

## Epigenetics

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RESEARCH PAPER

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## DNA methylation of loci within *ABCG1* and *PHOSPHO1* in blood DNA is associated with future type 2 diabetes risk

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### ABSTRACT

Identification of subjects with a high risk of developing type 2 diabetes (T2D) is fundamental for prevention of the disease. Consequently, it is essential to search for new biomarkers that can improve the prediction of T2D. The aim of this study was to examine whether 5 DNA methylation loci in blood DNA (*ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1*, and *TXNIP*), recently reported to be associated with T2D, might predict future T2D in subjects from the Botnia prospective study. We also tested if these CpG sites exhibit altered DNA methylation in human pancreatic islets, liver, adipose tissue, and skeletal muscle from diabetic vs. non-diabetic subjects. DNA methylation at the *ABCG1* locus cg06500161 in blood DNA was associated with an increased risk for future T2D (OR = 1.09, 95% CI = 1.02–1.16, *P*-value = 0.007, *Q*-value = 0.018), while DNA methylation at the *PHOSPHO1* locus cg02650017 in blood DNA was associated with a decreased risk for future T2D (OR = 0.85, 95% CI = 0.75–0.95, *P*-value = 0.006, *Q*-value = 0.018) after adjustment for age, gender, fasting glucose, and family relation. Furthermore, the level of DNA methylation at the *ABCG1* locus cg06500161 in blood DNA correlated positively with BMI, HbA1c, fasting insulin, and triglyceride levels, and was increased in adipose tissue and blood from the diabetic twin among monozygotic twin pairs discordant for T2D. DNA methylation at the *PHOSPHO1* locus cg02650017 in blood correlated positively with HDL levels, and was decreased in skeletal muscle from diabetic vs. non-diabetic monozygotic twins. DNA methylation of cg18181703 (*SOCS3*), cg11024682 (*SREBF1*), and cg19693031 (*TXNIP*) was not associated with future T2D risk in subjects from the Botnia prospective study.

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### Introduction


Type 2 diabetes (T2D) is a complex disease that results from genetic and environmental interactions that can be modified and/or mediated by epigenetic changes.<sup>1–3</sup> A number of genetic and non-genetic factors have been identified that increase the risk of T2D.<sup>4–9</sup> However, a healthier lifestyle, including proper diet and exercise, can potentially reduce the risk of T2D by almost 50 percent in high-risk groups.<sup>10,11</sup> Therefore, there is great interest and need to identify individuals that have a high risk of developing T2D. By postponing and/or preventing T2D and its complications, it may be possible to reduce T2D-associated mortality and the financial cost of treating the disease and its complications.

To date, more than 65 genetic variants have been identified that increase the risk of T2D by almost 10 percent.<sup>6</sup> However, genetic screening for T2D risk variants has not been

implemented in the clinics. Despite the potential value of such screening tests, a number of limitations have hindered their use, including their small effect size, their low discriminative ability, a small added value compared with the clinical risk factors, and a lack of models that take into account gene-gene and gene-environment interactions.<sup>12</sup> Thus, the search for new biomarkers is ongoing. There is great interest in epigenetic biomarkers such as DNA methylation, which, unlike the DNA sequence, can be influenced by the environment, and hence may potentially improve T2D prediction.

Recently, an epigenome-wide association study identified 5 DNA methylation loci (*ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1*, and *TXNIP*) in blood that were associated with future T2D. Furthermore, the study showed that a methylation score that combined the results from the 5 methylation loci was associated with

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**Table 1.** Clinical characteristics of study subjects from the Botnia prospective study at baseline and of monozygotic twin pairs discordant for T2D from whom blood samples and skeletal muscle biopsies were obtained.

