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Research Article

Candidate gene association study of type 2 diabetes in a nested case-control study of the EPIC-Potsdam cohort – Role of fat assimilation

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To search for common variants etiological for type 2 diabetes, we screened 15 genes involved in fat assimilation for sequence variants. Approximately 55 kb in promoter and coding regions, and intron/splice sites were sequenced by cycle sequencing. In the set of 15 genes, 71 single nucleotide polymorphisms (SNPs) were detected. 33 SNPs were presumed to be functionally significant and were genotyped in 192 incident type 2 diabetes subjects and 384 matched controls from the European Prospective Investigation into Cancer and Nutrition-Potsdam cohort. A total of 27 SNPs out of 15 genes showed no statistical association with type 2 diabetes in our study. Six SNPs demonstrated nominal association with type 2 diabetes, with the most significant marker (*FABP6* Thr79Met) having an adjusted odds ratio of 0.45 (95% CI 0.22 – 0.92) in homozygous Met allele carriers. Evidence for an association with disease status was also found for a novel Arg109Cys (g.2129C > T) variant of colipase, 5′UTR (rs2084202) and Met71Val (rs8192506) variants of diazepam-binding inhibitor, Arg298His (rs13283456) of *PTGES2*, and a novel promoter variant (g.-1324G > A) of *SLC27A5*. The results presented here provide preliminary evidence for the association of common variants in genes involved in fat assimilation with the genetic susceptibility of type 2 diabetes. However, they definitely need further verification.

Keywords: Candidate genes / Fatty acid metabolism / Nested case-control study / Type 2 diabetes

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1 Introduction

The genetic dissection of complex disorders has become one of the greatest challenges facing human geneticists. Even though genome-wide scans have become technically and economically more feasible [1, 2], candidate gene association studies still remain a favourable study design for the identification of common genetic variants involved in common diseases [3, 4].

We have conducted a *de novo* search for sequence variants using cycle-sequencing method in genes involved in fat assimilation such as fat sensing, and digestion, and fatty acid absorption, binding, and metabolism to identify novel variants that might be related to type 2 diabetes.

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Abbreviations: CLPS, colipase; **DBI,** diazepam-binding inhibitor; **EPIC,** European Prospective Investigation into Cancer and Nutrition; **FABP,** fatty acid-binding protein, **GIP,** glucose-dependent insulinotropic polypeptide; **MAF,** minor allele frequency; **OR,** odds ratio; **PNLIP,** pancreatic triacylglycerol lipase; **PTGES,** prostaglandin E synthase; **SNP,** single nucleotide polymorphism; **SLC27,** solute carrier family 27 (fatty acid transporter); **UTR,** untranslated region

Overall, 15 candidate genes from 7 gene families were screened.

The glucose-dependent gastric inhibitory polypeptide gene (*GIP*) encodes an incretin hormone and belongs to the glucagon superfamily [5]. The encoded protein is important for the maintenance of glucose homeostasis as it is a potent stimulator of insulin secretion following food ingestion and nutrient absorption [6]. GIP exerts its effects by binding to its specific receptor, the GIP receptor (GIPR), which is expressed in various tissues including pancreatic islets, adipose tissue and brain [7]. The pancreatic colipase (CLPS) is an essential protein for efficient dietary lipid hydrolysis, since the pancreatic triglyceride lipase (PNLIP) requires CLPS for explicating activity. Fatty acid transporters (solute carrier family 27, SLC27As) are polytopic integral transmembrane proteins that enhance the uptake of long chain and very long chain fatty acids into cells [8]. Members of the fatty acid-binding protein (FABP) family have been implicated in playing a role in fatty acid uptake into cells, storage, and export, as well as cholesterol and phospholipid metabolism. New insights into their functional role as inflammatory signalling and transcriptionally active proteins have arisen from FABP-deficient mouse models [9]. Diazepam-binding inhibitor (DBI) is also known as acyl-CoA-binding protein (ACBP) and is involved in several biological pathways. In intestinal and pancreatic cells, the enzyme is involved in fatty acid oxidation and lysophosphatidic acid synthesis in the mitochondria as well as the synthesis of phospholipids and cholesteryl ester in the ER. It has been described as a regulator of insulin release from pancreatic cells, a potent cholecystokinin-releasing peptide in the intestine and as a mediator in corticotropin-dependent adrenal steroidogenesis [10]. Genes encoding prostaglandin E synthases (*PTGES*s) were also included in the study because of their role in the synthesis of the antilipolytic compound prostaglandin E_2 [11].

