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## PAPER

# Role of the *FOXC2* –512C>T polymorphism in type 2 diabetes: possible association with the dysmetabolic syndrome

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**OBJECTIVE:** Overexpression of the human transcription factor *FOXC2* gene (*FOXC2*) protects against insulin resistance in mice and a common *FOXC2* polymorphism (–512C>T) has been suggested to be associated with insulin resistance in humans. Here, we addressed the potential role for *FOXC2* as a candidate gene for type 2 diabetes and associated phenotypes.

**MATERIALS AND METHODS:** A case–control study was performed in 390 type 2 diabetic patients and 307 control subjects. The number of patients was increased to a total of 768 subjects for further study of phenotypic differences relating to the dysmetabolic syndrome relative to genetic variation. The *FOXC2* –512C>T polymorphism was genotyped by a restriction fragment length polymorphism PCR assay.

**RESULTS:** *FOXC2* –512C>T allele and genotype distribution did not differ between patients with type 2 diabetes and control subjects, but the C/C genotype was associated with increased body mass index (BMI, kg/m<sup>2</sup>) ( $P_a=0.03$ ) among type 2 diabetic patients. The *FOXC2* –512C>T polymorphism was a significant independent predictor of BMI ( $P=0.001$ ) in a multiple regression model including age, gender and affection status. We found no significant association with type 2 diabetes-related metabolic parameters but that the C-allele ( $P=0.01$ ) and C/C and C/T genotypes ( $P=0.03$ ) were significantly over-represented in type 2 diabetic males with a concomitant diagnosis of dysmetabolic syndrome.

**CONCLUSION:** We conclude that *FOXC2* is associated with obesity and metabolic deterioration but does not contribute to an increased risk for type 2 diabetes.

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**Keywords:** type 2 diabetes; noninsulin-dependent diabetes; dysmetabolic syndrome; forkhead transcription factor; case–control association study

## Introduction

Along with the genes encoding PPAR- $\gamma$ ,<sup>1</sup> calpain-10,<sup>2</sup> adiponectin<sup>3,4</sup> and Kir6.2,<sup>5</sup> the human forkhead transcription factor *FOXC2* gene (*FOXC2*) is one of the most recently identified candidate genes for insulin resistance and type 2 diabetes.<sup>6,7</sup> Transgenic mice overexpressing human *FOXC2* in white adipose tissue exhibit increased insulin sensitivity, lower plasma glucose, insulin and triglyceride levels and appear relatively resistant to diet-induced insulin resistance and obesity.<sup>6</sup> In humans, *FOXC2* is expressed in both adipose tissue and skeletal muscle. The expression is sensitive to regulation by insulin and there is an inverse correlation

between the levels of *FOXC2* mRNA in both visceral fat and muscle and measures of insulin resistance.<sup>7</sup> The T/T genotype of a common polymorphism in the 5'untranslated region (5'UTR) of the gene (–512C>T) has been associated with increased mRNA expression in visceral compared to subcutaneous fat, and the T-allele with higher insulin sensitivity and lower triglyceride levels.<sup>7,8</sup> Although these data identified *FOXC2* as a candidate gene for insulin resistance and/or type 2 diabetes, three recent reports in Japanese, Pima Indians and Danish Caucasians have failed to identify an association between *FOXC2* and type 2 diabetes.<sup>8–10</sup> In addition, contrasting with previous data, the T-allele was weakly associated with increased waist-to-hip ratio (WHR), plasma triglycerides and measures of insulin resistance in the Danish study.<sup>10</sup>

Insulin resistance and additional features of the metabolic, or rather *dysmetabolic*, syndrome including abdominal obesity, hypertension and dyslipidemia are under strong genetic influence and cluster in families and they are the

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hallmark feature of type 2 diabetes.<sup>11</sup> A concomitant diagnosis of dysmetabolic syndrome is present in up to 80% of type 2 diabetic patients and is associated with a doubling of the risk for cardiovascular morbidity and mortality.<sup>12</sup> To address the question whether *FOXC2* is associated with type 2 diabetes or associated phenotypes, we performed a case-control study in Scandinavian patients with type 2 diabetes and nondiabetic control subjects without a family history of type 2 diabetes. The type 2 diabetic study material was extended and stratified for the number of dysmetabolic syndrome components for a more precise investigation of association to these features.

