

# Screening of 134 Single Nucleotide Polymorphisms (SNPs) Previously Associated With Type 2 Diabetes Replicates Association With 12 SNPs in Nine Genes

Cristen J. Willer,<sup>1</sup> Lori L. Bonnycastle,<sup>2</sup> Karen N. Conneely,<sup>1</sup> William L. Duren,<sup>1</sup> Anne U. Jackson,<sup>1</sup> Laura J. Scott,<sup>1</sup> Narisu Narisu,<sup>2</sup> Peter S. Chines,<sup>2</sup> Andrew Skol,<sup>1</sup> Heather M. Stringham,<sup>1</sup> John Petrie,<sup>2</sup> Michael R. Erdos,<sup>2</sup> Amy J. Swift,<sup>2</sup> Sareena T. Enloe,<sup>2</sup> Andrew G. Sprau,<sup>2</sup> Eboni Smith,<sup>2</sup> Maurine Tong,<sup>2</sup> Kimberly F. Doheny,<sup>3</sup> Elizabeth W. Pugh,<sup>3</sup> Richard M. Watanabe,<sup>4</sup> Thomas A. Buchanan,<sup>5</sup> Timo T. Valle,<sup>6</sup> Richard N. Bergman,<sup>7</sup> Jaakko Tuomilehto,<sup>6,8</sup> Karen L. Mohlke,<sup>9</sup> Francis S. Collins,<sup>2</sup> and Michael Boehnke<sup>1</sup>

More than 120 published reports have described associations between single nucleotide polymorphisms (SNPs) and type 2 diabetes. However, multiple studies of the same variant have often been discordant. From a literature search, we identified previously reported type 2 diabetes-associated SNPs. We initially genotyped 134 SNPs on 786 index case subjects from type 2 diabetes families and 617 control subjects with normal glucose tolerance from Finland and excluded from analysis 20 SNPs in strong linkage disequilibrium ( $r^2 > 0.8$ ) with another typed SNP. Of the 114 SNPs examined, we followed up the 20 most significant SNPs ( $P < 0.10$ ) on an additional 384 case subjects and 366 control subjects from a population-based study in Finland. In the combined data, we replicated association ( $P < 0.05$ ) for 12 SNPs: *PPARG* Pro12Ala and His447, *KCNJ11* Glu23Lys and rs5210, *TNF* -857, *SLC2A2* Ile110Thr, *HNF1A/TCF1* rs2701175 and GE117881\_360, *PCK1* -232, *NEUROD1* Thr45Ala, *IL6* -598, and *ENPP1* Lys121Gln. The replication of 12 SNPs of 114 tested was significantly greater than expected by chance under the null hypothesis

of no association ( $P = 0.012$ ). We observed that SNPs from genes that had three or more previous reports of association were significantly more likely to be replicated in our sample ( $P = 0.03$ ), although we also replicated 4 of 58 SNPs from genes that had only one previous report of association. *Diabetes* 56:256–264, 2007

Type 2 diabetes is a common disease characterized by insulin resistance and reduced insulin secretion. There are >120 published reports of association between genetic variants and type 2 diabetes (see Table S1 of the online appendix available at <http://diabetes.diabetesjournals.org>), but multiple studies of the same variant often show inconsistent findings (1). Such failures to replicate findings may reflect the fact that the original studies had false-positive results, that the replication studies were underpowered to detect the modest impact of individual loci on type 2 diabetes, or that there was etiologic heterogeneity across populations. In addition, different sampling schemes that ascertained subjects with more severe type 2 diabetes phenotypes or complications, a positive family history, or for other diseases such as obesity or hypertension may enrich for different susceptibility alleles (2).

Most of the published reports of association between a single nucleotide polymorphism (SNP) and type 2 diabetes involve markers in or around a candidate gene or linkage region. Candidate genes were typically examined because of their known or potential role in diabetes-related metabolic pathways (reviewed in ref. 3) or glucose metabolism (4), their location relative to linkage peaks, as with *Calpain-10* on chromosome 2 (5), and their involvement in maturity-onset diabetes of young (MODY) (6–9) or other Mendelian forms of diabetes (10).

