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Genetic variation within the *TRPM5* locus associates with prediabetic phenotypes in subjects at increased risk for type 2 diabetes

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ABSTRACT

The functional knockout of the calcium-sensitive, nonselective cation channel TRPM5 alters glucose-induced insulin secretion and glucose tolerance. We hypothesized that genetic variation in the *TRPM5* gene may contribute to prediabetic phenotypes, including pancreatic β-cell dysfunction. We genotyped 1798 white subjects at increased type 2 diabetes mellitus risk for 9 *TRPM5* single nucleotide polymorphisms (namely, rs2301696, rs800344, rs800345, rs800347, rs800348, rs2074234, rs2301698, rs886277, and rs2301699) and also performed correlational analyses with metabolic traits. An oral glucose tolerance test (OGTT) was conducted on all subjects, and a subset (n = 525) additionally underwent a hyperinsulinemic-euglycemic clamp. The 9 chosen single nucleotide polymorphisms cover 100% of the common genetic variation (minor allele frequency $\geq$0.05) within the *TRPM5* locus (D' = 1.0; $r^2 \geq 0.8$). Rs800344, rs800345, and rs2301699 were significantly associated with area under the curve (AUC) glucose during the OGTT in the additive and dominant models after adjustment for sex, age, and body mass index (all Ps $\leq$ .0025). Furthermore, rs800344 was significantly associated with 2-hour glucose in the dominant model (P = .0009). After stratification for sex, rs2301699 was significantly associated with the ratio of AUC insulin 0 to 30 minutes to AUC glucose 0 to 30 minutes in women (P = .0097), but not in men (P = .3), in the dominant model. Female minor allele carriers of rs2301699 showed significantly lower glucagon-like peptide–1 levels at 30 minutes during

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the OGTT compared with major allele homozygotes ($P = .0124$), whereas in male subjects, no significant differences were found ($P = .3$). In our German population, the common *TRPM5* variants are likely to be associated with prediabetic phenotypes; and this may in turn contribute to the development of type 2 diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus affects millions of people worldwide, and its chronic complications represent an important cause of death in many industrialized countries [1,2]. The underlying mechanisms in the development of type 2 diabetes mellitus comprise a complex interaction of genetic and environmental factors [3,4]. Recent genomewide association (GWA) studies have significantly improved knowledge of the genetic basis of common type 2 diabetes mellitus by identifying more than 30 mostly unsuspected loci with independent effects on type 2 diabetes mellitus [5-16]. Although most these type 2 diabetes mellitus risk alleles appear to be associated with pancreatic β-cell dysfunction, some of the genetic variants are also associated with insulin resistance [17]. However, the identified loci explain only a small proportion (<10%) of type 2 diabetes mellitus heritability [13], underlining the importance of the identification of other disease-causing variants [18].

One gene that might be involved in the modulation of glucose homeostasis-related phenotypes is *TRPM5*, which encodes the calcium-sensitive, nonselective transient receptor potential cation channel, subfamily M, member 5 (TRPM5; where *M* stands for melastatin). The TRPM subfamily consists of 8 members that have 6 putative transmembrane domains in common [19]. TRPM5 was firstly reported to be highly expressed in taste buds, where it is involved in the signal transduction for bitter, sweet, and umami tastes [20,21] and moreover mediates the thermal sensitivity of sweet taste [22]. However, recent work showed that TRPM5 is also widely distributed in the central nervous system and peripheral organs, with the highest expressions found in the intestine, pancreas, prostate, and pituitary [23]. TRPM5 is predominately expressed in the proximal small intestine where it plays a key role in nutrient sensing and underlies a dynamic metabolic control, with its expression being negatively correlated with blood glucose concentrations in diabetic patients [24]. The functional significance of TRPM5 has also been shown in pancreatic β-cells where activation of TRPM5 by rapid changes in cytosolic Ca^{2+} levels resulted in transient membrane depolarization [25]. In line with this, functional knockout of *Trpm5* attenuated high-frequency glucose-induced Ca^{2+} oscillations in mouse pancreatic islets and caused reductions in glucose- and arginine-induced insulin secretion and impaired glucose tolerance [26,27].