## Materials and methods

### Subjects

A case-control association study was performed in a random sample of 390 unrelated type 2 diabetic patients from Finland, one patient per family in the previously published Botnia study<sup>13</sup> and 307 nondiabetic spouses without a family history of type 2 diabetes (age 40 y or older with normal glucose tolerance assessed by a standard oral glucose tolerance test (75 g D-glucose)) to obtain an estimate of allele and genotype frequencies of the 5'UTR *FOXC2* -512C>T polymorphism and to study its potential role for phenotypes associated with type 2 diabetes. The groups were selected as to match for gender and age. To further investigate the association between *FOXC2* -512C>T and type 2 diabetes-related phenotypes clustering in the dysmetabolic syndrome, another random sample of 378 unrelated patients from both Finland and the South of Sweden was added to the study, amounting to a total number of 768 type 2 diabetic subjects. All subjects gave written informed consent and the study was conducted in accordance with the Declaration of Helsinki and approved by the local University ethics committee.

### Case-control association study

The following phenotypes were compared: age (y), total body weight (kg), body mass index (BMI, kg/m<sup>2</sup>), body fat mass (kg), lean body mass (kg), WHR, fasting plasma glucose (fP-Glucose, mmol/l), fasting serum insulin (fS-Insulin,  $\mu$ U/ml), homeostasis model assessment insulin resistance index (HOMA-IR),<sup>14</sup> fasting plasma triglycerides (fP-TG, mmol/l) and fasting plasma HDL cholesterol (fP-HDL; mmol/l). In all, 13 of the type 2 diabetic patients (3.3%) and none of the control subjects were treated with antihyperlipidemic agents. The mean duration of type 2 diabetes at the time for phenotypic characterization was 6.3 (2.4–13.1) y in this population.

### Stratification for the dysmetabolic syndrome

Sufficient data from a total of 768 type 2 diabetic subjects were available from 727 subjects (344 males and 383 females,

age 61.9 y (53.1–69.1), BMI 28.7 kg/m<sup>2</sup> (25.8–32.0)) that were stratified for a number of components of the dysmetabolic syndrome according to the following definitions complying with the updated WHO definition from 1999,<sup>15,16</sup> obesity: BMI >30 kg/m<sup>2</sup> or WHR >0.85 for females and >0.90 for males; dyslipidemia: fP-TG >1.7 mmol/l or fP-HDL-cholesterol <1.0 mmol/l for females and <0.9 mmol/l for males; and hypertension: a resting blood pressure >140/90 mmHg, a previous clinical diagnosis of hypertension or use of anti-hypertensive drugs. In all, 83% of the subjects met the criteria for obesity, 66% the criteria for hypertension and 52% the criteria for dyslipidemia. The number of subjects who did not meet any of the criteria was low ( $n=38$ ) and these subjects were therefore pooled with those meeting one criteria so that altogether 174 patients (25%; 84 males and 90 females) had no more than one component, 328 patients (45%; 149 males and 179 females) had two components and 225 patients (31%; 111 males and 114 females) fulfilled all three criteria. Of the type 2 diabetic subjects, 17% received insulin, 34% received tablets (metformin or sulfonylurea), 7% received both insulin and tablets and 34% had diet recommendations. From 8% of the subjects, we have no information of the treatment. In all, 3.4% of the type 2 diabetic patients received antihyperlipidemic drugs. The mean duration of type 2 diabetes at the time for phenotypic characterization was 6.8 (2.4–14) y in this extended population. Since insulin resistance in a control population is defined as the lowest quartile of measures of insulin sensitivity or highest quartile of fasting insulin or HOMA-IR index, the number of insulin-resistant control subjects was too low ( $n=77$ ) to allow for a meaningful analysis with enough power to identify differences in allele or genotype distribution, and was therefore not included (data not shown).

### Phenotypic characterization

All laboratory specimens were obtained after a 12 h overnight fast. Fasting concentrations of glucose, insulin and lipids were measured as described previously.<sup>13</sup> The HOMA-IR index (fS-Insulin multiplied by fP-Glucose divided by 22.5) was used to estimate the degree of insulin resistance.<sup>14</sup> Body weight, height, waist (measured with a soft tape midway between the lowest rib and the iliac crest), hip circumference (at the widest part of the gluteal region) and body composition by bioimpedance were measured with subjects in light clothing without shoes and BMI and WHR calculated accordingly. Blood pressure was measured (mean of at least two measures) in the supine position after a 10-min rest using a standard forearm manometer. Type 2 diabetes was diagnosed according to the WHO criteria from 1998<sup>16</sup> or by a previous diagnosis of diabetes and treatment with oral agents and/or insulin.