Here we describe an investigation of SNPs reported to be associated with type 2 diabetes in the literature before May 2005. We tested each SNP for type 2 diabetes association in a two-stage study based on a Finnish sample of 1,170 case subjects and 983 control subjects. Through our literature survey, we identified 147 SNPs in 76 genes for which associations with type 2 diabetes ( $P < 0.05$ ) had been reported. The 76 genes are involved in a wide range of biological processes, including fatty acid biosynthesis

From the <sup>1</sup>Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan; the <sup>2</sup>Genome Technology Branch, National Human Genome Research Institute, Bethesda, Maryland; the <sup>3</sup>Center for Inherited Disease Research, Johns Hopkins University School of Medicine, Baltimore, Maryland; the <sup>4</sup>Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; the <sup>5</sup>Department of Medicine, Division of Endocrinology, Keck School of Medicine, University of Southern California, Los Angeles, California; the <sup>6</sup>Diabetes and Genetic Epidemiology Unit, Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; the <sup>7</sup>Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California; the <sup>8</sup>Department of Public Health, University of Helsinki, Helsinki, Finland, and the South Ostrobothnia Central Hospital, Seinäjoki, Finland; and the <sup>9</sup>Department of Genetics, University of North Carolina, Chapel Hill, North Carolina.

Address correspondence and reprint requests to Michael Boehnke, PhD, Department of Biostatistics, School of Public Health, University of Michigan, 1420 Washington Heights, Ann Arbor, MI 48109-2029. E-mail: boehnke@umich.edu.

Received for publication 7 April 2006 and accepted in revised form 18 September 2006.

Additional information for this article can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

FUSION, Finland-United States Investigation of NIDDM Genetics; LD, linkage disequilibrium; MAF, minor allele frequency; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; SNP, single nucleotide polymorphism; WHR, waist-to-hip ratio.

DOI: 10.2337/db06-0461

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1  
Characteristics of the study sample

	Stage 1			Stage 2	
	FUSION case subjects	FUSION control subjects	Finrisk control subjects	Finrisk case subjects	Finrisk control subjects
No. of samples	786	375*	242	384	366
Sex (male/female)	437/349	155/220	169/73	232/152	214/152
Age at diagnosis (years)	51.0 (12.0)	NA	NA	59.0 (12.0)	NA
Age at examination (years)	64.3 (10.1)	69.8 (5.9)	64.6 (9.9)	60.5 (12.2)	60.5 (11.3)
BMI (kg/m <sup>2</sup> )	29.4 (6.1)	27.0 (5.4)	26.8 (4.3)	30.6 (6.2)	26.6 (4.5)
Fasting glucose (mmol)	9.7 (4.9)	5.0 (0.6)	5.6 (0.5)	7.3 (1.3)	5.6 (0.5)
Fasting insulin (pmol)	96.0 (84.0)	60.0 (42.0)	42.0 (28.8)	79.2 (61.8)	39.0 (27.6)

Data are median (interquartile range). \*Consists of 223 FUSION elderly control subjects and 152 spouse control subjects. NA, not applicable.

and metabolism, gluconeogenesis, glucose metabolism and transport, glycogen biosynthesis and metabolism, glycolysis, regulation of transcription, signal transduction, and apoptosis (Gene Ontology database). Among 114 SNPs examined in 71 genes, we observed significant type 2 diabetes association ( $P < 0.05$ ) for 12 SNPs in 9 genes: *PPARG* (2 SNPs), *KCNJ11* (2 SNPs), *TNF*, *SLC2A2*, *HNFA/TCF1* (2 SNPs), *PCK1*, *NEUROD1*, *IL6*, and *ENPP1*. We conclude that these previously reported type 2 diabetes-associated genes show an excess of type 2 diabetes association beyond that expected by chance, but at the same time, many of the previously reported association results may have been false-positives or of minor impact in the Finnish population.