	Botnia prospective study (Blood)		MZ twins discordant for T2D (Blood)		MZ twins discordant for T2D (Skeletal muscle)	
	Controls	Converters	Non-diabetic	T2D	Non-diabetic	T2D
N (male/female)	129 (62/67)	129 (65/64)	19 (11/8)		17 (10/7)	
Age (years)	51.4 ± 9.1	52.8 ± 12.3	66.5 ± 8.0		66.8 ± 7.3	
BMI (kg/m <sup>2</sup> )	27.6 ± 3.0	28.8 ± 4.3*	29.1 ± 6.1	30.9 ± 6.4*	29.9 ± 6.1	32.1 ± 6.1*
Fasting glucose (mmol/L)	5.1 ± 0.4	5.3 ± 0.6*	6.2 ± 0.6	9.7 ± 3.0*	6.1 ± 0.6	9.9 ± 2.5*
Fasting insulin (pmol/L)	59.7 ± 29.2	89.6 ± 71.5*	67.5 ± 51.7	122.2 ± 147.2	73.3 ± 59.0	136.2 ± 172.3
HbA1c						
percent	5.5 ± 0.5	5.7 ± 0.5*	5.9 ± 0.4	7.4 ± 1.7*	5.8 ± 0.4	7.6 ± 1.7*
mmol/mol	37.0 ± 5.5	39.0 ± 5.5*	41.0 ± 4.4	57.0 ± 18.6*	40.0 ± 4.4	60.0 ± 18.6*
Cholesterol (mmol/L)	6.1 ± 1.1	5.7 ± 1.0*	5.2 ± 0.9	4.7 ± 1.0	5.4 ± 0.9	4.9 ± 1.1
Triglycerides (mmol/L)	1.5 ± 0.8	1.6 ± 0.9	1.2 ± 0.6	1.5 ± 0.7	1.3 ± 0.6	1.8 ± 0.9
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.3 ± 0.3*	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4
LDL cholesterol (mmol/L)	—	—	3.2 ± 0.7	2.8 ± 0.8	3.4 ± 0.7	2.9 ± 0.8
Have family history record	119 (91.5)	109 (88.6)				
Leukocyte (× 10 <sup>9</sup> /L)	6.0 ± 1.5	6.7 ± 1.9*				
Follow up (years)	9.3 ± 3.5	6.9 ± 3.5*				
Normal glucose tolerance	84 (64.6)	50 (38.7)*				

Data are presented as mean ± SD or as number (%).

\**P*-value < 0.05, converters vs. controls and T2D vs. non-diabetic subjects.

Lipid and glucose phenotype data are based on 12 to 18 twin pairs.

LDL data is not available for the selected Botnia subjects at baseline.

future T2D incidence.<sup>8</sup> The aim of this study was to replicate the potential use of the 5 DNA methylation loci in blood DNA recently reported to be associated with T2D, in order to predict future T2D in subjects from the Botnia prospective study. Furthermore, we wanted to test if these loci and genes show altered DNA methylation and gene expression in primary target tissues for T2D from diabetic vs. non-diabetic subjects.

## Results

### Prediction of T2D using DNA methylation loci in blood DNA

For the prediction of T2D, we analyzed DNA methylation of 5 CpG sites: cg06500161 (*ABCG1*), cg02650017 (*PHOSPHO1*), cg18181703 (*SOCS3*), cg11024682 (*SREBF1*), and cg19693031 (*TXNIP*), which have recently been reported to predict T2D,<sup>8</sup> in blood DNA from 258 individuals from the Botnia prospective study who were all non-diabetic at baseline (Table 1). DNA methylation at the *ABCG1* locus cg06500161 was associated with a 9% increased risk for future T2D among people matched for age, gender, and fasting glucose levels (OR = 1.09, 95% CI = 1.02–1.16, *P*-value = 0.007, *Q*-value =

0.018), while DNA methylation at the *PHOSPHO1* locus cg02650017 was associated with a 15% decreased risk for future T2D among people matched for age, gender, and fasting glucose levels (OR = 0.85, 95% CI = 0.75–0.95, *P*-value = 0.006, *Q*-value = 0.018). Furthermore, DNA methylation levels of both loci were still associated with T2D risk after adjusting for leukocyte count: *ABCG1* locus (OR = 1.10, *P*-value = 0.004), and *PHOSPHO1* locus (OR = 0.85, *P*-value = 0.007). None of the other 3 loci (*SOCS3*, *SREBF1*, and *TXNIP*) were significantly associated with the development of T2D (Table 2). A methylation score combining the DNA methylation of the 5 CpG sites could not predict future T2D (*P*-value = 0.22).