### Genotyping

DNA was extracted from blood using a conventional method.<sup>17</sup> The *FOXC2* -512C>T region was amplified with

primers 5'-GTC TTA GAG CCG ACG GAT TCC TG-3' and 5'-TGG GGA CCA AGG TGG ACC CTC G-3'. PCR was carried out in a 20 µl volume containing 10 µM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 5% DMSO, 0.5 U *Taq* polymerase (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 10 pmol of each primer and 25 ng genomic DNA. The cycling conditions were 94°C for 5 min, 35 cycles of 94°C for 30 s, 63°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 10 min. PCR products were digested with *Bbs*I (New England Biolabs, Beverly, MA, USA) in 37°C overnight. The digested products were separated on 3% agarose gel.

### Statistical analyses

Allele and genotype frequencies were compared by  $\chi^2$  statistics. Continuous variables were compared by nonparametric Kruskal–Wallis one-way ANOVA unless exhibiting normal distribution. Unless predefined by previous data or hypothesis, further tests on differences concerning major variables for the phenotypes (BMI and HOMA-IR) between pooled groups of different genotype carriers were only performed when a significant difference between the groups was found by the initial ANOVA. This may be overly conservative since it does not fully allow us to test for a recessive effect. On the other hand, a significant difference in one group (control subjects or type 2 diabetic patients) was followed up by the reciprocal analysis in the other. Data are presented as median with interquartile range (25–75th percentile). Where applicable, data were adjusted for age and gender (weight, BMI, body fat mass, lean body mass and WHR), or age, gender and BMI (fP-Glucose, HbA<sub>1c</sub>, fS-Insulin, HOMA-IR, fP-HDL-cholesterol and fP-TG) using the general linear model. Adjusted *P*-values (*P*<sub>a</sub>) are presented along with unadjusted values. A *P*-value below 0.05 was considered statistically significant. Since only two major and highly interrelated phenotypic traits were investigated, body

composition and insulin resistance, two-tailed *P*-values are reported without correction for multiple testing. All statistical operations were performed using the Number Cruncher Statistical Software (NCSS, Kaysville, USA).

### Results

Clinical characteristics of the study subjects are summarized in Table 1 and allele and genotype frequencies for the FOX2 –512C>T polymorphism in type 2 diabetic patients and control subjects are presented in Table 2. All genotype frequencies were in Hardy–Weinberg equilibrium. There were no significant differences in allele or genotype frequency between the groups. Although the C-allele has originally been described as the wild-type allele for this single-nucleotide polymorphism (GenBank Accession no. NM\_005251), the more common allele in Scandinavian populations is T.<sup>7,10</sup>

Differences in type 2 diabetes-related phenotypes between genotype carriers are presented in Table 3. Unintentionally, there were significantly more females than males homozygous for the T-allele among the control subjects (47.6 vs 35.5% for female and male control subjects, respectively, *P*=0.04). Consequently, all data were at least adjusted for gender and age for the subsequent analyses.

There was a significant difference in weight between carriers of different –512C>T genotypes in both type 2 diabetic patients (*P*<sub>a</sub>=0.04) and control subjects (*P*<sub>a</sub>=0.04). In the type 2 diabetic patients, this difference was significant when comparing carriers of the T-allele with subjects homozygous for the C-allele; weight 78.0 (70.0–88.0) vs 85.0 (76.2–94.5) kg (*P*=0.005, *P*<sub>a</sub>=0.01). In contrast, in the control subjects the difference was significant when comparing carriers of the T/T vs C/C and C/T genotypes; weight 72.0 (64.0–83.7) vs 76.0 (71.8–86.6) kg (*P*=0.06, *P*<sub>a</sub>=0.02). More importantly, type 2 diabetic carriers of the C/C genotype

