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## Revealing the genetic roots of obesity and type 2 diabetes

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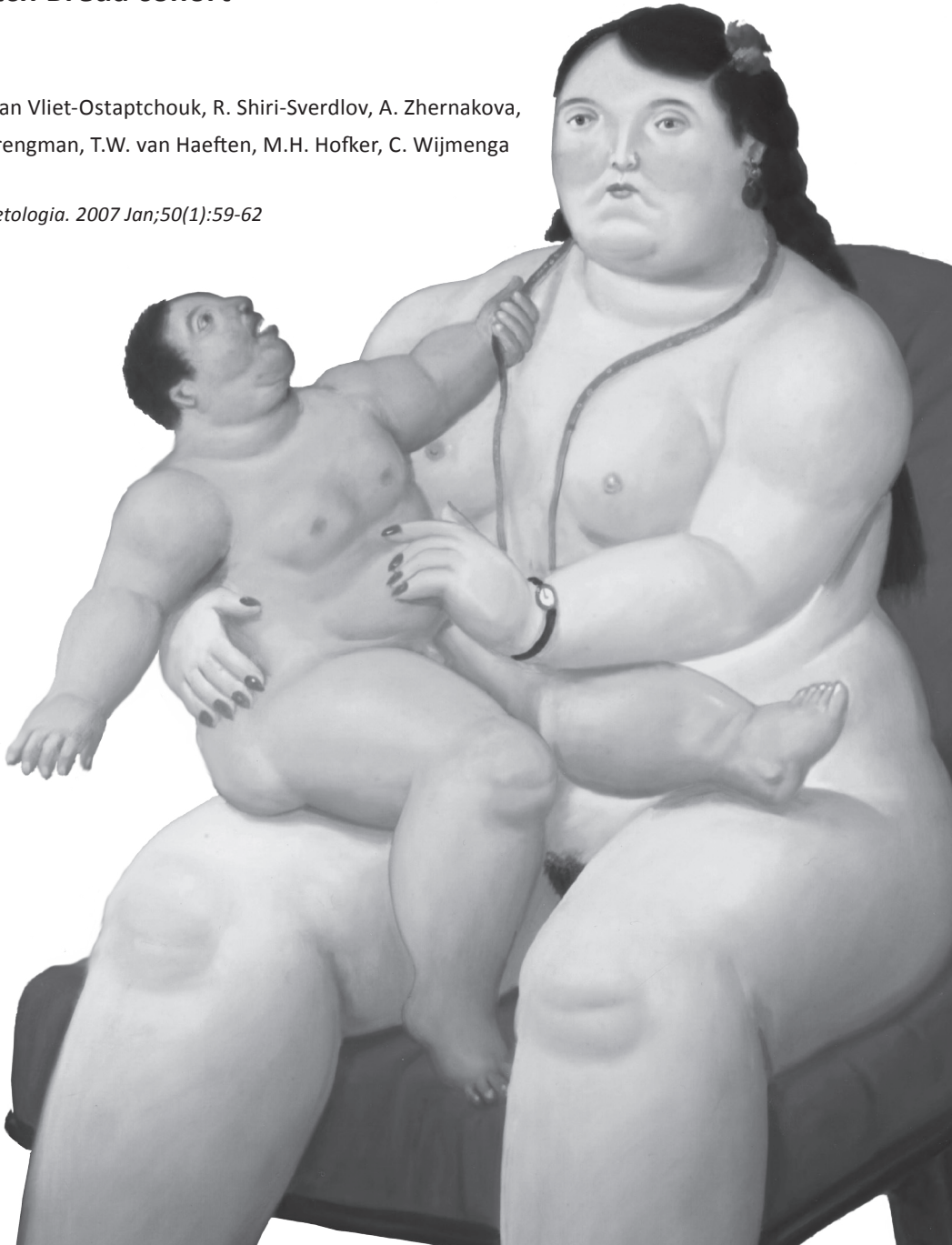
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# Chapter 5

## **Association of variants of transcription factor 7- like 2 (*TCF7L2*) with susceptibility to type 2 diabetes in the Dutch Breda cohort**

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## Abstract

**Aim/ hypothesis:** A strong association between susceptibility to type 2 diabetes and common variants of transcription factor 7-like 2 (*TCF7L2*), encoding an enteroendocrine transcription factor involved in glucose homeostasis, has been reported in three different populations (Iceland, Denmark and US) by Grant et al. We aimed to replicate these findings in a Dutch cohort.