Taken together, *TRPM5* appears to be an attractive prediabetes candidate gene. Therefore, in the present study, we tested the impact of common genetic variation within this locus on prediabetes phenotypes, including β-cell dysfunction and insulin resistance.

2. Patients and methods

2.1. Subjects

The 1798 nondiabetic subjects (subject characteristics shown in Supplementary Table 1) at an increased risk for type 2 diabetes mellitus (family history of diabetes, history of gestational diabetes, impaired glucose tolerance, or overweight) were recruited from the ongoing Tübingen Family Study for type 2 diabetes mellitus. All subjects were metabolically characterized by an oral glucose tolerance test (OGTT), and a subgroup of 525 subjects was additionally characterized by a hyperinsulinemic-euglycemic clamp. The participants gave informed written consent to the study. The protocol was approved by the Ethics Committee of the University of Tübingen.

2.2. Analysis of the *TRPM5* gene and selection of tagging single nucleotide polymorphisms for genotyping

Using the publicly available phase II data of the International HapMap Project derived from a population of Utah residents with ancestry from northern and western Europe (release #24, November 2008, http://www.hapmap.org/index.html.en [28]), we screened in silico the complete *TRPM5* gene spanning 18.5 kilobases (kb) (24 exons, located on human chromosome 11p15.5) as well as 3 kb of its 5′-flanking region and 0.5 kb of its 3′-flanking region (Supplementary Fig. 1). Only 500 base pairs of the 3′-flanking region were chosen to avoid inclusion of the promoter region of the adjacent *TSSC4* (tumor suppressing subtransferable candidate 4) gene. Among 14 informative single nucleotide polymorphisms (SNPs), 9 SNPs were chosen as representative covering 100% of the common genetic variation (minor allele frequency [MAF] $\geq$0.05) of the locus with $D' = 1.0$ and $r^2 \geq 0.8$.

As shown in Supplementary Fig. 1, the 9 genotyped SNPs were rs2301696 C/G, rs800344 G/A, rs800345 C/T, rs800347 A/G, rs800348 T/C, rs2301698 T/G, rs2301699 C/G (all located in noncoding regions of the gene locus), rs2074234 T/C (located in exon 17, resulting in the synonymous mutation T836T), and rs886277 T/C (located in exon 5, resulting in the missense mutation N235S).

2.3. Genotyping of the study population

DNA was isolated from whole blood, for genotyping, using a commercial DNA isolation kit (NucleoSpin; Macherey & Nagel, Düren, Germany). Genotyping was done using the Mass Array system and iPLEX SNP genotyping software from Sequenom (Hamburg, Germany). The average genotyping success rate

Table 1 – Associations of *TRPM5* SNPs rs2301696, rs800344, and rs800345 with indices of insulin secretion and insulin sensitivity