**Table 1** Clinical characteristics of subjects in the case–control association study

	Type 2 diabetic patients <sup>a</sup>	Control subjects	<i>P</i> -value
<i>n</i>	390	307	
Sex (M/F)	167/223	144/163	0.3
Age (y)	62.0 (54.8–67.9) ( <i>n</i> =388)	60.3 (53.4–67.2) ( <i>n</i> =306)	0.2
Weight (kg)	79.0 (70.5–89.0) ( <i>n</i> =389)	74.7 (67.0–84.0) ( <i>n</i> =301)	<0.0001
BMI (kg/m <sup>2</sup> )	28.9 (25.9–31.8) ( <i>n</i> =389)	26.4 (24.1–29.2) ( <i>n</i> =300)	<0.0001
Body fat mass (kg)	23.1 (19.3–28.5) ( <i>n</i> =230)	20.8 (17.1–24.6) ( <i>n</i> =213)	<0.0001
WHR	0.94 (0.87–0.99) ( <i>n</i> =382)	0.88 (0.82–0.95) ( <i>n</i> =298)	<0.0001
fP-Glucose (mmol/l)	9.4 (7.6–11.7) ( <i>n</i> =388)	5.4 (5.0–5.8) ( <i>n</i> =307)	<0.0001
HbA <sub>1c</sub> (%)	7.3 (6.4–8.9) ( <i>n</i> =220)	5.5 (5.3–5.8) ( <i>n</i> =192)	<0.0001
fS-Insulin (µU/ml)	12.1 (7.5–20.7) ( <i>n</i> =374)	7.5 (4.9–10.0) ( <i>n</i> =295)	<0.0001
HOMA-IR	5.0 (2.9–9.6) ( <i>n</i> =372)	1.8 (1.2–2.5) ( <i>n</i> =295)	<0.0001
fP-Cholesterol (mmol/l)	5.77 (5.11–6.68) ( <i>n</i> =375)	5.94 (5.21–6.74) ( <i>n</i> =276)	0.3
fP-HDL (mmol/l)	1.19 (1.02–1.42) ( <i>n</i> =363)	1.36 (1.16–1.66) ( <i>n</i> =277)	<0.0001
fP-TG (mmol/l)	1.59 (1.17–2.25) ( <i>n</i> =375)	1.22 (0.89–1.68) ( <i>n</i> =276)	<0.0001

Data are median (interquartile range). *P*-values refer to Mann–Whitney nonparametric statistics, except for the gender comparison where it refers to  $\chi^2$  statistics. <sup>a</sup>In all, 15% of the type 2 diabetic subjects received insulin treatment, 36% received oral agents (metformin or sulfonylurea), 8% received both insulin and tablets and 32% were on diet restrictions. From 9% of the patients no information about treatment was available.

displayed an increased BMI compared to carriers of the C/T and T/T genotypes ( $P=0.01$ ,  $P_a=0.01$ ); 30.1 (28.0–33.3) vs 28.6 (25.8–31.7) kg/m<sup>2</sup>. The observed differences in weight and BMI were explained by an increased fat mass (Table 3), but there were no significant differences between type 2 diabetic genotype carriers concerning either percent body fat (not shown) or distribution (WHR; Table 3). There was no difference in BMI between different genotype carriers among the control subjects, neither a difference in body composition, but a borderline significant increase in WHR ( $P=0.04$ ,  $P_a=0.09$ ) that was significant when comparing carriers of the T/T vs C/C and C/T genotypes; WHR 0.86 (0.82–0.93) vs 0.90 (0.82–0.96) ( $P=0.02$ ,  $P_a=0.03$ ). There was no significant difference in the distribution of treatments in the different –512C>T genotype groups (11% insulin, 39% oral agents, 4.5% insulin and oral agents, 36% diet restrictions and 9.5% unknown treatment among the C/C carriers vs 20% insulin, 31% oral agents, 8% insulin and oral agents, 31% diet restrictions and 10% unknown treatment among the C/T carriers vs 12% insulin, 39% oral agents, 8% insulin

and oral agents, 31% diet restrictions and 10% unknown treatment among the T/T carriers,  $P=0.5$ ).

In light of the minor but significant association between the FOXC2 –512C>T polymorphism, body weight and BMI, we compared the upper and lower BMI quartiles of the type 2 diabetic patients as an association study for obesity. The quartiles were gender neutral, that is, composed of the upper and lower male and female BMI quartiles, respectively: 96 subjects in each quartile; BMI 24.5 (23.2–25.3) vs 33.8 (32.7–37.0) kg/m<sup>2</sup> ( $P<0.0001$ ). The C-allele was more common in the upper BMI quartile (40.1 vs 29.7% in the upper and lower BMI quartile, respectively;  $P=0.04$ ) as was the C/C vs C/T and T/T genotype (18.8 vs 8.3% in the upper and lower BMI quartile, respectively,  $P=0.03$ ). In fact, FOXC2 –512C>T genotype ( $P=0.001$ ), age ( $P=0.01$ ) and affection status (type 2 diabetics vs controls,  $P<0.0001$ ) were all significant independent predictors of BMI in the whole study material in a multiple regression model including gender ( $P=0.08$ ), although the model only explained about 10% of the variability in BMI ( $R^2=0.099$ ).