### Correlations between DNA methylation in blood DNA and T2D risk factors

Next, for the 258 Botnia subjects who were non-diabetic at baseline, we correlated DNA methylation at the 5 investigated DNA methylation loci with risk factors for T2D, as well as glucose and lipid phenotypes. DNA methylation at the *ABCG1* locus cg06500161 correlated positively with BMI, HbA1c, fasting insulin, and triglyceride levels, while

**Table 2.** Associations between DNA methylation in CpG sites annotated to *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP* and future T2D in subjects from the Botnia prospective study.

CpG site	Gene	OR	95% CI	<i>P</i> -value	<i>Q</i> -value*	DNA methylation (%)			N	Gene region
						Controls	Converters	Delta		
cg06500161	<i>ABCG1</i>	1.09	1.02–1.16	0.007	0.018	65.9 ± 4.2	67.2 ± 5.7	1.3	216	Body
cg02650017	<i>PHOSPHO1</i>	0.85	0.75–0.95	0.006	0.018	2.7 ± 2.2	1.9 ± 2.1	−0.8	244	Body
cg18181703	<i>SOCS3</i>	0.97	0.93–1.02	0.22	0.275	52.4 ± 6.2	51.3 ± 6.0	−1.1	238	Body
cg11024682	<i>SREBF1</i>	0.99	0.95–1.03	0.68	0.680	49.4 ± 6.4	49.7 ± 6.4	0.2	239	Body
cg19693031	<i>TXNIP</i>	0.96	0.91–1.02	0.16	0.267	70.2 ± 5.5	69.6 ± 5.4	−0.6	247	3'UTR

Logistic regression is corrected for age, gender, fasting glucose, and family relation.

\*FDR was calculated for 5 tests.

Methylation data is presented as mean ± SD. OR, odds ratio; CI, confidence interval. UTR, untranslated region.

**Table 3.** Correlations between DNA methylation and glucose and lipid phenotypes with  $P$ -value < 0.05 in blood from subjects from the Botnia prospective study.

CpG site	Gene	Pearson corr.	$P$ -value	Q-value*	N	Phenotype
cg06500161	<i>ABCG1</i>	0.21	0.001	0.008	224	BMI (kg/m <sup>2</sup> )
cg06500161	<i>ABCG1</i>	0.14	0.044	0.155	205	HbA1c (%)
cg06500161	<i>ABCG1</i>	0.23	0.001	0.006	218	Fasting Insulin (pmol/l)
cg06500161	<i>ABCG1</i>	0.22	0.001	0.006	209	Triglycerides (mmol/l)
cg02650017	<i>PHOSPHO1</i>	0.19	0.004	0.029	235	HDL (mmol/l)
cg18181703	<i>SOCS3</i>	-0.18	0.005	0.050	244	Age (years)
cg18181703	<i>SOCS3</i>	-0.13	0.041	0.125	245	BMI (kg/m <sup>2</sup> )
cg11024682	<i>SREBF1</i>	0.24	0.0002	0.006	246	Age (years)
cg11024682	<i>SREBF1</i>	0.15	0.022	0.099	247	BMI (kg/m <sup>2</sup> )
cg11024682	<i>SREBF1</i>	0.15	0.022	0.099	247	Fasting glucose (mmol/l)
cg11024682	<i>SREBF1</i>	0.13	0.050	0.155	228	HbA1c (%)
cg19693031	<i>TXNIP</i>	-0.13	0.039	0.155	239	Triglycerides (mmol/l)

\*FDR was calculated for 40 tests.

DNA methylation at the *PHOSPHO1* locus cg02650017 correlated positively with HDL levels (Table 3). Also, methylation at the *SOCS3*, *SREBF1*, and *TXNIP* loci correlated with risk factors for T2D (Table 3).