SNP	rs2301696			P_{add}	P_{dom}	rs800344			P_{add}	P_{dom}	rs800345			P_{add}	P_{dom}
Genotype	CC	CG	GG			GG	GA	AA			CC	CT	TT		
n	521	876	385	–	–	1540	243	13	–	–	1505	261	18	–	–
BMI (kg/m^2)	29.9 ± 9.8	30.3 ± 9.1	30.1 ± 8.5	.9	.6	30.1 ± 9.3	30.1 ± 8.5	32.4 ± 10.3	.5	.6	30.2 ± 9.4	29.7 ± 8.3	31.9 ± 10.0	.6	.8
Waist circumference (cm)	95 ± 20	97 ± 19	96 ± 18	.4	.2	96 ± 19	96 ± 19	102 ± 22	.3	.5	96 ± 19	96 ± 19	100 ± 21	.5	.8
Fasting glucose (mmol/L)	5.11 ± 0.53	5.15 ± 0.56	5.14 ± 0.56	.6	.3	5.12 ± 0.55	5.20 ± 0.54	5.11 ± 0.43	.05	.0158	5.13 ± 0.55	5.16 ± 0.54	5.32 ± 0.62	.10	.07
Glucose 120 min OGTT (mmol/L)	6.24 ± 1.55	6.33 ± 1.65	6.41 ± 1.80	.7	.5	6.28 ± 1.65	6.63 ± 1.69	6.36 ± 1.75	.0038	.0009	6.29 ± 1.64	6.45 ± 1.76	6.75 ± 1.91	.15	.06
AUC glucose OGTT (mmol/L)	14.55 ± 3.05	14.73 ± 3.15	14.89 ± 3.20	.4	.3	14.62 ± 3.13	15.29 ± 3.16	14.66 ± 2.28	.0012	.0003	14.64 ± 3.13	15.13 ± 3.09	15.65 ± 3.18	.0012	.0003
HOMA-IR (U)	2.60 ± 2.30	2.84 ± 2.56	2.73 ± 2.52	.6	.3	2.70 ± 2.43	2.97 ± 2.69	3.42 ± 2.88	.10	.0375	2.72 ± 2.46	2.84 ± 2.56	3.32 ± 2.55	.2	.10
ISI, OGTT (U)	16.1 ± 10.4	15.4 ± 10.9	15.1 ± 10.5	.5	.2	15.7 ± 10.7	14.3 ± 10.2	15.5 ± 13.1	.0115	.0039	15.6 ± 10.6	14.8 ± 10.7	14.2 ± 11.5	.0208	.0057
ISI, clamp (U) [a]	0.080 ± 0.041	0.092 ± 0.061	0.078 ± 0.054	.7	.7	0.087 ± 0.054	0.073 ± 0.052	–	.2	.14	0.087 ± 0.055	0.072 ± 0.049	–	.15	.07
AUC insulin 0-30 min/AUC glucose 0-30 min (·10^{-9})	42.9 ± 31.2	45.7 ± 34.3	42.6 ± 28.9	.8	1.0	43.6 ± 32.0	46.5 ± 34.4	54.2 ± 54.4	.3	.15	43.6 ± 31.4	47.6 ± 38.2	49.8 ± 47.2	.6	.5
AUC C-peptide/AUC glucose (·10^{-9})	316 ± 103	330 ± 110	314 ± 93	.0279	.3	322 ± 104	325 ± 107	326 ± 125	.3	.16	321 ± 104	330 ± 112	314 ± 116	.4	.7

Data are given as means ± SD. For statistical analysis, data were $\log_e$-transformed. Indices of insulin sensitivity were adjusted for sex, age, and BMI. Indices of insulin secretion were adjusted for sex, age, BMI, and insulin sensitivity. ISI indicates insulin sensitivity index; add, additive model; dom, dominant model.

[a] The ISI (clamp) data were available for 525 subjects.

Table 2 – Associations of *TRPM5* SNPs rs800347, rs800348, and rs2074234 with indices of insulin secretion and insulin sensitivity