This model was also applied to data on body composition (total body fat) that were available for a subset of the study subjects (230 type 2 diabetic patients and 213 control subjects). There was a significantly increased total body fat mass in type 2 diabetic patients homozygous for –512C ( $P=0.04$ ). FOXC2 –512C>T genotype was an independent predictor ( $P=0.002$ ) of this variable in a multiple regression model including gender ( $P<0.0001$ ) and age ( $P=0.3$ ) explaining about half of the variation in body fat percent ( $R^2=0.53$ ) in type 2 diabetic patients. The model did not perform in control subjects due to skewed distribution of residuals (data not shown).

There were no differences concerning metabolic parameters including insulin sensitivity and plasma lipids (Table 3).

**Table 2** Allele and genotype frequencies of FOXC2 –512C>T in Scandinavian Caucasians

	Type 2 diabetic subjects	Control subjects	P-value
<i>Allele</i>			
C	274 (35.3)	214 (35.0)	0.9
T	502 (64.7)	398 (65.0)	
<i>Genotype</i>			
C/C	46 (11.9)	37 (12.1)	1.0
C/T	182 (46.9)	140 (45.8)	
T/T	160 (41.2)	129 (42.2)	

Data are *n* (%). In all, 0.4% of the genotypes ( $n=3$ ) could not be obtained for technical reasons. *P*-values refer to  $\chi^2$  statistics.

**Table 3** Comparison of clinical characteristics according to FOXC2 –512C>T genotype

	Type 2 diabetic subjects			P-value	$P_a$	Control subjects			P-value	$P_a$
	C/C	C/T	T/T			C/C	C/T	T/T		
Age (y)	60.5 (53.3–66.9)	63.0 (55.2–67.9)	61.2 (54.0–68.3)	0.5	NA	56.8 (51.6–63.6)	60.4 (53.6–67.2)	62.1 (53.4–68.2)	0.1	NA
Duration (y)	5.2 (1.9–10.1)	6.9 (2.5–14.4)	6.3 (2.4–12.5)	0.5	NA	NA	NA	NA	NA	NA
Sex (M/F)	21/25	79/103	66/94	0.8	NA	17/20	76/64	50/79	<b>0.04</b>	NA
Weight (kg)	85.0 (76.2–94.5)	78.1 (71.0–87.8)	77.1 (69.0–88.4)	<b>0.02</b>	<b>0.04</b>	77.0 (71.8–86.6)	76.0 (67.0–84.0)	72.0 (64.0–83.7)	0.1	<b>0.04</b>
BMI (kg/m <sup>2</sup> )	30.1 (28.0–33.3)	28.9 (26.1–31.8)	28.2 (25.7–31.6)	<b>0.02</b>	<b>0.03</b>	26.7 (24.8–29.5)	26.5 (24.2–29.4)	25.9 (23.4–28.9)	0.3	0.3
Body fat mass (kg)	26.6 (22.2–30.4)	23.6 (19.4–28.4)	22.4 (18.0–28.2)	<b>0.01</b>	<b>0.04</b>	21.0 (18.3–25.3)	20.2 (17.2–24.4)	21.5 (17.0–24.8)	0.7	0.4
Lean body mass (kg)	56.2 (51.6–67.0)	55.0 (47.5–63.5)	51.3 (46.3–59.6)	0.2	0.5	55.9 (50.7–61.8)	55.6 (47.7–64.7)	52.3 (44.8–59.2)	0.06	0.06
WHR	0.94 (0.86–1.02)	0.94 (0.87–0.99)	0.92 (0.87–0.98)	0.5	0.6	0.90 (0.84–0.95)	0.91 (0.82–0.96)	0.86 (0.82–0.93)	<b>0.04</b>	<b>0.09</b>
fP-Glucose (mmol/l)	9.7 (8.1–12.1)	9.7 (7.7–12.1)	9.0 (7.5–11.4)	0.4	0.2	5.4 (5.1–5.9)	5.5 (5.0–5.9)	5.5 (5.1–5.8)	0.6	0.5
HbA <sub>1c</sub> (%)	7.2 (6.4–9.4)	7.2 (6.3–8.6)	7.5 (6.5–9.3)	0.5	0.4	5.7 (5.3–5.8)	5.5 (5.3–5.8)	5.5 (5.2–5.7)	0.8	<b>0.98</b>
fS-Insulin ( $\mu$ U/ml)	13.0 (7.9–23.0)	12.7 (7.9–21.5)	11.1 (7.1–18.9)	0.4	0.2	6.4 (4.3–9.6)	8.1 (5.3–10.5)	6.8 (4.6–9.4)	0.1	0.07
HOMA-IR	5.4 (3.1–11.8)	5.3 (2.9–10.4)	4.9 (2.9–8.0)	0.3	0.2	1.4 (1.0–2.5)	2.0 (1.3–2.6)	1.7 (1.1–2.4)	0.2	0.3
fP-HDL (mmol/l)	1.1 (1.0–1.4)	1.2 (1.0–1.4)	1.2 (1.0–1.6)	0.3	0.3	1.3 (1.1–1.7)	1.4 (1.2–1.6)	1.4 (1.2–1.7)	0.6	0.3
fS-TG (mmol/l)	1.8 (1.4–2.3)	1.6 (1.2–2.2)	1.6 (1.1–2.3)	0.3	0.2	1.2 (0.8–1.6)	1.2 (0.9–1.6)	1.3 (0.9–1.7)	0.5	0.6

