Lack of association between metabolic traits and the −512 polymorphism in *FOXC2* in German people with normal glucose tolerance

To the Editor: A recent paper reported an association between the −512C>T promoter polymorphism in the forkhead box C2 (*FOXC2*) gene and increased serum C-peptide and hypertriglyceridaemia in normal-glucose-tolerant subjects from Denmark [1]. An association with Type 2 diabetes was not observed in this population. Previous papers had found conflicting data on this common polymorphism regarding an association with obesity and diabetes-related traits [2, 3].

We genotyped this polymorphism in 732 subjects (264 male, 468 female; aged 36±12 years [SD]) with normal glucose tolerance according to WHO criteria, and studied any associations with metabolic traits. The subjects were participants of the ongoing Tübingen family study of Type 2 diabetes in the south-west of Germany. About one half of the subjects had a family history of Type 2 diabetes. All subjects underwent a 2-h oral glucose tolerance test. Informed written consent was obtained from all participants and the studies were approved by the local ethics committee. For statistical analyses, the parameters were logarithmically transformed. With the exception of BMI and percentage body fat, all parameters were tested for association with the genotype using a multivariate linear regression model to adjust for age, sex and BMI.

As shown in Table 1, we found no association between this polymorphism and any of the metabolic traits studied. Since a previous study [2] found a sex-specific effect on triglycerides, we performed the analysis in men and women separately, but also found no significant association with the −512 genotype. In particular, no associations with plasma insulin, C-peptide or serum triglycerides were observed. Absence or presence of family history of Type 2 diabetes had no influence on the results.

One explanation for the lack of differences between the genotypes is that there are differences in sample selection. For example, in comparison with the Danish report [1], we studied younger subjects with a higher proportion of positive family history of Type 2 diabetes. The Danish study reported marginally higher fasting insulin and significantly higher fasting C-peptide in carriers of the T-allele. This was interpreted as an indication of insulin resistance. It is worth noting, however, that in this paper the carriers of the T-allele had a somewhat higher WHR and this variable was not included in the multivariate regression analysis. Although the WHR difference was not quite significant, it may have contributed to the borderline

Table 1. Association of the −512C>T *FOXC2* variant with diabetes-related traits in non-diabetic German people undergoing an oral glucose tolerance test

Genotype	Men				Women				All			
	CC (*n*=42)	*CT* (*n*=122)	*TT* (*n*=100)	p^{a}	*CC* (*n*=73)	*CT* (*n*=230)	*TT* (*n*=165)	p^{a}	*CC* (*n*=115)	*CT* (*n*=352)	*TT* (*n*=265)	p^{a}
BMI (kg/m^2)	24.8±0.6	25.4±0.4	25.7±0.5	0.6	26.5±0.7	25.9±0.4	26.2±0.5	0.7	25.9±0.5	25.7±0.3	26.0±0.4	0.9
Body fat (%)	20.7±1.1	20.8±0.6	20.8±0.7	0.9	32.1±1.1	31.3±0.6	31.5±0.7	0.9	27.9±1.0	27.6±0.5	27.5±0.6	1.0
Fasting plasma glucose (mmol/l)	5.0±0.1	5.0±0.04	5.0±0.1	1.0	5.0±0.1	4.9±0.04	4.9±0.04	0.8	5.0±0.05	4.9±0.03	5.0±0.03	0.7
2-h plasma glucose(mmol/l)	5.4±0.2	5.2±0.1	5.6±0.1	0.2	6.0±0.2	5.7±0.1	5.7±0.1	0.2	5.8±0.1	5.5±0.07	5.7±0.08	0.09
Fasting plasma insulin (pmol/l)	42±3	46±2	46±3	0.6	57±4	53±2	55±4	0.4	51±3	51±2	52±3	0.3
30-min plasma insulin (pmol/l)	367±31	399±27	394±34	0.8	430±32	380±16	393±25	0.2	407±23	387±14	393±20	0.3
120-min plasma insulin (pmol/l)	245±31	224±18	264±27	0.7	367±36	347±31	342±23	0.7	322±26	304±22	312±18	0.6
Fasting plasma C-peptide (pmol/l)	609±73	600±31	633±36	0.6	624±34	628±22	637±31	0.5	619±18	618±34	635±23	0.8
30-min plasma C-peptide (pmol/l)	2166±114	2325±87	2370±103	0.9	2300±97	2190±54	2268±86	0.7	2252±75	2236±47	2306±66	0.9
120-min plasma C-peptide (pmol/l)	2265±161	2326±106	2600±152	0.2	2817±121	2760±78	2911±97	0.5	2619±100	2609±63	2794±84	0.1
Fasting NEFA (mmol/ml)	495±33	427±19	480±19	0.09	516±23	541±15	546±19	0.9	509±17	502±12	520±14	0.6
First phase insulin secretion (pmol/l)^{b}	917±65	1046±57	1018±69	0.9	1154±60	1129±35	1131±53	0.7	1066±46	1099±30	1088±42	0.9
ISI (U)^{c}	22.0±1.9	20.6±1.0	21.8±1.3	0.8	17.6±1.2	19.7±0.5	20.5±0.9	0.4	19.2±1.2	20.1±0.7	20.9±1.0	0.4
Triglycerides (mmol/l)	1.64±0.28	1.71±0.19	2.05±0.37	0.6	1.38±0.14	1.15±0.06	1.16±0.06	0.3	1.47±0.14	1.36±0.08	1.49±0.15	0.9
Cholesterol (mmol/l)	4.97±0.16	5.10±0.10	5.15±0.13	0.6	5.15±0.13	4.99±0.05	4.99±0.08	0.3	5.10±0.10	5.04±0.05	5.04±0.08	0.5

ISI, insulin sensitivity index; [a] with the exception of BMI and percentage body fat all parameters were adjusted for BMI, age and sex; [b] estimated from the OGTT [5]; [c] estimated from the OGTT [6]

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difference in insulin resistance. In addition, it should be mentioned that other studies have found just the opposite, namely an association of the T-allele with enhanced insulin sensitivity [3] and lower plasma triglycerides [2, 3].

To detect a 10% difference between genotypes, the statistical power of our data set was more than 80% for the relevant parameters tested. Therefore, we should have been able to detect differences between genotypes in insulin sensitivity, serum fasting C-peptide or triglycerides, which were found to be 10 to 20% in the Danish paper.

In summary, although animal and in vitro studies have suggested a role of *FOXC2* in obesity, insulin resistance and Type 2 diabetes [4], the common −512 promoter polymorphism in this gene does not seem to have a major metabolic effect in the German population.

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