Here, we report our findings of the sequencing effort comprising all exons, intron-splice junctions and 5′flanking sites of these genes and, furthermore, give an overview of the association analyses of 33 single nucleotide polymorphisms (SNPs) selected by their putative functional role and reasonable minor allele frequency (MAF) regarding risk of type 2 diabetes in a nested case-control study of 192 incident type 2 diabetes subjects and 384 controls of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort. Some of the latter results have already been published elsewhere [12].

2 Materials and methods

2.1 Subjects

The EPIC-Potsdam is a longitudinal study embedded in the multicenter EPIC-study comprising 520 000 study participants. For EPIC-Potsdam, 27 548 participants aged 35 – 65 years at study entry were recruited from the general population of the Potsdam area. The baseline examinations and blood collections took place between 1994 and 1998 [13]. During the first follow-up period, 2 – 3 years after recruitment, 192 incident cases of type 2 diabetes were medically proven by confirmation of the primary care physician and additionally confirmed by GAD65 and IA-2 antibody measurements. Cases were matched with two control subjects each by age and sex ($n = 384$). Gender distribution was 59% male and 41% female subjects with a mean (±SD) age of 55.5 (6.8) years. BMI, HbA1c, HDL-C, CRP, and adiponectin were 30.8 (±4.8) and 26.7 (±3.6) kg/m^2 ($p < 0.0001$), 6.4 (±2.2) and 4.7 (±0.7)% ($p < 0.0001$), 0.90 (±0.2) and 1.06 (±0.3) mmol/L ($p < 0.0001$), 4.1 (±5.1) and 2.5 (±4.4) µg/mL ($p < 0.0001$), 6.0 (±3.5) and 8.0 (±4.6) µg/mL ($p < 0.0001$) in cases and controls, respectively. Further details of the study group have been published elsewhere [14, 15].

2.2 Sequencing and SNP-genotyping

Sequences were determined on the basis of GenBank reference sequence accession numbers: *CLPS* (NT_007592), *PNLIP* (NT_030059), *GIP* (NT_010783), *GIPR* (NT_011109), *FABP1* (NT_022184), *FABP2* (NT_016354), *FABP3* (NT_032977), *FABP4* (NT_008183), *FABP6* (NT_023133), *SLC27A1* (NT_011295), *SLC27A2* (NT_010194), (NT_008470), *SLC27A5* (NT_011109), *SLC27A4 and PTGES1* and *PTGES2* (NT_008470). The promoter (within 1 kb upstream), splice-junctions and exons including untranslated regions (UTRs) of all candidate genes were sequenced in 47 genomes from unrelated male Caucasian individuals taken from the Metabolic Intervention Cohort Kiel (MICK) [12] using cycle-sequencing method and high-throughput 96-CE analyses (ABI PRISM 3700 DNA Analyser). Sequences of primers are available on request. SNP nomenclature followed the international nomenclature system described by [16]. Polymorphisms with a putative functional relevance and MAF greater than or equal to 2% were selected for further testing. Additionally, some SNPs newly annotated during study process were selected from public databases. Genotyping of selected polymorphisms was performed using ABI TaqMan primers and technology (ABI Prism 7900 HT) as described before [17]. Genotyping success rate was 99.1 (±0.6)%. Genotyping error was ≤0.5% in 576 replicates and a similar result was obtained by resequencing of three SNPs.

2.3 Statistical analysis

Power calculations were performed with the program of Jim Gauderman and John Morrison (QUANTO, version 1.0, 2005) (available at http://hydra.usc.edu/gxe). Assuming a disease prevalence of 0.1, and an allele frequency of 0.2,

Table 1. Power calculations of the matched case-control study

Allele frequency	OR		
	Recessive IM	Dominant IM	Additive IM
0.05	>10.0 (<0.1)	2.3 (0.3)	2.2 (0.3)
0.1	6.5 (<0.1)	2.0 (0.4)	1.9 (0.4)
0.2	3.2 (0.1)	1.8 (0.5)	1.6 (0.55)
0.3	2.4 (0.3)	1.8 (0.55)	1.6 (0.6)
0.4	2.0 (0.4)	1.9 (0.55)	1.5 (0.65)