Data are median (interquartile range). In all, 0.4% of the genotypes ( $n=3$ ) could not be obtained for technical reasons. *P*-values refer to ANOVA, before (*P*) and after ( $P_a$ ) adjusting for relevant covariates (see Materials and methods for details), except for the gender comparison where they represent  $\chi^2$  statistics. Duration (y), duration of type 2 diabetes at the time for phenotypic characterization. NA, not applicable. Bold indicates statistically significant.

To evaluate a putative association between *FOXC2* C-512T and the dysmetabolic syndrome, we increased the number of type 2 diabetic subjects and stratified the material for a number of additional components of the syndrome: obesity, hypertension and dyslipidemia. Throughout the strata there were nonsignificant increasing and decreasing trends for the prevalence of the C- and T-alleles, respectively ( $P=0.4$ ) (Table 4). In males, both allele ( $P=0.01$ ) and genotype ( $P=0.03$ ) distributions differed significantly comparing patients with (three components) and without (none or one component) a concomitant dysmetabolic syndrome diagnosis.

## Discussion

*FOXC2* is a major metabolic regulator of adipocyte metabolism. It has been shown that transgenic mice overexpressing human *FOXC2* in white adipose tissue exhibit enhanced insulin sensitivity and that they are relatively resistant to weight gain during high fat feeding.<sup>6</sup> In humans, *FOXC2* mRNA levels in both visceral adipose tissue and skeletal muscle correlate with insulin sensitivity.<sup>7</sup> We recently

identified *FOXC2* as a candidate gene for insulin resistance in a study using a family-based approach: analysis of genotype discordant nondiabetic siblings with a family history of type 2 diabetes, where the C-allele of the 5'UTR  $-512C>T$  polymorphism was found to be associated with insulin resistance (increased HOMA-IR) and increased plasma triglyceride levels. Our findings were largely confined to female siblings.<sup>7</sup> In the present investigation, a case-control association study-based approach was used to study the potential role for *FOXC2* in type 2 diabetes. We found no association between the *FOXC2*  $-512C>T$  polymorphism and type 2 diabetes, but an association between the C-allele and obesity in type 2 diabetic subjects.

Three previous studies have investigated the potential role of *FOXC2* in type 2 diabetes. In agreement with our data, none of these studies performed in Japanese,<sup>9</sup> Pima Indians<sup>8</sup> and Danish Caucasians,<sup>10</sup> respectively, could show an association between *FOXC2* and type 2 diabetes. However, also in agreement with our present data, a significant association between the  $-512C>T$  variant and BMI ( $P=0.03$ ) and measures of body composition (percent body fat;  $P=0.02$ ) was noted in Pima Indians.<sup>8</sup> Furthermore, the previously suggested gender-specific effect on triglycerides and insulin sensitivity was confirmed in the Pima Indian population, where  $-512C>T$  seems to be involved in the regulation of basal glucose turnover and plasma triglyceride levels in women.<sup>8</sup>