## RESEARCH DESIGN AND METHODS

Our sample consisted of 2,153 Finnish individuals: 1,170 type 2 diabetic case subjects and 983 control subjects with normal glucose tolerance (NGT). We adopted a two-stage design in which we genotyped 786 case subjects and 617 control subjects in stage 1 and followed up SNPs with associated  $P < 0.10$  in an additional 384 case subjects and 366 control subjects in stage 2. We excluded SNPs from stage 2 genotyping if they were in strong linkage disequilibrium (LD) ( $r^2 > 0.8$ ) with another SNP selected for stage 2 genotyping. This two-stage design together with joint analysis of all the data generally provides nearly the same power as the more expensive alternative of genotyping all SNPs in the full sample (11). By use of this strategy, our sample provides at least 78% power to detect type 2 diabetes association with odds ratios (ORs)  $\geq 1.3$ , given a minor allele frequency (MAF)  $\geq 10\%$  under a multiplicative disease model with a type I error rate of 0.05. The power to detect markers with ORs  $\geq 1.2$  was 55, 85, and 100% for markers with 10, 20, and 50% MAFs, respectively. We determined the power of our two-stage study using the power calculator CaTS (11) (available at <http://www.sph.umich.edu/csg/abecasis/CaTS/>).

For stage 1 type 2 diabetic case subjects, we selected one individual from each of 786 unrelated families ascertained for type 2 diabetes sibling pairs in the Finland-United States Investigation of NIDDM Genetics (FUSION) study (12,13); type 2 diabetes in all of these case subjects was diagnosed as determined by World Health Organization 1985 criteria (14), and in 767 of these case subjects (97.6%), diabetes had been diagnosed after the age of 35 years. Stage 1 control subjects comprised 617 unrelated individuals with NGT: 152 spouses of FUSION type 2 diabetic subjects (spouse control subjects) (12,13), who had NGT (14), 223 unrelated individuals who had NGT (14) by an oral glucose tolerance test at ages 65 and 70 years (elderly control subjects), and 242 individuals with NGT (15) selected from the population-based Finrisk 2002 study (16). The stage 2 samples of 384 type 2 diabetic case subjects and 366 control subjects with NGT as determined by World Health Organization 1999 criteria (15) also came from the Finrisk 2002 study (16). Stage 1 and 2 case subjects and control subjects were approximately matched for age, sex, and province of birth (Table 1). Study protocols for the FUSION and Finrisk 2002 studies were approved by local ethics committees and/or institutional review boards of each participating recruitment or analysis site, and informed consent was obtained from all study participants.

**SNP selection and genotyping.** We performed literature reviews, updated in May 2005, first with search terms "genetic" and "association" and "diabetes" and second with terms "SNP" and "diabetes" (using [www.pubmed.gov](http://www.pubmed.gov)). We

reviewed abstracts to identify SNPs significantly associated with type 2 diabetes at a level of  $P < 0.05$  or at more stringent thresholds proposed in the original studies and identified 147 SNPs representing 76 genes (see Table S1 of the online appendix). We determined the dbSNP identifier from the original manuscript when available. In several instances we identified the associated SNP from surrounding sequence information or restriction fragment length polymorphism data included in the original manuscript. Of the 147 type 2 diabetes-associated SNPs identified from the literature, we were unable to design assays for 5 SNPs: *CAPN10* rs3842570, *IL6R* rs2228146, *HSPA1B* (*HSP70-2*) rs1061581, *LIPC* rs2070895, and *MGEA5* LLY-MGEA5-14 (see Table S1 of the online appendix). An additional five SNPs were not successfully genotyped: *HNFA/TCF1* rs1169305, *NOS3* rs1799984, *HTR2C* rs3813928, *PIK3R1* rs8192680, and *HSD11B1* rs846910. Three additional SNPs in *PTPN1* (rs3787348, rs754118, and rs2282147) were not genotyped because they were in complete LD ( $r^2 = 1$ ) in the HapMap CEU sample with other SNPs we genotyped.