Maximal relative risk estimates in a case-control study of 192 cases and matched controls with an estimated 80% power assuming a disease prevalence of 0.1, and a type 1 error rate of 0.05 for different allele frequencies concerning different inheritance models (IMs) using QUANTO version 1.0 (available at http://hydra.usc.edu/gxe).

the nested case-control sample afforded an estimated 80% power at $p < 0.05$ for a genotype relative risk of 1.6 (0.55) in additive (codominant), 1.8 (0.5) in dominant and 3.2 (0.1) in recessive genetic models calculated (Table 1). Minor allele frequencies were calculated for each SNP and agreement with Hardy-Weinberg equilibrium (HWE) was tested using a χ^2 'goodness-of-fit' test in controls and type 2 diabetes subjects separately. If HWE was violated in controls, the respected variant was removed from further analysis. Comparisons of genotype frequencies in cases and controls were performed using χ^2 test. Comparison of allele frequencies were performed with Cochran-Armitage's trend test, which tests for additive allele effects on disease penetrance. Corrected *p*-values were obtained by applying Sidak's multiple-testing adjustment to the smoothed *p*-values [18]. Association between type 2 diabetes and each SNP under dominant, codominant and recessive models was determined by conditional logistic regression analysis. Multivariate regression analyses implicated several polymorphic sites of genes that were significantly associated with type 2 diabetes. We tested interactions between pairwise genotype combinations of these SNPs with a crossproduct term in the main effects model. Analyses were adjusted for BMI (kg/m^2), waist-hip-ratio, sports activities (h/week), total energy (kJ/d), alcohol intake (g/d) and other noncontinuous covariates (*i. e*. use of drugs, and comorbidities). A *p*-value of $0.1 \geq p \geq 0.05$ was assessed to indicate borderline significance and a *p*-value of <0.05 significance. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, North Carolina, USA).

3 Results

Fifteen candidate genes were screened for sequence variants in 94 homologous chromosomes from male subjects. In Table 2, genes are listed according to their functional involvement in fat sensing, and digestion, fatty acid transport and absorption and fat metabolism. The total genomic DNA length screened was 15.7 kb in promoter regions, 15.9 kb in intron/splice-site regions, 0.6 kb in 5′-/3′-UTRs and 22.3 kb in coding regions. The number of identified SNPs was 71 (Table 2) corresponding to an average SNP density of one SNP *per* 770 basepairs. Of these, 62% were located in noncoding regions and 38% in coding regions. Overall 33 SNPs were selected for genotyping based on their putative function (splice-site mutation, nonsynonymous coding), localisation (putative 5′promoter region) and minor allele frequencies. For the *PNLIP* gene, we found no SNP with any functional implication.

Polymorphisms were genotyped in a nested case-control study of 192 incident type 2 diabetes patients and twice the number of sex- and age-matched controls. Minor allele frequencies and χ^2 statistics of genotype comparisons are shown in Table 2. Eleven SNPs (33%) had a MAF below 5%, 3 (9%) had a MAF between 5 and 10%; and 19 (58%) had a MAF greater than or equal to 10%. Three SNPs (*FABP*3 g.-152 G > T, *DBI* rs3731607, *DBI* rs2289948) showed departure from Hardy–Weinberg equilibrium ($p < 0.05$) in controls and were discarded from further analysis. As shown in Table 2 borderline significant ($p \leq 0.1$) and significant ($p < 0.05$) results of trend statistics were obtained for *CLPS* Ala109Cys ($p = 0.1$), *PTGES2* Arg298His ($p = 0.08$), *SLC27A5* rs4801275 ($p = 0.08$), *SLC27A5* g.-1324G > A ($p = 0.05$), *DBI* rs2084202 ($p = 0.02$), *DBI* rs8192506 ($p = 0.03$) and *FABP6* Thr79Met ($p = 0.01$). Multiple-testing adjustment of *p*-values by Sidak's method removed significance.