### Epigenetic dysregulation of DNA methylation in target tissues for T2D

To test if DNA methylation of *ABCG1*, *PHOSPHO1*, *SREBF1*, *SOCS3*, and *TXNIP* is dysregulated in primary target tissues for T2D, we studied DNA methylation of all CpG sites annotated to these 5 genes on the Infinium 450K array in T2D case-control studies from human pancreatic islets and liver, and from adipose tissue, skeletal muscle and blood obtained from monozygotic twins discordant for T2D.<sup>13-17</sup> Based on  $P < 0.05$ , we found that DNA methylation of cg06500161 (*ABCG1*) was increased in adipose tissue and blood from subjects with T2D, DNA methylation of cg02650017 (*PHOSPHO1*) was decreased in T2D skeletal muscle, DNA methylation of cg11024682 (*SREBF1*) was increased in T2D pancreatic islets, and DNA methylation of cg19693031 (*TXNIP*) was decreased in pancreatic islets, skeletal muscle, and blood from subjects with T2D (Table 4). In addition, a number of surrounding CpG sites in these genes was also differentially methylated in human pancreatic islets, liver, adipose tissue, skeletal muscle, and blood from diabetic vs. non-diabetic subjects (Supplementary Table 2).

**Table 4.** Differential DNA methylation of the 5 CpG sites annotated to *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP* in T2D compared with non-diabetic subjects in pancreatic islets, adipose tissue, skeletal muscle, and blood.

CpG	Gene	DNA methylation (%)			$P$ -value	Q-value*	Tissue	Ref.
		Non-diabetic	T2D	Delta				
cg06500161	<i>ABCG1</i>	56.7 ± 2.3	57.9 ± 2.8	1.2	0.032	0.135	Blood	
cg06500161	<i>ABCG1</i>	43.9 ± 5.3	47.7 ± 5.2	3.8	0.025	0.123	Adipose tissue	Nilsson et al.
cg02650017	<i>PHOSPHO1</i>	26.3 ± 5.7	24.2 ± 5.4	-2.1	0.020	0.123	Skeletal muscle	
cg11024682	<i>SREBF1</i>	52.0 ± 5.8	59.5 ± 8.4	7.5	0.045	0.160	Pancreatic islets	Dayeh et al.
cg19693031	<i>TXNIP</i>	63.4 ± 4.3	59.4 ± 6.6	-4.0	0.020	0.123	Skeletal muscle	
cg19693031	<i>TXNIP</i>	67.6 ± 4.5	64.7 ± 6.5	-3.0	0.023	0.123	Blood	
cg19693031	<i>TXNIP</i>	56.7 ± 10.4	44.8 ± 10.4	-11.9	0.0006	0.016	Pancreatic islets	Dayeh et al.

\*FDR was calculated for 25 tests.

### Correlations between DNA methylation and gene expression in human tissues

We also wanted to investigate if the association between DNA methylation and metabolic phenotypes/T2D potentially could be mediated via altered expression of the nearest gene. We correlated DNA methylation at the 5 studied CpG sites in pancreatic islets, liver, adipose tissue, and skeletal muscle with gene expression from the same tissue and subjects, using available microarray data.<sup>13-17</sup> We found that DNA methylation of cg18181703 correlated positively with *SOCS3* expression in human pancreatic islets (Spearman's rho = 0.19,  $P$ -value = 0.033, Q-value = 0.16,  $n = 34$ ), and negatively with *SOCS3* expression in adipose tissue (Regression coefficient = -0.028,  $P$ -value = 0.002, Q-value = 0.011,  $n = 25$ ). Additionally, expression of *ABCG1* and *TXNIP* was altered in muscle from subjects with T2D, while expression of *SREBF1* was altered in both diabetic muscle and liver (Supplementary Table 3). It should be noted that expression data from blood was not available from subjects in the Botnia prospective study or the T2D discordant twins.

### Correlation between DNA methylation in blood with methylation in adipose tissue and skeletal muscle from the same subject

Finally, we investigated whether DNA methylation in blood could be used as a surrogate marker for DNA methylation in target tissues for T2D. DNA methylation at the 5 studied loci

in blood was correlated with DNA methylation of the corresponding CpG site in adipose tissue and skeletal muscle obtained from the same subject. We found that DNA methylation of cg18181703 (*SOCS3*) and cg11024682 (*SREBF1*) in blood correlated with the degree of methylation in adipose tissue (Regression coefficient = 0.31 and 0.40, *P*-value = 0.010 and 0.052, *n* = 28).