In our stage 1 sample, we successfully genotyped 42 SNPs at the Center for Inherited Disease Research using the Illumina GoldenGate Assay (17,18) and 92 SNPs at the National Human Genome Research Institute using the Sequenom homogeneous MassEXTEND Assay. The 134 successfully genotyped SNPs had an average call rate of 98.1% and reproducibility rates of 99.91 and 99.94% for the Sequenom and Illumina systems, respectively. All but 1 of these 134 SNPs had call rates of  $>95\%$  (SNP rs8192692 had a call rate of 93.7%), and all were consistent with Hardy-Weinberg expectations ( $P > 0.001$ ).

The 134 successfully genotyped SNPs were from 71 genes (Table 2). We excluded 20 SNPs in strong LD ( $r^2 > 0.8$ ) with another genotyped SNP; thus, we tested 114 SNPs for association with type 2 diabetes. Of the 114 SNPs assessed for type 2 diabetes association in the stage 1 sample, we genotyped in the stage 2 sample 20 SNPs with  $P < 0.1$  after stage 1. We present type 2 diabetes association results in the combined stage 1 and 2 samples of 1,170 type 2 diabetes case subjects and 983 NGT control subjects for these 20 SNPs (see Table S2 of the online appendix and Table 3).

Association results for SNPs in *RETN* (rs1862513) and the *MODY* genes *NEUROD1*, *IPF1*, and *HNFA/TCF1* (rs1801262, rs1169288, rs1800574, rs1920792, rs1169289, rs2701175, GE117881\_360, GE117884\_349, rs2071197, rs736824, *IPF1* Asp76Asn, rs2178463, rs2393792, rs2144908, and rs1799884) have previously been reported by our group in nearly equivalent samples (19,20). We included these SNPs in this report to estimate an overall ability to replicate all previous associations identified from our literature survey. Four additional SNPs (Pro12Ala [rs1801282] in *PPARG*, rs4994 in *ADRB3*, and rs3792267 and rs5030952 in *CAPN10*) have been previously reported by our group in smaller samples (21–23), and results after genotyping a larger sample are included here. We present new data for 114 SNPs not previously genotyped by our group.

**Type 2 diabetes-SNP association.** We tested for type 2 diabetes association and estimated ORs and 95% CIs using logistic regression under dominant, recessive, and multiplicative genetic models. Four SNPs were monomorphic in our sample (rs4148628, *ACDC* Ile164Thr, rs1800561, and rs2233578). Of the 110 polymorphic SNPs examined, the previously reported risk alleles for 99 SNPs were unambiguously determined, and we used a one-sided test of association to that same allele; for the remaining 11 SNPs, we used a two-sided test. We tested all SNP model combinations for which there were  $>10$  total individuals (case subjects and control subjects) in a genotype class using logistic regression. We calculated the minimum  $P$  value from up to three models tested (dominant, recessive, and multiplicative) and corrected for this maximization with a modified Bonferroni adjustment (24), which accounts for the correlation between tests (K.C., M.B., unpublished data). We verified analytically derived  $P$  values by permutation testing and observed nearly