**Methods:** We analyzed the genotypes of two intronic single nucleotide polymorphisms (SNPs) in *TCF7L2* gene in 502 unrelated type 2 diabetes patients and in a set of healthy controls (n=920). The two SNPs showed almost complete linkage disequilibrium ( $D'=0.91$ ).

**Results:** We were able to replicate the previously reported association in our Breda cohort. The minor alleles of both variants were significantly over-represented in cases [odds ratio (OR) 1.29, 95% confidence interval (CI) 1.09 to 1.52,  $p=3 \times 10^{-3}$  for rs12255372; OR 1.41, 95% CI 1.19 to 1.66,  $p=4.4 \times 10^{-5}$  for rs7903146]. In addition, *TCF7L2* haplotypes were analyzed for association with the disease. The analysis of haplotypes did not reveal any strong association beyond that expected from analyzing individual SNPs. The *TT*-haplotype carrying the minor alleles was more frequent among cases (OR 1.38,  $p=2.7 \times 10^{-3}$ ).

**Conclusions/interpretation:** Our data strongly confirm that variants of the *TCF7L2* gene contribute to the risk of type 2 diabetes. The population attributable risk from this factor in the Dutch type 2 diabetes population is 10%.

## Introduction

The *TCF7L2* gene encodes for an enteroendocrine transcription factor that has a role in the Wnt signaling pathway, which is one of the key developmental and growth regulatory mechanisms of the cell. One of the peptide hormones produced by enteroendocrine cells and transcriptionally regulated by *TCF7L2* is glucagon-like peptide 1 (GLP-1). Hence, variants of the *TCF7L2* gene could influence the susceptibility to type 2 diabetes by altering levels of the insulinotropic hormone GLP-1. In concert with insulin, GLP-1 exerts a critical effect on blood glucose homeostasis by stimulating early insulin production from the pancreatic beta cells (1).

Recently, Reynisdottir et al (2) found suggestive linkage of type 2 diabetes to chromosome 10q in the Icelandic population. Fine-mapping with 228 microsatellite markers in Icelandic individuals with type 2 diabetes and controls throughout a 10.5 Mb interval on 10q identified one microsatellite marker, DG10S478 in intron 3 of the *TCF7L2* gene, as being strongly associated with type 2 diabetes ( $p=2.1 \times 10^{-9}$ ) [(3). This association was replicated in both a Danish cohort ( $p=4.8 \times 10^{-3}$ ) and a US cohort ( $p=3.3 \times 10^{-9}$ ). A combined analysis showed that individuals heterozygous for the risk allele (38% of the population) have a modest risk of 1.45 of developing type 2 diabetes, whereas individuals homozygous for the risk allele (7% of the population) have a higher risk (2.41) of developing type 2 diabetes.

In the current study we examined whether variants of the *TCF7L2* gene contribute to the risk of type 2 diabetes in a Dutch population, using 502 unrelated type 2 diabetes patients from the Breda cohort and a set of 920 healthy controls.

## Subjects and methods

### Breda cohort

DNA was available from 502 type 2 diabetes subjects from the Breda cohort (**Table 1**) (4). All patients were diagnosed according to the WHO criteria (random plasma glucose level  $>11.1$  mmol/l or a fasting plasma glucose level  $>7.0$  mmol/l). The clinical characteristics of the patients (HbA1c, plasma cholesterol, HDL cholesterol and triglycerides) were available, as well as the level of obesity in each individual given by the body mass index (BMI; defined as weight (in kilograms) divided by height (in meters) squared). The control cohort (n=920) was comprised of healthy blood bank donors of Dutch Caucasian origin (5). Informed written consent was obtained from all

individuals. The Breda study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

**Table 1:** Descriptive statistics for study subjects from the type 2 diabetes Breda cohort and the control cohort.

Trait	Breda cohort	Control cohort
<i>n</i> (female/male)	502 (272/229 <sup>a</sup> )	920 (354/557 <sup>b</sup> )
Age-at-study (years)	70.8 ± 9.9	47.8 ± 12.7
Age-at-diagnosis (years)	63.0 ± 11.3	-
BMI (kg/m <sup>2</sup> )	27.8 ± 4.1	NA
HbA1C (%)	7.3 ± 1.1	NA
HDL cholesterol (mmol/l)	1.2 ± 0.3	NA
Total cholesterol (mmol/l)	5.3 ± 1.0	NA
Triglycerides (mmol/l)	1.9 ± 1.0	NA

The data are presented as mean ± SD. BMI: Body Mass Index. HbA1c: haemoglobin A1c = glucose bound to haemoglobin. HDL: high density lipoprotein. NA: not available. <sup>a</sup> not available for 1 subject, <sup>b</sup> not available for 9 subjects.