In the next stage, we assessed disease associations employing conditional logistic regression analysis for each SNP. First, we calculated crude odds ratios (Ors), then we adjusted the risk estimates for BMI (continuous variable) and, finally, we applied a multiple adjusted model. Six SNPs in five different genes showed significant associations with disease status ($p < 0.05$) under at least one model (Table 3). The newly detected variant of the *CLPS* gene (Ala109Cys) was significantly more frequent in cases than in controls and the heterozygous genotype was associated with increased disease risk (adjusted OR = 5.83, 95% CI 1.26–27.0). No homozygous carrier of the 109Cys variant was present in our study group. Evidence for an association with disease status was found for a frequent g.-132A > G polymorphism (crude OR 0.63, 95% CI 0.41–0.96) and a rare nonsynonymous coding (Met71Val) SNP of *DBI* (crude OR 1.89, 95% CI 1.03–3.47). Adjustment of covariates removed the significant association of the heterozygous Met71Val genotype, but did not change the association of the g.-132A > G SNP of *DBI*. The rare allele of the *PTGES2* Arg298His polymorphism showed borderline significantly decreased disease risk if analysis was adjusted for BMI (OR 0.64, 95% CI 0.41–1.01) and a *SLC27A5* promotor (g.-1324G > A) polymorphism was associated with type 2 diabetes only in the multivariable-adjusted model (OR

Table 2. Number of SNPs identified by direct sequencing and SNPs genotyped in 192 incident type 2 diabetes subjects and 384 controls

Functional role	No. of identified SNPs	Gene symbol	Gene ID	NCBI dbSNP ID	Localisation	Base change	AA change	MAF	χ^2 *p*	Trend test *p*	p_{correc}*
Fat sensing	20	*GIP*	2695	rs2291726	Intron 2	C > T		0.470	0.27	0.96	1.0
				rs2291725	Exon 3	C > T	Ser103Gly	0.492	0.13	0.71	1.0
		GIPR	2696	rs1800437	Exon 12	G > C	Glu354Gln	0.220	0.45	0.45	1.0
Fat digestion	11	*PNLIP*	5406	–	–	–	–	–	–	–	–
		CLPS	1208	g.2129C > T[a)]	Exon 3	C > T	Ala109Cys	0.013	0.10	**0.10**	1.0
Fat absorption	16	*SLC27A1*	376497	rs15401	3′UTR	G > A		0.082	0.63	0.38	1.0
				rs2278280	3′UTR	A > G		0.396	0.75	0.49	1.0
				rs3746318	Intron 8, −43	C > T		0.377	0.77	0.57	1.0
		SLC27A2	11001	g.53741T > A[b)]	3′UTR, +72	T > A		0.009	0.38	0.38	1.0
				g.276C > T[b)]	Exon 1	C > T	Ala92	0.125	0.77	0.80	1.0
				rs17415911	Intron 3, −142	A > G		0.169	0.36	0.97	1.0
		SLC27A4	10999	rs2240953	Exon 3	G > A	Gly209Ser	0.047	0.56	0.99	0.9
		SLC27A5	10998	rs4801275	Intron 3, −165	G > A		0.033	0.08	**0.08**	0.9
				g.-1324G > A[c)]	Promotor, −1324	G > A		0.162	0.15	**0.05**	0.9
Fat metabolism	24	*FABP1*	2168	rs2241883	Exon 3	A > G	Thr94Ala	0.364	0.59	0.34	0.9
		FABP2	2169	rs1799883	Exon 2	G > A	Ala54Thr	0.303	0.65	0.62	0.9
				rs2282688	Promotor, −471	G > A		0.442	0.64	0.39	1.0
				rs6857641	Promotor, −260	G > A		0.443	0.61	0.37	1.0
				rs10034579	Promotor, −778	G > T		0.445	0.54	0.32	1.0
		FABP3	2170	rs11588069	Exon 2	A > G	Lys53Arg	0.040	0.58	0.91	1.0
				g.-373G > A[d)]	Promotor, −373	G > A		0.108	0.72	0.47	1.0
				g.-152G > T[d)]	Promotor, −152	G > T		0.017	nd	nd	nd
		FABP4	2167	g.-133T > C[e)]	Promotor, −133	T > C		0.024	0.40	0.40	0.6
		FABP6	2172	rs2277954	Intron 3	T > C		0.092	0.77	0.65	0.6
				rs1130435	Exon 2	C > T	Thr79Met	0.413	0.02	**0.01**	0.6
		DBI	1622	rs2084202	5′UTR, −132	A > G		0.157	0.07	**0.02**	0.6
				rs3731607	Intron/promotor	C > T		0.199	nd	nd	nd
				rs8192501	Intron/5′UTR	G > A		0.026	0.68	0.68	0.9
				rs8192504	Exon 2	A > G	Asn56Asp	0.004	0.20	0.20	0.9
				rs8192506	Exon 4	A > G	Met71Val	0.039	0.03	**0.03**	0.9
				rs2289948	3′flanking	G > C		0.198	nd	nd	0.9
		PTGES1	9536	rs11792431	Exon 1	A > G	Ala15	0.058	0.11	0.30	0.9
		PTGES2	80142	rs13283456	Exon 6	C > T	Arg298His	0.180	0.21	**0.08**	1.0
				g.-417G > T[f)]	Promotor, c.-417	G > T		0.027	0.40	0.85	0.9