TABLE 2  
SNPs assessed for association in the Finnish case-control sample

Gene	dbSNP ID	Gene	dbSNP ID	Gene	dbSNP ID
<i>ABCA1</i>	rs2020927	<i>HNFI1/TCF1</i>	GE117881_360	<i>PKLR</i>	rs1052176†
<i>ABCC8</i>	rs1799854		GE117884_349		rs1052177†
	rs1799858		rs1169289†		rs2071053†
	rs1801261		rs1800574‡		rs3020781
	rs2074308		rs1920792	<i>PPARG</i>	rs1801282‡
	rs2283257		rs2178463†		rs3856806
	rs4148628*		rs2393792†	<i>PPARGC1A</i>	rs3755863†
	rs4148643		rs2701175		rs8192678‡
	rs4148646†	<i>HSD11B1</i>	rs12086634	<i>PPP1R3A</i>	rs1799999‡
	rs757110‡†	<i>HTR2C</i>	rs3813929	<i>PRKCZ</i>	rs436045
	rs8192692	<i>IDE</i>	rs4646953	<i>PTGS2</i>	rs20417
<i>ABCC9</i>	rs1283802	<i>IL6</i>	rs1800795		rs2066826
<i>ACDC</i>	lle164Thr*‡		rs1800797†	<i>PTPN1</i>	rs2282146
	rs1501299	<i>IL6R</i>	rs8192284‡		rs16995309*‡
	rs16861194	<i>INS</i>	rs3842752		rs718630
	rs17300539	<i>INSR</i>	rs1799816‡		rs2206656
	rs2241766		rs2860177		rs3787345†
	rs266729		rs2860178		rs4811078
<i>ADCYAP1</i>	rs2856966‡		rs7252268		rs178049†
<i>ADRB2</i>	rs1042711	<i>IPF1</i>	ipfl_2*‡		rs941798
	rs1042714‡†	<i>IRS1</i>	rs12053536		rs718050†
<i>ADRB3</i>	rs4994‡		rs13306465	<i>PYY</i>	rs162430
<i>AKR1B1</i>	rs759853		rs1801123	<i>RETN</i>	rs1862513
<i>ALDOB</i>	rs506571		rs1801278‡	<i>SLC2A1</i>	rs841853
<i>APOD</i>	rs2280520	<i>IRS2</i>	rs1805097‡	<i>SLC2A2</i>	rs5398
<i>BCHE</i>	rs1803274‡	<i>ITGB3/GPIIIa</i>	rs5918		rs5400‡
<i>CAPN10</i>	rs2975760	<i>KCNJ11</i>	rs5210		rs5404
	rs3792267		rs5218†		rs5406†
	rs5030952		rs5219‡		rs6785803†
<i>CASQ1</i>	rs2275703	<i>KCNJ9</i>	rs2180752	<i>SORBS1</i>	rs2281939‡
	rs617599†		rs2737705	<i>SOS1</i>	rs27577088
	rs617698		rs2753268	<i>SPINK</i>	rs17107315‡
<i>CD38</i>	rs1800561*‡	<i>LEP</i>	rs2278815	<i>SREBF1</i>	rs2297508
<i>COG2</i>	rs1051038	<i>LIPC</i>	rs8192701	<i>TNF</i>	rs1799724†
<i>CRP</i>	rs2794521	<i>LPL</i>	rs285		rs1800610
<i>ENPP1</i>	rs1044498‡	<i>LTA</i>	rs1041981‡		rs1800629
<i>FABP2</i>	rs1397613	<i>MCP1</i>	rs3760399	<i>UCP1</i>	rs10011540
<i>FRDA</i>	rs2498429	<i>MET</i>	rs38848		rs2270565‡†
<i>GCGR</i>	rs1801483*‡		rs41736	<i>UCP2</i>	rs659366
<i>GFPT2</i>	rs2303007‡	<i>NEUROD1</i>	rs1801262‡		rs660339‡
<i>GNB3</i>	rs5443	<i>NOS3</i>	rs1799983	<i>UCP3</i>	rs1800849
<i>GYS1</i>	rs8103451	<i>PAX4</i>	rs2233578*‡	<i>UTS2</i>	rs2890565‡
<i>HFE</i>	rs1799945‡	<i>PCK1</i>	rs2071023	<i>VLDLR</i>	rs2242103
	rs1800562‡			<i>WFS1</i>	rs1801208‡
					rs734312‡
				<i>WNT5B</i>	rs2270031
					rs2270036

\*SNPs that were monomorphic or had MAFs <0.005 in our sample. †SNPs in  $r^2 > 0.8$  with another genotyped SNP; only one SNP of each pair in  $r^2 > 0.8$  is indicated. ‡Nonsynonymous SNPs. Table S1 of the online appendix provides more information about each SNP and the original association reference and Table S3 of the online appendix provides  $D'$  and  $r^2$  values for SNPs with  $r^2 > 0.8$  with another genotyped SNP.