## Genotyping

SNPs were genotyped using Taqman assay-on-demand (Applied Biosystems). Assays were performed according to the manufacturer's specifications (assays C\_29347861\_10, C\_291484\_20). The sequence information for all primers and probes is available upon request. The genotypes were analyzed using a TaqMan 7900HT (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). The DNA samples were processed in 384 well plates. Each plate contained 8 negative controls and 16 genotyping controls (four duplicates of four different samples obtained from the Centre d'Etude du Polymorphisme Humain (CEPH)). The genotype success rates were 97.8% for rs12255372 and 98.6% for rs7903146. There were no discordances in the genotypes of any of the CEPH samples. The controls were in agreement with Hardy-Weinberg equilibrium ( $\chi^2 = 2.72$ ,  $p = 0.10$  for rs12255372 and  $\chi^2 = 0.72$ ,  $p = 0.40$  for rs7903146).

## Statistical analysis

The genotype frequencies were tested for Hardy-Weinberg equilibrium by  $\chi^2$  analysis. Differences in allele, genotype and haplotype distribution in cases and controls were tested for significance using a 95% two-sided  $\chi^2$  test. Odds ratios (OR) and the confidence intervals (CI) were calculated using Woolf's method with Haldane's correction (6). To

test for association of genotypes and type 2 diabetes, genotype-based ORs with 95% CIs were calculated using logistic regression model, where individuals homozygous for the common allele were the reference group. The association between genotypes of two SNPs and the cohort's clinical characteristics and BMI was determined using linear regression analysis, including age and sex as explanatory variables in the models. Haplotypes of two polymorphisms were estimated using the COCAPHASE package of UNPHASED program (7). The  $D'$  and  $r^2$  between the *TCF7L2* SNPs were calculated with the same package. Population attributable risk (PAR) was calculated for rs7903146 using the corresponding genotype frequencies for this marker as explained by Greenland et al (8). Power calculation was completed using the UCLA's Department of Statistics Power Calculator (available at <http://calculators.stat.ucla.edu/powercalc>). All statistical analyses were performed using SPSS program, version 13.0 for Windows (SPSS, Chicago, IL, USA) unless otherwise stated.

## Results

We genotyped two SNPs (rs12255372, rs7903146) that were shown to be most strongly associated with type 2 diabetes by Grant et al (3). Their study showed that the rs12255372\**T* allele is perfectly correlated with the DG10S478 risk allele. The rs12255372 variant was successfully genotyped in 492 individuals from the Breda cohort and in 901 controls. The *T*-allele was significantly more frequent in patients than controls (34% versus 29%,  $p=0.003$ ), and the frequencies of the *GT*- and minor *TT*-genotypes were also increased in type 2 diabetes patients ( $p=0.012$ ) (**Table 2**). However, this association was weaker than that observed with rs7903146.

The variant rs7903146 in the *TCF7L2* gene could be genotyped in 496 cases and 907 controls. The *T* allele was over-represented in the type 2 diabetes cohort compared to the control group (37% versus 29%,  $p=4.4 \times 10^{-5}$ ) (**Table 2**), which is compatible with Grant et al's results. The *CT*- and *TT*-genotypes were more prevalent in the type 2 diabetes patients with a frequency of 45% and 15% in cases, respectively, compared to a frequency of 40% of *CT*-genotype and 9% of *TT*-genotype in controls ( $p=0.0003$ ).

Risks were consistently higher for homozygous than for heterozygous persons for both SNPs (OR 1.53, 95% CI 1.05 – 2.21 and OR 1.35, 95% CI 1.07 – 1.71 for rs12255372 and OR 1.96, 95% CI 1.37 – 2.80 and OR 1.37, 95% CI 1.08 – 1.73 for rs7903146, respectively) (**Table 2**). Moreover, the test for trend showed even stronger association

of the disease with an increased number of minor alleles, especially for rs7903146 ( $p=6.6 \times 10^{-5}$ ), suggesting an allele-dose effect.