SNP	rs800347			P_{add}	P_{dom}	rs800348			P_{add}	$P_{dom.}$	rs2074234			P_{add}	$P_{dom.}$
Genotype	AA	AG	GG			TT	TC	CC			TT	TC	CC		
n	586	865	345	–	–	577	880	338	–	–	738	816	242	–	–
BMI (kg/m^2)	30.5 ± 9.3	29.8 ± 9.1	30.3 ± 9.4	.5	.3	30.4 ± 9.5	30.1 ± 9.0	29.7 ± 9.1	.6	.5	30.3 ± 9.3	30.2 ± 9.0	29.4 ± 9.4	.4	.4
Waist circumference (cm)	97 ± 19	96 ± 19	96 ± 19	.8	.7	96 ± 19	96 ± 19	95 ± 20	.9	.9	97 ± 20	96 ± 18	94 ± 19	.6	.7
Fasting glucose (mmol/L)	5.17 ± 0.56	5.11 ± 0.56	5.14 ± 0.53	.9	.7	5.15 ± 0.53	5.14 ± 0.57	5.09 ± 0.54	.6	.6	5.13 ± 0.55	5.14 ± 0.55	5.12 ± 0.57	.3	.18
Glucose 120 min OGTT (mmol/L)	6.41 ± 1.62	6.25 ± 1.66	6.36 ± 1.74	.8	.5	6.37 ± 1.69	6.36 ± 1.69	6.15 ± 1.52	.4	.8	6.25 ± 1.60	6.37 ± 1.69	6.42 ± 1.75	.0401	.0475
AUC glucose OGTT (mmol/L)	14.93 ± 3.15	14.58 ± 3.14	14.69 ± 3.08	.9	.6	14.90 ± 3.09	14.78 ± 3.21	14.21 ± 2.97	.0242	.09	14.62 ± 3.06	14.79 ± 3.14	14.76 ± 3.34	.07	.0468
HOMA-IR (U)	2.92 ± 2.75	2.62 ± 2.33	2.74 ± 2.30	.3	.15	2.80 ± 2.54	2.78 ± 2.51	2.54 ± 2.23	.2	.5	2.66 ± 2.52	2.80 ± 2.44	2.78 ± 2.42	.0409	.0344
ISI, OGTT (U)	14.8 ± 10.2	16.1 ± 11.1	15.4 ± 10.2	.4	.16	14.7 ± 9.6	15.4 ± 10.8	17.1 ± 11.9	.08	.2	15.4 ± 10.2	15.6 ± 11.1	15.6 ± 10.4	.14	.09
ISI, clamp (U)[a]	0.087 ± 0.057	0.085 ± 0.054	0.082 ± 0.048	.4	.4	0.082 ± 0.051	0.084 ± 0.055	0.092 ± 0.056	1.0	1.0	0.083 ± 0.051	0.085 ± 0.055	0.092 ± 0.063	.5	.9
AUC insulin 0-30 min/AUC glucose 0-30 min (·10^{-9})	44.0 ± 30.5	43.8 ± 32.8	45.0 ± 35.2	.7	.6	44.8 ± 33.7	43.6 ± 30.7	44.3 ± 35.2	.4	.6	44.9 ± 32.7	43.7 ± 32.2	43.2 ± 33.6	.07	.0320
AUC C-peptide/AUC glucose (·10^{-9})	326 ± 104	322 ± 107	318 ± 100	.9	.7	325 ± 103	320 ± 102	325 ± 114	.3	.7	325 ± 108	320 ± 103	322 ± 103	.4	.19

Data are given as means ± SD. For statistical analysis, data were $\log_e$-transformed. Indices of insulin sensitivity were adjusted for sex, age, and BMI. Indices of insulin secretion were adjusted for sex, age, BMI, and insulin sensitivity.

[a] The ISI (clamp) data were available for 525 subjects.

Table 3 – Associations of *TRPM5* SNPs rs2301698, rs886277, and rs2301699 with indices of insulin secretion and insulin sensitivity.