AA: amino acid; MAF: minor allele frequency; nd: not determined.
a) refseq: NT_007592.
b) refseq: NT_010194.
c) refseq: NT_011109.
d) refseq: NT_032977.
e) refseq: NT_008183.
f) refseq: NT_008470.
* Sidak's method applied on smoothed *p*-trend values.

0.54, 95% CI 0.33–0.9). The strongest statistical evidence for disease association was for a nonsynonymous coding (Thr79Met) SNP in the *FABP6* gene. 21% of the study subjects were carriers of the homozygous mutant allele. The Met allele of the Thr79Met substitution showed decreased risk of type 2 diabetes in the codominant and dominant inheritance models in crude and adjusted logistic regression analyses. Homozygosity for the rare allele was associated with disease with OR = 0.5 ($p = 0.01$) in the codominant model calculated. The ORs remained significant if other covariates were included in the model (OR 0.45, 95% CI 0.22–0.92). In our last model, we calculated the ORs of those SNPs primarily associated with type 2 diabetes simultaneously and adjusted for other confounding variables (Table 4). Pairwise interaction between each polymorphism defined as binary variables was also tested, but none of the interaction terms turned out to be significant. Therefore, interaction terms were removed from the statistical model.

Table 3. Gene variants significantly associated with type 2 diabetes

Gene	SNP	IM tested	No. of cases/controls	OR (95% CI)					
				Crude	*p*	Model 1	*p*	Model 2	*p*
CLPS	g.2129C > T (Ala109Cys)	CC *vs.* CT	184/377 *vs.* 8/7	2.29 (0.83–6.30)	0.11	**3.75 (1.13–12.49)**	**0.03**	**5.83 (1.26–27.0)**	**0.02**
SLC27A5	g.-1324G>A (promoter)	GG *vs.* GA	140/251 *vs.* 46/114	0.75 (0.51–1.10)	0.14	0.71 (0.45–1.11)	0.13	**0.54 (0.32–0.90)**	**0.02**
		GG *vs.* AA	140/251 *vs.* 2/9	0.39 (0.08–1.92)	0.25	0.64 (0.13–3.16)	0.58	0.59 (0.10–3.59)	0.57
		GG *vs.* GA+AA	140/251 *vs.* 48/123	0.72 (0.50–1.06)	0.09	0.70 (0.45–1.09)	0.12	**0.54 (0.33–0.90)**	**0.02**
		GG + GA *vs.* AA	186/365 *vs.* 2/9	0.42 (0.09–2.03)	0.28	0.70 (0.14–3.45)	0.66	0.65 (0.11–3.91)	0.63
FABP6	rs1130435 (Thr79Met)	CC *vs.* CT	80/118 *vs.* 86/189	**0.66 (0.44–0.99)**	**0.04**	0.69 (0.43–1.09)	0.11	**0.53 (0.31–0.91)**	**0.02**
		CC *vs.* TT	80/118 *vs.* 25/73	**0.50 (0.29–0.86)**	**0.01**	**0.47 (0.25–0.88)**	**0.02**	**0.45 (0.22–0.92)**	**0.03**
		CC *vs.* CT + TT	80/118 vs111/262	**0.61 (0.42–0.89)**	**0.01**	**0.62 (0.40–0.94)**	**0.03**	**0.50 (0.31–0.83)**	**0.01**
		CC + CT *vs.* TT	166/307 *vs.* 25/73	0.63 (0.38–1.03)	0.07	0.57 (0.32–1.01)	0.05	0.62 (0.32–1.19)	0.15
DBI	rs2084202 (g.-132A>G)	AA *vs.* AG	145/255 *vs.* 39/97	0.68 (0.44–1.05)	0.08	0.69 (0.43–1.12)	0.13	0.57 (0.32–1.00)	0.05
		AA *vs.* GG	145/255 *vs.* 3/16	0.30 (0.09–1.08)	0.06	0.34 (0.09–1.32)	0.12	0.35 (0.08–1.56)	0.17
		AA *vs.* AG + GG	145/255 *vs.* 42/113	**0.63 (0.41–0.96)**	**0.03**	0.65 (0.40–1.04)	0.07	**0.54 (0.31–0.94)**	**0.03**
		AA + AG *vs.* GG	184/352 *vs.* 3/16	0.34 (0.10–1.20)	0.09	0.38 (0.10–1.50)	0.17	0.40 (0.09–1.86)	0.24
	rs8192506 (Met71Val)	AA *vs.* AG	169/355 *vs.* 21/23	**1.89 (1.03–3.47)**	**0.04**	1.97 (0.96–4.05)	0.07	1.92 (0.87–4.24)	0.11
PTGES2	rs13283456 (Arg298His)	CC *vs.* CT	135/241 *vs.* 49/112	0.78 (0.52–1.16)	0.21	0.66 (0.41–1.05)	0.08	0.71 (0.43–1.19)	0.19
		CC *vs.* TT	135/241 *vs.* 4/15	0.44 (0.14–1.41)	0.17	0.52 (0.14–1.88)	0.31	0.32 (0.08–1.35)	0.12
		CC *vs.* CT + TT	135/241 *vs.* 53/127	0.74 (0.50–1.09)	0.12	**0.64 (0.41–1.01)**	**0.05**	0.66 (0.40–1.07)	0.09
		CC + CT *vs.* TT	184/353 *vs.* 4/15	0.48 (0.15–1.51)	0.21	0.57 (0.16–2.01)	0.38	0.34 (0.08–1.40)	0.14