## Discussion

In this study, we replicated the recently reported association between DNA methylation at cg06500161 (*ABCG1*) and future T2D in subjects from the Botnia prospective study. Furthermore, DNA methylation at cg06500161 in blood correlated positively with BMI, HbA1c, fasting insulin, and triglyceride levels, and it was increased in adipose tissue and blood from the diabetic twin among monozygotic twin pairs discordant for T2D.

*ABCG1* encodes a member of the ATP-binding cassette (ABC) protein family, which plays a role in the homeostasis of glucose and lipids. These proteins do so by removing excess cholesterol from peripheral tissues and transporting it to the liver.<sup>18</sup> The HDL-mediated increase in insulin secretion is dependent on *ABCG1*. Loss of both *ABCA1* and *ABCG1* results in sterol accumulation, impaired glucose-stimulated insulin secretion, and inflammation of pancreatic  $\beta$ -cells.<sup>19</sup>

Recently, DNA methylation at cg06500161 (*ABCG1*) in blood was associated negatively with HDL-C levels and positively with triglyceride levels and myocardial infarction. In addition, methylation of 2 CpG sites in *ABCG1* in adipose tissue was also positively associated with triglyceride levels.<sup>7</sup> Another study showed that DNA methylation of cg06500161 in CD4<sup>+</sup> T cells was associated positively with fasting insulin levels and HOMA-IR.<sup>20</sup> Importantly, our *ABCG1* methylation data are in line with these studies.

DNA methylation at cg06500161 in blood has been shown to correlate negatively with *ABCG1* expression in blood.<sup>7,8</sup> These authors showed that the strength of the association varied for the 6 different *ABCG1* transcripts. Furthermore, an electrophoretic mobility shift assay (EMSA) showed that methylated cg06500161 had a lower binding affinity to an unknown protein complex compared with unmethylated cg06500161.<sup>7</sup> A recent study showed that the relationship between BMI and expression of *ABCG1* in monocytes is partially mediated through DNA methylation.<sup>21</sup> Interestingly, *ABCG1* has shown allelic imbalance of expression in human pancreatic islets, and in lymphoblastoid cell lines, suggesting that altered allelic expression of *ABCG1* may result from both genetic and epigenetic mechanisms.<sup>22,23</sup> Accordingly, we found that *ABCG1* expression was reduced in skeletal muscle from subjects with T2D. Taken together, these findings point toward the importance of taking into consideration the different *ABCG1* transcripts that might be expressed in different tissues and genetic variation.

Genetic variation in coding SNPs in *ABCG1* has not previously been associated with increased risk of T2D.<sup>24</sup> However, a recent study integrated regional *ABCG1* SNP data with methylation of cg06500161 and identified a number of methylation

quantitative trait loci (cis-meQTLs), some of which were associated with insulin and HOMA-IR.<sup>20</sup> It will be interesting to examine whether combining genetic, clinical, and epigenetic information provides us with better prediction models for diabetes.

*PHOSPHO1* encodes a phosphatase that is highly expressed in skeletal muscle and plays a role in skeletal mineralization. Under certain circumstances, it may also cause vascular mineralization.<sup>25-27</sup> Cardiovascular calcification is a common consequence of aging, diabetes, and hypercholesterolemia.<sup>28</sup> *PHOSPHO1*, is considered to be an attractive target for cardiovascular therapy.<sup>26</sup> Interestingly, we found that DNA methylation at the *PHOSPHO1* locus cg02650017 in blood correlated positively with HDL levels. Furthermore, DNA methylation at the *PHOSPHO1* locus cg02650017 was decreased in skeletal muscle from diabetic vs. non-diabetic monozygotic twins, while its expression was not altered.