Obesity, particularly abdominal obesity, usually precedes insulin resistance and type 2 diabetes<sup>13,18</sup> and genetic factors are considered to explain 60% of the variance in abdominal fat.<sup>19,20</sup> We found that type 2 diabetic carriers of the C/C genotype displayed an increased BMI compared to carriers of the T-allele. One should be aware of the fact that BMI and other metabolic measurements of a diabetic subject are influenced by treatment, degree of glycemic control and duration of diabetes. However, we found no significant difference in the distribution of treatments or duration of type 2 diabetes in the different genotype groups. We also found a weak association to WHR in the control subjects, but we view this association with caution since the gender distribution among the different genotype carriers in the control group was skewed and the significance of the association disappeared after adjusting for gender and age. In addition, a recent study in glucose tolerant Danish Caucasians found increased triglyceride levels ( $P=0.03$ ), fasting serum C-peptide ( $P=0.009$ ), insulinogenic index ( $P=0.04$ ) and, restricted to females, WHR ( $P=0.01$ ) in carriers of the T-allele compared to homozygous carriers of the C-allele.<sup>10</sup> We have no obvious explanation for this discrepancy other than methodological differences or that the polymorphism in the Danes rose on a different ancestral haplotype. This would imply that the  $-512C>T$  polymorphism is not the causal one. Although differences in gene expression due to allelic variation are relatively common in humans,<sup>21</sup> it must be stressed that it is presently not known whether the *FOXC2* 5'UTR polymorphism is of direct

**Table 4** Allele and genotype frequencies of *FOXC2*  $-512C>T$  in patients with type 2 diabetes with and without a concomitant diagnosis of dysmetabolic syndrome

	Dysmetabolic syndrome components (n)			P-value	P-value*
	0-1	2	3		
<b>Allele</b>					
All subjects					
C	110 (31.6)	219 (33.4)	162 (36.0)	0.4	0.2
T	238 (68.4)	437 (66.6)	288 (64.0)		
Males				0.04	0.01
C	45 (26.8)	101 (33.9)	87 (39.2)		
T	123 (73.2)	197 (66.1)	135 (60.8)		
Females				0.7	0.5
C	65 (36.1)	118 (33.0)	75 (32.9)		
T	115 (63.9)	240 (67.0)	153 (67.1)		
<b>Genotype</b>					
All subjects					
C/C	13 (7.5)	37 (11.3)	30 (13.3)	0.4	0.2
C/T	84 (48.3)	145 (44.2)	102 (45.3)		
T/T	77 (44.3)	146 (44.5)	93 (41.3)		
Males				0.1	0.03
C/C	5 (6.0)	17 (11.4)	16 (14.4)		
C/T	35 (41.7)	67 (45.0)	55 (49.5)		
T/T	44 (52.4)	65 (43.6)	40 (36.0)		
Females				0.4	0.2
C/C	8 (8.9)	20 (11.2)	14 (12.3)		
C/T	49 (54.4)	78 (43.6)	47 (41.2)		
T/T	33 (36.7)	81 (45.3)	53 (46.5)		

Data are n (%). P-values refer to  $\chi^2$  statistics; P\*-value for comparison between groups with 0-1 and 3 components of the dysmetabolic syndrome, respectively.

functional importance. It is also possible that the polymorphism may show different manifestations in different phenotypes or populations.

We were unable to confirm an association between the C-allele of  $-512C>T$  and insulin resistance or hypertriglyceridemia in the present investigation, but we observed a significant association between *FOXC2*  $-512C$  and a concomitant diagnosis of the dysmetabolic syndrome in male type 2 diabetic subjects. The gender-specific effects are more evident in females in previous reports, whereas the effect is stronger in males in this study. This is an interesting difference, but although it is conceivable that males and females react differently to environmental risk factors given the same *FOXC2* genotype, we feel that it is premature to draw any conclusions from it. We cannot exclude that the differences are due to lack of power in this and previous studies. Alternative explanations such as, for example, duration at risk and gender preference in choice of treatment may also be of importance. Clearly, there is a need for larger studies addressing this issue. It should be noted that the three component dysmetabolic syndrome strata in our material do not correspond to the WHO or NCEP definitions of the syndrome as only two additional criteria in addition to type 2 diabetes are required for this diagnosis.<sup>15</sup> Applying these criteria, three quarters of the type 2 diabetic subjects in the present investigation have a concomitant dysmetabolic syndrome, which is in accordance with previous estimates in the Botnia study.<sup>12</sup> Whereas a theoretical link between *FOXC2*, obesity and dyslipidemia can be easily envisioned,<sup>6,7,22</sup> there is no evident functional explanation for a role for *FOXC2* in hypertension, but the dysmetabolic syndrome is a complex trait and this issue certainly requires confirmation in population-based prospective studies.