**Table 2:** Comparison of genotype and allele frequencies in the Breda cohort and control subjects.

rs12255372					
genotype	T2D patients (n=502)	Controls (n=920)	OR	95% CI	p-value
GG (%)	216 (44)	469 (52)	-	-	-
GT (%)	217 (44)	348 (39)	1.35	1.07 – 1.71	0.01
TT (%)	59 (12)	84 (9)	1.53	1.05 – 2.21	0.03
p-value genotype					0.012
G allele (%)	649 (66)	1286 (71)	1.29	1.09 – 1.52	0.003
T allele (%)	335 (34)	516 (29)			
rs7903146					
genotype	T2D patients (n=502)	Controls (n=920)	OR	95% CI	p-value
CC (%)	203 (41)	459 (51)	-	-	-
CT (%)	221 (45)	365 (40)	1.37	1.08 – 1.73	0.009
TT (%)	72 (15)	83 (9)	1.96	1.37 – 2.80	0.0002
p-value genotype					0.0003
C allele (%)	627 (63)	1283 (71)	1.41	1.19 – 1.66	0.00004
T allele (%)	365 (37)	531 (29)			

The two SNPs were found to be in strong LD with each other ( $D'=0.91$ ,  $r^2=0.76$ ) in our control population. The *TT*- haplotype, which carries the two minor alleles of both SNPs, was more common among patients than controls (33% versus 26%,  $p=0.00027$ ). As the haplotype does not perform better than the rs7903146 SNP located in intron 3 of *TCF7L2*, this SNP can be used as a marker for type 2 diabetes risk. The allele frequency data for rs7903146 revealed that individuals carrying the *T* allele (37% frequency in patients compared to 29% in controls) have a modest but significantly higher risk of developing type 2 diabetes (OR 1.41, 95% CI 1.19 – 1.66), with a population attributable risk of 10% (95% CI 0.054-0.155). These results strongly suggest that *TCF7L2* is associated with type 2 diabetes in the Dutch Breda cohort.

## Discussion

Recently Grant et al (3) demonstrated a convincing association between susceptibility to type 2 diabetes and common variants in *TCF7L2*. In order to replicate these findings, two *TCF7L2* SNPs (rs12255372 and rs7903146) were genotyped in the Dutch Breda cohort and a control group. Our study had power of 80% at  $p=0.05$  to detect effects with an OR >1.39 for the examined variants. Consistent with Grant et al's observation, we found the minor alleles of both variants were significantly over-represented in Dutch type 2 diabetes patients. The frequency of heterozygous and homozygous carriers of the at-risk alleles, compared with non-carriers, was also increased in type 2 diabetes patients. In line with Grant et al, we also revealed a negative, borderline-significant association with BMI (data not shown), suggesting that BMI does not influence the risk variants.

Despite serious efforts to identify genetic variants that predispose to common forms of type 2 diabetes, till now only a few genes, such as *KCNJ11*, *PPARG* and *HNF4A*, have been reproducibly associated with this complex disease in a variety of large-scale studies performed in the different populations (9, 10). Our replication study shows that the *TCF7L2* gene is an important candidate gene contributing to susceptibility to type 2 diabetes. Since the most strongly associated SNP is within an intron of the *TCF7L2* gene, this SNP is not likely to be the true disease variant. However, the intronic variants may affect the level of gene expression by altering e.g. transcription, splicing or message stability. Hence, full sequence analysis of the gene and a comparison of the allelic architecture among different type 2 diabetes populations are needed to identify the causative variant. The *TCF7L2* gene probably contributes to the complex nature of type 2 diabetes by affecting the blood glucose homeostasis, since *TCF7L2* is known to control the activity of a number of genes, including the proglucagon gene that encodes the insulinotropic hormone GLP-1. GLP-1, along with insulin, plays a role in blood glucose homeostasis, and GLP-1 therapies for type 2 diabetes are currently in clinical development. As long as the exact role of these gene polymorphisms remains unclear, it is difficult to assess its functional role in the development of type 2 diabetes.

It will be of great interest to learn the molecular mechanisms of action of the *TCF7L2* gene in the pathogenesis of type 2 diabetes and to identify potential targets for drugs that could be used for treatment and prevention of the disease due to the gene variants. It is tempting to speculate on a future genetic test that could identify the individuals carrying the at-risk *TCF7L2* gene variants, so that they could be offered a preventive health care program (special diet and exercises).



In conclusion, we have confirmed that variants of the *TCF7L2* gene modulate susceptibility to type 2 diabetes in a Dutch population.

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