SNP	rs2301698			P_{add}	P_{dom}	rs886277			P_{add}	P_{dom}	rs2301699			P_{add}	P_{dom}
Genotype	TT	TG	GG			TT	TC	CC			CC	CG	GG		
n	489	865	441	–	–	708	837	245	–	–	879	729	168	–	–
BMI (kg/m^2)	30.3 ± 9.2	29.9 ± 9.0	30.4 ± 9.6	.6	1.0	30.2 ± 9.3	30.1 ± 9.1	29.9 ± 9.2	1.0	.9	30.4 ± 9.6	29.9 ± 8.6	30.0 ± 9.7	.5	.2
Waist circumference (cm)	96 ± 19	96 ± 19	97 ± 19	.6	.6	96 ± 19	96 ± 19	96 ± 19	.9	.7	97 ± 19	96 ± 18	95 ± 20	.7	.4
Fasting glucose (mmol/L)	5.17 ± 0.55	5.14 ± 0.56	5.09 ± 0.54	.15	.3	5.18 ± 0.56	5.12 ± 0.55	5.07 ± 0.51	.07	.0320	5.12 ± 0.55	5.15 ± 0.56	5.11 ± 0.54	.5	.2
Glucose 120 min OGTT (mmol/L)	6.35 ± 1.67	6.32 ± 1.65	6.28 ± 1.66	.9	.8	6.39 ± 1.65	6.31 ± 1.68	6.19 ± 1.61	.6	.4	6.23 ± 1.61	6.41 ± 1.70	6.51 ± 1.78	.0195	.0073
AUC glucose OGTT (mmol/L)	14.76 ± 3.13	14.63 ± 3.19	14.81 ± 3.00	.2	.6	14.86 ± 3.20	14.59 ± 3.12	14.75 ± 3.00	.3	.4	14.52 ± 3.04	14.94 ± 3.20	14.93 ± 3.24	.0025	.0006
HOMA-IR (U)	2.77 ± 2.44	2.73 ± 2.53	2.72 ± 2.39	.7	.5	2.80 ± 2.46	2.70 ± 2.47	2.74 ± 2.53	.4	.2	2.76 ± 2.59	2.71 ± 2.31	2.81 ± 2.38	.4	.2
ISI, OGTT (U)	15.4 ± 10.5	15.6 ± 10.7	15.6 ± 10.8	.9	.7	15.3 ± 10.8	15.6 ± 10.4	16.0 ± 11.0	.2	.09	15.6 ± 10.6	15.4 ± 10.8	14.7 ± 9.6	.06	.0319
ISI, clamp (U)[a]	0.086 ± 0.052	0.083 ± 0.048	0.088 ± 0.066	.7	.9	0.086 ± 0.049	0.084 ± 0.052	0.086 ± 0.069	1.0	.9	0.089 ± 0.058	0.079 ± 0.046	0.082 ± 0.064	.13	.0489
AUC insulin 0-30 min/AUC glucose 0-30 min ($\cdot 10^{-9}$)	45.0 ± 33.3	43.4 ± 31.2	44.8 ± 34.4	.4	.18	44.9 ± 32.6	43.5 ± 32.0	44.1 ± 34.5	.4	.4	44.5 ± 32.6	43.4 ± 32.3	45.2 ± 33.9	.4	.2
AUC C-peptide/AUC glucose ($\cdot 10^{-9}$)	324 ± 114	322 ± 97	324 ± 111	.7	.7	324 ± 107	320 ± 100	327 ± 114	.9	.7	326 ± 109	319 ± 101	325 ± 102	.3	.13

Data are given as means ± SD. For statistical analysis, data were $\log_e$-transformed. Indices of insulin sensitivity were adjusted for sex, age, and BMI. Indices of insulin secretion were adjusted for sex, age, BMI, and insulin sensitivity.

[a] The ISI (clamp) data were available from 525 subjects.

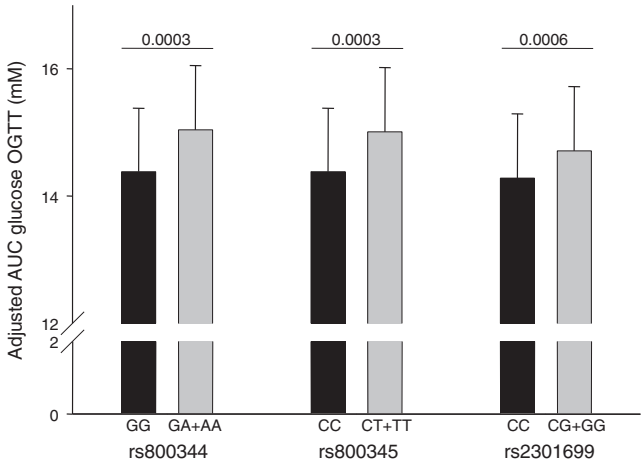

Fig. 1 – Associations of *TRPM5* SNPs rs800344, rs800345, and rs2301699 with AUC glucose during the OGTT. Data are presented as means ± SEM. Data adjusted for sex, age, and BMI are shown. The AUCs were determined by the trapezoidal method. To approximate normal distribution, $\log_e$-transformation of metabolic variables was performed before multiple linear regression analyses. In multiple linear regression models, AUC glucose was chosen as dependent variable and sex, age, BMI, and genotype were tested as independent variables in the dominant model. *P* values are given above the columns.

was 99.6% (rs2301696: 99.1%, rs800344: 99.9%, rs800345: 99.2%, rs800347: 99.9%, rs800348: 99.8%, rs2074234: 99.9%, rs2301698: 99.8%, rs886277: 99.6%, and rs2301699: 98.8%).