identical results (data not shown). We assessed the three SNPs with MAFs <0.005 (rs1801483, rs16995309, and *IPF1* Asp76Asn) using Fisher's exact test because of small cell counts. Given multiple associated SNPs in the same gene, we assessed the independent contribution of each SNP to an association signal ( $P < 0.05$ ) by including the more significant SNP as a covariate in logistic regression and reassessing the evidence for association. To determine the overall significance of the study, we used the binomial distribution to estimate the probability of observing at least as many significant results given the number tested. To estimate the significance of the study given the presence of LD between SNPs tested, we estimated the equivalent number of independent SNPs significantly associated and tested (25) and estimated the significance using the most nearly corresponding binomial distribution. We estimated the false-positive report probability (26) using type I and type II

error rate estimates of 0.05 and 0.22 and a priori probability that each SNP is a true positive of 0.05 or 0.01.

**Quantitative trait-SNP association.** We performed tests for association with quantitative traits in the combined sample of 152 spouse control subjects and 223 elderly control subjects with NGT ( $n = 375$ ). We excluded affected individuals from the assessment of quantitative traits because it is difficult to interpret quantitative traits for glucose and insulin metabolism in affected individuals. Different treatment regimens and responses to treatment may also contribute to difficulties in measuring these traits in affected individuals. The following traits were available for individuals with NGT: fasting and 2-h glucose, fasting and 2-h insulin, fasting and 2-h free fatty acid, fasting HDL cholesterol, LDL cholesterol, total triglycerides, systolic and diastolic blood pressure, BMI, and waist-to-hip ratio (WHR). The following additional traits

TABLE 3  
SNPs exhibiting significant association with type 2 diabetes in the combined stage 1 and 2 samples

Gene	SNP	Risk/non-risk allele	Frequency of risk allele		<i>P</i> value	Best model	OR (95% CI)
			Case subjects	Control subjects			
<i>PPARG</i>	rs1801282 Pro12Ala	C/G	0.853	0.817	0.0019	Mult	1.30 (1.10–1.53)
	rs3856806 His447His	C/T	0.822	0.797	0.013*	Rec	1.26 (1.05–1.51)
<i>KCNJ11</i>	rs5219 Glu23Lys	A/G	0.494	0.445	0.0019	Mult	1.22 (1.08–1.38)
	rs5210 3p + 215	G/A	0.659	0.618	0.0046†	Mult	1.21 (1.06–1.37)
<i>TNF</i>	rs1800610	A/G	0.069	0.049	0.0073	Mult	1.42 (1.10–1.82)
<i>SLC2A2</i>	rs5400 Ile110Thr	G/A	0.883	0.859	0.014	Dom	2.98 (1.31–6.79)
<i>HNF1A/TCF1</i>	rs2701175	C/A	0.423	0.419	0.015	Rec	1.33 (1.06–1.68)
	GE117881_360	G/A	0.469	0.456	0.040‡	Rec	1.25 (1.01–1.55)
<i>PCK1</i>	rs2071023	C/G	0.558	0.533	0.031	Dom	1.27 (1.02–1.57)
<i>NEUROD1</i>	rs1801262 Ala45Thr	T/C	0.368	0.336	0.033	Mult	1.15 (1.01–1.30)
<i>IL6</i>	rs1800795 –174	G/C	0.473	0.447	0.038	Rec	1.25 (1.02–1.55)
<i>ENPP1</i>	rs1044498 Lys121Gln	C/A	0.145	0.123	0.038	Dom	1.24 (1.01–1.51)

\**P* value was not significant after correcting for *PPARG* Pro12Ala. †*P* value was not significant after correcting for *KCNJ11* Glu23Lys. ‡*P* value was not significant after correcting for *HNF1A/TCF1* rs2701175. Table S2 of the online appendix provides complete genotype counts, ORs, allele frequencies, and *P* values for all SNPs tested. Dom, dominant genetic model; Mult, multiplicative genetic model; Rec, recessive genetic model.

