includes Danish subjects from the Inter99 study, from the out-patient clinic at Steno Diabetes Center (Steno), and from the Anglo-Danish-Dutch Study of Intensive Treatment In People with Screen Detected Diabetes in Primary Care (ADDITION) study. The complete description of all the cohorts can be found in the Supporting Information file. n_{max}: maximum number of available subjects in the cohort.