In conclusion, our present data suggest that *FOXC2*, a key regulator of adipocyte metabolism, is weakly but consistently associated with obesity and features of the dysmetabolic syndrome, but that genetic variability in the gene does not contribute to the increased risk for type 2 diabetes.

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#### References

- Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SE, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common

- PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; **26**: 76–80.
- Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000; **26**: 163–175.
- Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tmoita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941–946.
- Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasada K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Kimura S, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 2002; **51**: 536–540.
- Love-Gregory L, Wasson J, Lin J, Skolnick G, Suarez B, Permutt MA. An E23K single nucleotide polymorphism in the islet ATP-sensitive potassium channel gene (Kir6.2) contributes as much to the risk of type II diabetes in Caucasians as the PPAR $\gamma$ /Pro12Ala variant. *Diabetologia* 2002; **43**: 136–137.
- Cederberg A, Grönning LM, Ahrén B, Taskén K, Carlsson P, Enerbäck S. *FOXC2* is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 2001; **106**: 563–573.
- Ridderstråle M, Carlsson E, Klannemark M, Cederberg A, Kösters C, Tornqvist H, Storgaard H, Vaag A, Enerbäck S, Groop L. *FOXC2* mRNA and a 5'UTR polymorphism of the gene are associated with insulin resistance. *Diabetes* 2002; **51**: 3354–3660.
- Kovacs P, Lehn-Stefan A, Stumvoll M, Bogardus C, Baier L. Genetic variation in the human winged helix/forkhead transcription factor gene *FOXC2* in Pima Indians. *Diabetes* 2003; **52**: 1292–1295.
- Osawa H, Onuma H, Murakami A, Ochi M, Nishimiya T, Kato K, Shimizu I, Fujii Y, Ohashi J, Makino H. Systematic search for single nucleotide polymorphisms in the *FOXC2* gene. The absence of evidence for the association of three frequent single nucleotide polymorphisms and four common haplotypes with Japanese type 2 diabetes. *Diabetes* 2003; **52**: 562–567.
- Yanagisawa K, Hingstrup-Larsen L, Andersen G, Drivsholm T, Cederberg A, Westergren R, Borch-Johnsen K, Pedersen O, Enerbäck S, Hansen T. The *FOXC2*  $-512C>T$  variant is associated with hypertriglyceridaemia and increased serum C-peptide in Danish Caucasian glucose-tolerant subjects. *Diabetologia* 2003; **46**: 1576–1580.
- Groop L, Orho-Melander M. The dysmetabolic syndrome. *J Intern Med* 2001; **250**: 105–120.
- Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001; **24**: 683–689.
- Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M, Ehrnström BO, Forsén B, Isomaa B, Snickars B, Taskinen MR. Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. *Diabetes* 1996; **45**: 1585–1593.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985 Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
- World Health Organisation. *Report of a WHO Consultation: Definition of Metabolic Syndrome in Definition, Diagnosis, and*

- Classification of Diabetes Mellitus and its Complications. I. Diagnosis and Classification of Diabetes Mellitus.* World Health Organisation, Department of Non communicable Disease Surveillance: Geneva; 1999.
- 16 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetes Med* 1998; **15**: 539–553.
  - 17 Vandenplas S, Wiid I, Grobler-Rabie A, Brebner K, Ricketts M, Wallis G, Bester A, Boyd C, Mathew C. Blot hybridisation analysis of genomic DNA. *J Med Genet* 1984; **21**: 164–172.
  - 18 Beck-Nielsen H, Groop LC. Metabolic and genetic characterization of prediabetic states. Sequence of events leading to non-insulin-dependent diabetes mellitus. *J Clin Invest* 1994; **94**: 1714–1721.
  - 19 Carey DG, Nguyen TV, Campbell LV, Chisholm LV, Kelly P. Genetic influences on central abdominal fat: a twin study. *Int J Obes Relat Metab Disord* 1996; **20**: 722–726.
  - 20 Samaras K, Spector TD, Nguyen TV, Baan K, Campbell LV, Kelly P. Independent genetic factors determine the amount and distribution of fat in women after the menopause. *J Clin Endocrinol Metab* 1997; **82**: 781–785.
  - 21 Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW. Allelic variation in human gene expression. *Science* 2002; **297**: 1143.
  - 22 Grønning LM, Cederberg A, Miura N, Enerbäck S, Taskén K. Insulin and TNF $\alpha$  induce expression of the forkhead transcription factor gene *Foxc2* in 3T3-L1 adipocytes via PI3-kinase. *Mol Endocrinol* 2002; **16**: 873–883.