were available in the spouse control subjects only ( $n = 152$ ): glucose effectiveness, insulin sensitivity, acute insulin response, and disposition index. All traits were transformed to approximate univariate normality and adjusted for sex, age, and BMI (except for weight-related traits). Analyses were performed using recessive, dominant, and multiplicative genetic models if >10 individuals were in each genotype class. To consider the cumulative evidence for association with all traits for each SNP, we used the truncated product method (27). We calculated our test statistic as the sum of  $\ln(P_{\text{uncorrected}})$ , where  $P_{\text{uncorrected}}$  is the uncorrected *P* value for each trait-model combination, for all tests with a  $P_{\text{uncorrected}} < 0.05$ . To estimate the significance of this combined set of tests ( $P_{\text{corrected}}$ ) while accounting for the multiple phenotypes and models tested, we compared our test statistic to test statistics obtained by permuting the vector of phenotypes against the SNP genotypes.

**LD measures.** We estimated LD measures  $D'$  and  $r^2$  for all pairs of SNPs <2 Mb apart using LDmax (28).

## RESULTS

After genotyping 134 SNPs, we tested 114 for type 2 diabetes association and found 12 SNPs (10.5%) in 9 genes that were significantly associated with type 2 diabetes ( $P < 0.05$ ) (Table 3) in our combined stage 1 and 2 samples of 1,170 type 2 diabetes case subjects and 983 NGT control subjects (Table 1). These were among 20 SNPs selected for stage 2 genotyping based on evidence for association in stage 1 samples ( $P < 0.10$ ). Genotype counts, frequencies, *P* values, and ORs for all three genetic models are shown in Table S2 of the online appendix for all 134 SNPs. We used a one-sided test of association when the previously associated allele was known and a two-sided test otherwise. For the 12 SNPs that were associated, 1 was analyzed with a two-sided test because of conflicting reports with regard to the risk allele (*SLC2A2*). The other 11 SNPs were assessed with a one-sided test. The replication of 12 SNPs of the 114 tested was significantly greater than expected by chance (one-sided  $P = 0.012$ ). To verify this result while correcting for the LD between the SNPs tested, we determined that there were the equivalent of 10.5 independent SNPs in the 12 associated SNPs and 105.5 independent SNPs in the 114 SNPs tested, resulting in similar evidence for an excess of significant results (one-sided  $P = 0.017$ ). Genes containing SNPs that were associated with type 2 diabetes in our sample were *PPARG*

(two SNPs), *KCNJ11* (two SNPs), *TNF*, *SLC2A2*, *HNF1A/TCF1* (two SNPs), *PCK1*, *NEUROD1*, *IL6*, and *ENPP1*.

We also performed exploratory tests for association between these SNPs and available quantitative traits in unaffected individuals. These were not specifically an attempt to replicate previous association results with quantitative traits because our focus in this article is type 2 diabetes, but they did provide an opportunity to assess possible mechanisms of action for the associated SNPs. For the quantitative trait analysis, we excluded 8 SNPs with MAFs <0.005 in 375 individuals with NGT. Based on combined evidence across all quantitative traits assessed, 5 of the 106 SNPs had evidence of association ( $P < 0.05$ ), which was not in significant excess of the expected number of 5.3. Of the 12 SNPs exhibiting an association with type 2 diabetes ( $P < 0.05$ ,  $\text{MAF} > 0.005$ ,  $r^2 < 0.8$ ), 2 showed evidence for association with the quantitative traits (see Table S2 of the online appendix and Table 4), which was not a significant excess over expectation (one-sided  $P = 0.11$ ). Genes with SNPs that were associated with the diabetes-related quantitative traits in our NGT control subjects were *TNF*, *UCP3*, *IL6*, *ABCC8*, and *VLDLR* (Table 4).