2.4. *OGTT and hyperinsulinemic-euglycemic clamp*

Both assays were performed as formerly described [29].

2.5. *Determination of blood parameters*

Plasma glucose, insulin, and C-peptide concentrations were measured as recently described [29]. Glucagon-like peptide–1 (GLP-1) immunoreactivity was determined using a radioimmunoassay specific for the C-terminus of the peptide [30].

2.6. *Body composition and body fat distribution*

Body mass index (BMI) and waist circumference were measured as described [29].

2.7. *Calculations*

Insulin sensitivity from the OGTT (in arbitrary units) and clamp-derived insulin sensitivity (in arbitrary units) were calculated as reported earlier [29]. Insulin secretion in the OGTT was assessed by calculating the area under the curve (AUC) for C-peptide divided by the AUC for glucose. The AUCs were determined by the trapezoidal method. Insulin secretion was also assessed with the ratio of AUC insulin 0 to 30 minutes to AUC glucose 30 minutes, calculating (fasting insulin levels + insulin levels at 30 minutes during OGTT) divided by (fasting glucose levels + glucose levels at 30 minutes during OGTT).

2.8. *Statistical analyses*

Data are normally shown as means ± SD; however, to approximate normal distribution, $\log_e$-transformation of metabolic variables was performed before simple and multiple linear regression analyses. In multiple linear regression models, the trait was chosen as the dependent variable; and sex, age, BMI, and genotype were tested as independent variables. The OGTT-derived insulin sensitivity was added as an additional independent variable when indices of insulin secretion were chosen as dependent variable. To account for the number of SNPs analyzed (n = 9) and the number of inheritance models (n = 2), a Bonferroni-corrected α level of $P < .0028$ was considered statistically significant. Bonferroni correction was not performed for the number of traits given that the traits were not independent. Analysis of interaction effects between genotypes on the one hand and sex, age, BMI, and glucose tolerance status on the other hand on measures of insulin sensitivity and insulin secretion was performed by analysis of covariance (ANCOVA). To account for the number of SNPs that significantly associated with measures of glucose tolerance and were, subsequently, analyzed by ANCOVA (n = 3), a Bonferroni-corrected α level of $P < .017$ was considered statistically significant. Given that these

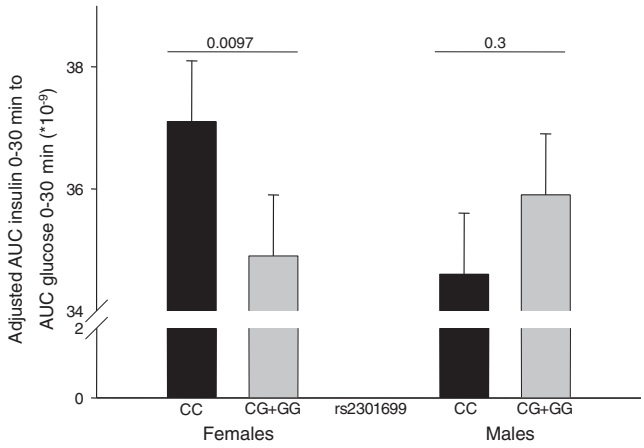

Fig. 2 – Association of *TRPM5* SNP rs2301699 with insulin secretion in female and male subjects. Data are presented as means ± SEM. Data adjusted for age, BMI, and OGTT-derived insulin sensitivity are shown. Insulin secretion was assessed by the ratio AUC insulin 0 to 30 minutes to AUC glucose 0 to 30 minutes during the OGTT. The AUCs were determined by the trapezoidal method. To approximate normal distribution, $\log_e$-transformation of metabolic variables was performed before multiple linear regression analyses. In multiple linear regression models, AUC insulin 0 to 30 minutes to AUC glucose 0 to 30 minutes was chosen as dependent variable and age, BMI, OGTT-derived insulin sensitivity, and genotype were tested as independent variables in the dominant model. *P* values are given above the columns.

analyses were performed in the dominant inheritance model, but not in the additive inheritance model, Bonferroni correction was performed only for the number of SNPs. The statistical software package JMP 7.0 (SAS Institute, Cary, NC) was used. In the dominant inheritance model, the OGTT study was sufficiently powered ($1 - \beta > 0.8$) to detect effect sizes greater than or equal to 19%; and the hyperinsulinemic-euglycemic clamp study, to detect effect sizes greater than or equal to 36% (2-tailed *t* test). Power calculation was performed using G*power software available at http://www.psycho.uni-duesseldorf.de/aap/projects/gpower. Hardy-Weinberg equilibrium was tested using χ^2 test.