Model 1: adjusted for BMI; Model 2: adjusted for BMI, waist-hip ratio, alcohol consumption (g/d), total energy intake (kJ/d), smoking (present, former and nonsmoking), sports activity (h/w), hyperlipoproteinaemia, hypertension, lipid-lowering drug intake and antihypertensive medication; IM, inheritance model.

Table 4. Risk estimates for disease associated SNPs in a simultaneously adjusted model

SNP			OR	95% CI	*p*
CLPS	Ala109Cys	CC *vs.* CT	4.08	0.82–20.47	0.09
SLC27A5	g.–1324G>A	GG *vs.* GA + AA	0.61	0.35–1.04	0.07
FABP6	Thr79Met	CC *vs.* CT + TT	**0.64**	**0.44–0.93**	**0.02**
DBI	g.–132A>G	AA *vs.* AG + GG	**0.53**	**0.29–0.94**	**0.03**
PTGES2	Arg298His	CC *vs.* CT + TT	0.65	0.41–1.01	0.06

Conditional logistic regression analyses of each SNP associated with type 2 diabetes adjusted for BMI, waist-hip ratio, alcohol consumption (g/d), total energy intake (kJ/d), smoking (present, former and nonsmoking), sports activity (h/w), hyperlipoproteinemia, hypertension, lipid-lowering drug intake, antihypertensive medication and other SNPs associated with type 2 diabetes in the study.

In this model, significant associations with risk of type 2 diabetes remained for *FABP*6 Thr79Met ($p = 0.02$), and DBI g.-132A > G ($p = 0.03$).

4 Discussion

To our knowledge, this is the fourth study on genetic susceptibility of type 2 diabetes in several pathway-related genes by *de novo* sequencing and case-control association study. Similar to and in fact more comprehensively than our study, Barroso *et al.* [19] investigated a large number of SNPs in 71 candidate genes involved in β-cell function and insulin action, using two independent populations, and analysing haplotypes in addition to individual SNPs. Genetic variants in five genes were related to type 2 diabetes risk. Also in 2003, a large-scale study was performed by Daimon *et al.* [20] in 120 candidate genes for type 2 diabetes in a small population-based case-control study consisting of 148 diabetic cases and 227 controls from Japan. Recently, Yokoi *et al.* [21] investigated 33 SNPs with MAF <0.10 from 12 genes including transcription factors and β-cell K_{ATP} channel subunits *KCNJ11* and *ABCC8* in a large Japanese case-control study of type 2 diabetes following resequencing of several target genes.

Here, we found nominal associations for *SLC27A5* g.-1324G > A, *FABP6* Thr79Met, *DBI* rs2084202, and rs8192506, *PTGES2* Arg298His and *CLPS* Arg109Cys. Among these genes, *FABP6* and *DBI* seemed to have the strongest effects. Because these genes are important for fatty acid transport (*FABP6*) and fatty acid activation (*DBI*), a perturbation of fat assimilation caused by either of its functional variants may increase the duration of higher postprandial fatty acid concentrations in the intestine. This may evoke the secretion of the gastric inhibitory polypeptide GIP which induces insulin secretion [22]. Therefore, we hypothesise that a perturbed fat assimilation enforces the negative effects of saturated fatty acids on insulin secretion *via* increased release of nutrient sensors such as GIP.