Epigenetic alterations have been reported for numerous genes in pancreatic islets, liver, adipose tissue, and skeletal muscle from subjects with T2D compared with non-diabetic controls.<sup>13-15,17</sup> However, the potential use of T2D-associated DNA methylation patterns in target tissues for disease prediction is hindered by the inaccessibility of these tissues, and will only become feasible if we could find biomarkers in easily accessible tissues that reflect the changes in internal and inaccessible tissues. In this study, we found that DNA methylation of cg06500161 (*ABCG1*) was increased in adipose tissue and blood from the same individual and that DNA methylation of cg19693031 (*TXNIP*) was decreased in pancreatic islets, skeletal muscle, and blood from subjects with T2D compared to controls. Furthermore, DNA methylation at cg18181703 (*SOCS3*) and cg11024682 (*SREBF1*) in blood correlated with DNA methylation levels in adipose tissue from the same individual. Recently, we have shown that age-associated DNA methylation changes in blood mirror DNA methylation changes in human adipose tissue from elderly compared with young subjects.<sup>29</sup> We found that methylation of a number of CpG sites in and surrounding the 5 investigated loci was altered in pancreatic islets, adipose tissue, skeletal muscle, and blood from subjects with T2D compared with non-diabetic controls. These epigenetic dysregulations at the same loci, but in different tissues from the same individual, may be mediated by so-called ‘metastable epialleles’; that is, “alleles that are variably expressed in genetically identical individuals due to epigenetic modifications that are established during early development”.<sup>30</sup> Despite the stochastic nature of these epigenetic modifications, they might be inherited transgenerationally, altered by the environment, and can affect all tissues of the body.<sup>30</sup>

The use of DNA methylation biomarkers in the clinic is still limited, and has been hindered by insufficient sensitivity and specificity. However, there are a number of advantages of using DNA methylation as a biomarker. For example, DNA methylation is quite stable over time, since it is covalently attached to the DNA. In addition, the degree of DNA methylation is independent of the amount of starting material, since most methods used for DNA methylation analysis determine the ratio of methylated to unmethylated CpG sites.<sup>31</sup>



Our inability to replicate 3 of the associations reported by Chambers et al.<sup>8</sup> could be explained by our modest sample size and differences in study design. Performing a power calculation revealed that we would need a larger sample size to be able to detect the effect size reported by Chambers et al. In addition, a larger sample size would be needed to compare the specificity and sensitivity of the identified DNA methylation biomarkers in blood in comparison to clinical and genetic screening.

## Conclusion

In this study, we have shown that DNA methylation at 2 CpG sites in *ABCG1* and *PHOSPHO1* are associated with future T2D risk in the Botnia prospective cohort. Furthermore, in healthy subjects, DNA methylation at both loci in blood DNA was associated with a number of T2D risk factors, such as BMI, HbA1c, fasting insulin levels, triglycerides, and HDL levels. Interestingly, we found that the altered DNA methylation observed in blood was also found in human target tissues for T2D: pancreatic islets, liver, adipose tissue, and skeletal muscle. Our data suggest that DNA methylation biomarkers in blood might be used as surrogate markers for DNA methylation in inaccessible target tissues. More importantly, the occurrence of altered DNA methylation in more than one human tissue at the same locus could be mediated by so-called ‘metastable epialleles’. The search for T2D-associated DNA methylation biomarkers is still in its infancy. The few studies conducted to date have screened less than 2 percent of CpG sites in the human genome and it is possible that many more DNA methylation biomarkers will be identified in the coming years. The hope is that they perform better than existing biomarkers.

## Materials and methods

### Study subjects

#### Botnia prospective study

For the prediction of T2D, we selected samples from the Botnia prospective study. This family-based study includes 2,770 participants from the Botnia region in Finland, who were all non-diabetic at baseline. Participants were followed prospectively, using repeated oral glucose tolerance tests (OGTTs) to detect progression toward T2D. The cohort has been previously described.<sup>32</sup> DNA was extracted from whole blood taken at baseline. In this study, we used DNA from 129 participants who developed T2D (converters) over a mean follow-up time of  $8.1 \pm 3.7$  years, and 129 participants who did not develop T2D (controls) over the same follow-up time. The controls were matched with converters for age and gender at baseline. Clinical characteristics of study subjects are shown in Table 1. At baseline, the converters had significantly higher BMI, fasting glucose levels, fasting insulin levels, HbA1c levels, leukocyte counts, and lower cholesterol and HDL levels compared with controls.