3. Results

3.1. Genotyping of the study population

The HapMap and the observed MAF are shown in Supplementary Fig. 1 and Supplementary Table 2. The minor differences between the observed MAF and the MAF published by HapMap might be due to genuine differences between our German population and HapMap's cohort of Utah residents with northern and western European ancestry. All 9 SNPs were found to be in Hardy-Weinberg equilibrium ($P > .05$).

3.2. Association of genetic variation in TRPM5 with glucose tolerance

All SNPs were tested in the additive and dominant inheritance models. As shown in Tables 1 and 3 (raw data) and Fig. 1 (adjusted data), rs800344, rs800345, and rs2301699 were significantly associated with AUC glucose during the OGTT in both the additive and dominant models after adjustment for sex, age, and BMI (all $Ps \leq .0025$). In addition, rs800344 was nominally associated with glucose at 120 minutes during the OGTT in the additive model and significantly associated in the dominant model ($P = .0038$ and $P = .0009$, respectively; Table 1). Rs2074234 was nominally associated with glucose at 120 minutes during the OGTT in the additive and dominant models and with AUC glucose during the OGTT in the dominant model (all $Ps < .05$; Table 2). During the OGTT, minor alleles of all 4 SNPs were found to be associated with increased glucose levels.

A stepwise regression analysis was performed and included sex, age, BMI, rs800344, rs800345, and rs2301699 as independent variables and AUC glucose during the OGTT as a dependent variable. Sex ($P = .0001$), age ($P < .0001$), BMI ($P < .0001$), rs800345 ($P = .0001$), and rs2301699 ($P = .0005$), but not rs800344 ($P = .5$), were found to de independently associated with AUC glucose during the OGTT.

3.3. Association of genetic variation in TRPM5 with insulin secretion and insulin sensitivity

Rs800344, rs800345, and rs2301699 were nominally associated with OGTT-derived insulin sensitivity in the dominant model after adjustment for sex, age, and BMI ($P = .0039$, $P = .0057$, and $P = .0319$, respectively), with minor allele carriers showing reduced insulin sensitivity. In addition, rs2301699 was nominally associated with clamp-derived insulin sensitivity in the dominant model; and rs2074234 was nominally associated with homeostasis model assessment of insulin resistance (HOMA-IR) in the additive and dominant models (all $Ps < .05$).

In contrast, genetic variants in *TRPM5* were not reliably associated with measures of adiposity or with indices of insulin secretion in the overall cohort (all $Ps \geq .03$).

3.4. Interaction effects of genetic variation in TRPM5 and anthropometric and metabolic phenotypes on insulin sensitivity and secretion

To test whether associations between *TRPM5* variants and metabolic traits may be attributable to distinct subgroups, we analysed interaction effects between genotypes (rs800344, rs800345, and rs2301699) on the one hand and sex, age, BMI, and glucose tolerance status on the other hand on measures of insulin sensitivity and insulin secretion by ANCOVA. We found an interaction effect between rs2301699 and sex on different indices of insulin secretion, including the ratio of AUC insulin 0 to 30 minutes to AUC glucose 0 to 30 minutes ($P = .0016$). After stratification for sex and adjustment for age, BMI, and OGTT-derived insulin sensitivity, rs2301699 was found to be significantly associated with the AUC insulin 0 to 30 minutes to AUC glucose 0 to 30 minutes ratio. Furthermore, in the dominant model, rs2301699 tended to associate with the ratio AUC C-peptide to AUC glucose during the OGTT in women ($P = .0097$ and $P = .09$, respectively), but not in men ($P = .3$ and $P = .6$, respectively; Fig. 2).