One major constraint of our analyses was that we could not rule out size effects smaller than OR 1.5 (or 0.65) within the sample size employed in our study. Therefore, the possibility remains that some of the analysed variants are associated with type 2 diabetes, albeit with weak genetic effects, such as OR of less than 1.5 that are likely to contribute to complex diseases [23]. Barroso *et al.* [19] illustrated sample size ranges which would be appropriate to determine small risk effects in case-control studies more precisely. For example, studies attaining 80% power to confess an OR of 1.2 of a low frequent variant (MAF = 0.2) with a type 1 error rate of 0.01% would require approximately 8000 cases. Obviously, such sample sizes will only be achieved in large-scale population studies such as the EPIC-Study with 520 000 participants. In the upcoming INTERACT PROJECT of EPIC, which has recently been financed by the European Commission, approximately 12 000 incident type 2 diabetes cases will be included. Nevertheless, if a type 1 error rate of 5% is assumed, our study provided efficient statistical power for genetic variants with a MAF of 0.2 to detect significant effects of ORs $\geq$ 1.6 or *vice versa* OR $\leq$ 0.55.

Many positive associations of common genetic variants with type 2 diabetes have been reported, but subsequent replication has proven the exception rather than the rule [24]. Candidate gene screening has identified the widespread Pro12Ala variant of the peroxisome proliferative activated receptor-gamma (*PPARγ*) and the common Glu23Lys variant of the ATP-sensitive potassium channel, Kir6.2 (*KCNJ11*) as diabetes-susceptibility markers which showed high reproducibility so far [25]. Within the task of our project, we meanwhile replicated the association finding for the *CLPS* Arg109Cys variant [12] and initiated complete haplotype analyses and independent replication of the *DBI* and *PTGES2* genes. We have performed functional analyses of the *FABP2* promotor polymorphism and found a significant association with type 2 diabetes in men [26]. We cannot exclude some false-positive findings because OR calculations had been undertaken without correction for multiple testing in this study. Primary adjustment of allelic associations for the 33 tests undertaken was performed, but the results are biased since some of the variants are not independent. Further comments on the justification of multiple-testing adjustments applied in studies as the one reported here have been given elsewhere [19]. Even so, we acknowledge that the results presented here should be interpreted with caution and definitely require replication through other studies. Also, despite some variants showed nominal association with disease risk, the functional implication of a susceptibility genotype has to be ruled out by *in vitro* analysis. A query using PolyPhen software (available at http://genetics.bwh.harvard.edu/pph/data/index.html) [27] of the functional impact of the Thr79Met variant of ileal FABP (Il-FABP) predicted no effect on the structure and function of the mature protein. Otherwise, residue 78 – 80 (lysine-threonine-phenylanaline) constitute a potential phosphorylation site for protein kinase C as predicted by PROSITE (www.expasy.org/prosite/) [28, 29]. Therefore, the replacement of threonine with methionine might result in a different post-translational modification of Il-FABP *via* diminished phosphorylation of that site. Nielsen *et al.* [30] first showed that FABPs isolated from lactating mammalian epithelial cells were phosphorylated upon insulin stimulation. As seen from adipocyte-FABP (A-FABP), posttranslational phosphorylation by insulin receptor tyrosine kinase destabilises the protein and changes its ligand-binding properties [31]. Phosphorylation hindered binding of the fluorescent fatty acid analogue 12-(9-anthroyloxy)oleic acid. Consequently, fatty acid-binding activity was recovered upon dephosphorylation with protein tyrosine phosphatase. Obviously, this binding modification – binding but also release of fatty acid – did not effect protein translocation into the nucleus [32] where it serves as mediator for PPARγ induced gene expression, but the cellular lipid repartitioning might well be affected through the phosphorylation status of FABPs. In any case, there is some theoretical plausibility for a functional relevance of the *FABP6* Thr79Met polymorphism.

At this stage, we are presenting preliminary data that provide provisional evidence for some common variants in genes involved in fat assimilation that contribute to genetic susceptibility of type 2 diabetes.

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