#### Monozygotic twin pairs discordant for T2D

Adipose tissue (14 pairs), skeletal muscle (17 pairs), and blood cells (19 pairs) from monozygotic twins discordant for T2D

were used in this study. Adipose tissue, skeletal muscle, and blood from the same individual were available for 9 of the twin pairs. Clinical characteristics for the 14 twin pairs from whom adipose tissue was obtained have been previously described,<sup>13</sup> and skeletal muscle from some of the twins have been investigated in several studies.<sup>15,16,33</sup> Clinical characteristics of the twin pairs from whom skeletal muscle and blood were obtained are shown in Table 1. Diabetic twins had significantly higher BMI, fasting glucose levels, and HbA1c levels compared with non-diabetic twins.

#### Human pancreatic islets

In this study, we used human pancreatic islets from 15 T2D and 34 non-diabetic donors. The clinical characteristics for these donors have been previously described.<sup>14</sup> T2D donors had higher HbA1c levels and lower levels of glucose-stimulated insulin secretion.

#### Human liver

In this study, we used human liver DNA methylation data from participants of the Kuopio Obesity Surgery Study (KOBS)—35 with T2D and 60 without. The clinical characteristics for these subjects have been previously described.<sup>17</sup> As expected, subjects with T2D had significantly higher fasting plasma glucose levels and fasting insulin levels than non-diabetic subjects.

#### DNA methylation analysis

Pyrosequencing was used to analyze DNA methylation of 5 different CpG sites in *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1*, and *TXNIP* in blood DNA from the Botnia subjects as previously described.<sup>14</sup> Primer sequences used for pyrosequencing are included in Supplementary Table 1. Genome-wide DNA methylation analysis of human pancreatic islets, liver and from adipose tissue, skeletal muscle and blood from monozygotic twins discordant for T2D was performed using the Infinium Human-Methylation450 BeadChips (Illumina), according to the manufacturer’s instructions. The genome-wide DNA methylation data from human pancreatic islets, liver, adipose tissue, and skeletal muscle from some of the subjects has been previously published.<sup>13,14,16,17</sup>

#### Gene expression analysis

mRNA expression data of the human pancreatic islets, adipose tissue and skeletal muscle were analyzed using the GeneChip Human Gene 1.0 ST array from Affymetrix (Santa Clara, CA, USA) according to the manufacturer’s recommendations. mRNA expression from liver from a subset of subjects (19 T2D and 23 non-diabetic subjects) was analyzed using the HumanHT-12 Expression BeadChip from Illumina according to the manufacturer’s recommendations. mRNA expression data from pancreatic islets, liver, and adipose tissue have previously been published.<sup>13,14,17</sup>

#### Statistics

To test if DNA methylation of the 5 analyzed CpG sites in *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1*, and *TXNIP* was

associated with future T2D in subjects from the Botnia prospective study we performed logistic regression analysis for each CpG site using a robust variance estimator in order to take care of possible dependency within families using STATA (StataCorp). Covariates included in the logistic regression model were age, sex, and fasting glucose at baseline. For each participant, standardized values of each “methylation level” (expressed in SD units from the mean) were summed to produce the methylation score; methylation score = ZTXNIP + ZSREBF1 + ZSOCS3 + ZPHOSPHO1 + ZABCG1, where  $ZTXNIP = (TXNIP - \text{mean}(TXNIP)) / SD(TXNIP)$  and so on. For the Botnia cohort, we used Pearson correlations to determine whether DNA methylation at the 5 CpG sites in blood was correlated to T2D risk factors, and glucose and lipid phenotypes. For discordant twins, we used regression analysis to correlate expression and methylation, taking twin pair relationship into account. We also used regression analysis to correlate DNA methylation between tissues from the same individual. We used a paired Wilcoxon test to compare DNA methylation and gene expression between monozygotic twins discordant for T2D. We used a false discovery rate (FDR) analysis to correct for multiple testing.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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