Interestingly, female minor allele carriers of rs2301699 showed significantly lower GLP-1 levels at 30 minutes during the OGTT compared with major allele homozygotes (CC: 39.9 ± 28.9 pmol/L vs CG + GG: 33.4 ± 24.4 pmol/L, $P = .0124$), whereas in male participants, no significant differences were found (CC: 38.3 ± 19.3 pmol/L vs CG + GG: 32.6 ± 17.5 pmol/L, $P = .3$).

No interaction effects were detected between *TRPM5* variants on the one hand and age, BMI, and glucose tolerance status on the other hand on indices of insulin sensitivity and insulin secretion (data not shown).

4. Discussion

Using the publicly available HapMap data, we identified 9 tagging SNPs covering 100% of the common genetic variation of the *TRPM5* locus.

TRPM5 SNP rs2301699 was significantly associated with insulin secretion in women, but not in men. The association between genetic variation in *TRPM5* and indices of insulin secretion is, in general, in agreement with previous animal studies supporting a key function of TRPM5 in glucose-stimulated insulin release [26,27], although the mechanisms underlying the sex specificity are currently unknown. Our findings are in line with the growing body of evidence supporting sex-gene interactions in the etiology of type 2 diabetes mellitus [31,32] and predisposing factors [33]. Possible mechanisms of sex-gene interactions comprise the effect of sex hormones, although alternative mechanisms underlying these interactions should also be considered [34]. Although the association between rs2301699 and glucose-stimulated

GLP-1 levels clearly needs further investigation, our findings may point to a potential mechanism of how variants in *TRPM5* affect insulin secretion. This assumption would be in agreement with the finding that TRPM5 is abundantly expressed in the gastrointestinal tract [24] and that α-gustducin, which colocalizes with TRPM5 [35], may regulate secretion of GLP-1 from L cells [36,37]. Previous studies have pointed to altered incretin signals as potential underlying mechanisms of some of the novel type 2 diabetes mellitus risk loci [38]. Whereas variants in *TCFL7* [39], *WFS1* [40], and *GIPR* [41] appear to affect incretin action, genetic variation in *KCNQ1* altered, similar to our findings in *TRPM5*, incretin secretion [42].

Although the data of the present study may indicate an involvement of *TRPM5* in glucose-dependent insulin secretion, genetic variants in *TRPM5* have not been reported in the recent GWA studies on the risk for type 2 diabetes mellitus and impaired fasting glucose [13,15]. One explanation could be that, in these GWA studies, in general, no stratification for sex was performed and, therefore, the association between *TRPM5* variants and prediabetic phenotypes in female subjects may have been overlooked.

Furthermore, we found that 3 *TRPM5* SNPs—rs800344, rs800345, and rs2301699—were significantly associated with glucose levels during the OGTT, with minor allele carriers showing increased values. Moreover, the 3 SNPs were nominally associated with OGTT-derived insulin sensitivity, with minor allele carriers depicting reduced insulin sensitivity. It is worth noting that the SNPs rs800345 and rs2301699 independently associated with glucose levels during the OGTT in a stepwise regression analysis, indicating that they may represent 2 independent causative polymorphisms. These findings were unexpected, and the underlying mechanisms of how *TRPM5* variants affect insulin sensitivity are currently elusive. Given that TRPM5 is abundantly expressed in the pituitary gland [23] and that the pituitary has a key function in the regulation of central and peripheral insulin sensitivity [43], one could speculate that TRPM5 is involved in the pituitary-dependent modulation of insulin sensitivity.

The present study has certain limitations that need to be taken into account. First, further replications in other cohorts are clearly needed to confirm the impact of genetic variation within this gene on insulin sensitivity, insulin secretion, and plasma GLP-1 levels. Second, we were not able to detect effect sizes smaller than 19% with sufficient power (80%). Thus, small effects of *TRPM5* variants on insulin secretion and insulin sensitivity possibly remained undetected in this study.

In conclusion, in our population of European ancestry, genetic variation in *TRPM5* was associated with prediabetic phenotypes and may, therefore, contribute to the development of type 2 diabetes mellitus. Its role as a type 2 diabetes mellitus gene remains to be assessed in case-control studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2011.02.002.

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