The two most significant type 2 diabetes association results in the combined stage 1 and 2 samples were for the widely replicated type 2 diabetes-associated variants *PPARG* Pro12Ala (rs1801282) ( $P = 0.0019$ ) (Table 3) and *KCNJ11* Glu23Lys (rs5219) ( $P = 0.0019$ ) (Table 3). *PPARG* encodes a transcription factor involved in adipocyte differentiation and accumulation of triglycerides and is involved in glucose-induced insulin secretion (29). Many studies have shown association with the *PPARG* Pro12Ala allele, including a previous report by our group based on approximately half the samples described here (21). There also have been negative reports (reviewed in ref. 30). In our current sample, the multiplicative model provided the strongest evidence for association, and the common Pro risk allele had frequencies of 0.853 in case subjects and 0.816 in control subjects (OR 1.30 [95% CI 1.10–1.53]). A synonymous coding SNP (His447, rs3856806) in *PPARG* was also associated with type 2 diabetes under a dominant

TABLE 4  
SNPs with significant association to quantitative phenotypes in 375 control subjects with NGT

Gene	SNP	Type 2 diabetes risk/nonrisk allele	$P_{\text{corrected}}$	Traits associated with the type 2 diabetes risk allele	Trait $P_{\text{uncorrected}}$
<i>TNF</i>	rs1800610	A/G	0.002	Increased BMI	0.000010
				Increased WHR	$0.00028 \times 10^{-4}$
<i>UCP3</i>	rs1800849	G/A	0.014	Decreased 2-h glucose levels	$1.0 \times 10^{-4}$
				Decreased fasting glucose	0.0042
				Increased 2-h insulin	0.027
				Decreased BMI	0.035
<i>IL6</i>	rs1800795	G/C	0.022	Decreased fasting free fatty acids	0.0010
				Increased disposition index	0.0078
				Decreased HDL	0.0092
				Increased glucose effectiveness	0.014
<i>ABCC8</i>	rs8192692	G/A	0.024	Decreased fasting insulin	0.0017
				Increased LDL	0.0023
				Decreased triglycerides	0.0035
<i>VLDLR</i>	rs2242103	A/T	0.041	Increased fasting free fatty acids	0.0045
				Decreased triglyceride	0.015
				Decreased LDL	0.035
				Increased insulin sensitivity	0.035

$P_{\text{corrected}}$  was determined using the truncated product method (see RESEARCH DESIGN AND METHODS). This  $P$  value was corrected for the number of genetic models and traits examined but was not corrected for the number of SNPs examined.

model (OR 1.26 [1.05–1.51],  $P = 0.013$ ) but was no longer significant after adjustment for the Pro12Ala variant (OR 1.11 [0.89–1.38],  $P = 0.064$ ). Prior meta-analysis of genotypes from ~25,000 individuals provided strong evidence for a modest effect for the Pro12Ala variant (OR 1.27,  $P < 2 \times 10^{-8}$ ) (30).

*KCNJ11* encodes the Kir6.2 potassium channel subunit which, together with the sulfonylurea receptor *ABCC8* (*SUR1*), is responsible for maintaining the  $\beta$ -cell transmembrane potential required for insulin secretion. Mutations in *KCNJ11* cause permanent neonatal diabetes, hypoglycemia of infancy, and hyperinsulinemia (31). The common Glu23Lys polymorphism has been associated with type 2 diabetes in multiple studies, but not in others (reviewed in ref. 3), and together with an *ABCC8* risk haplotype was a predictor of progression from impaired glucose tolerance to type 2 diabetes in a Finnish sample (32). The *KCNJ11* Glu23Lys polymorphism (rs5219) was strongly associated with type 2 diabetes in our sample ( $P = 0.0019$ ) (Table 3). The multiplicative model provided the strongest evidence for association; the lysine risk allele had frequencies of 0.494 in case subjects and 0.445 in control subjects (OR 1.22 [95% CI 1.08–1.38]). Prior meta-analysis of 13 studies resulted in an OR of 1.12 ( $P = 0.0017$ ) (33). Nearby SNPs rs5210 in *KCNJ11* and rs757110 in *ABCC8* also showed evidence for association in the stage 1 sample ( $P = 0.045$  and 0.060, respectively). However, both SNPs were in substantial LD with Glu23Lys in our sample ( $r^2 = 0.50$  and 0.88) and were no longer significant after accounting for the Glu23Lys association (rs5210: OR 1.07 [0.89–1.28],  $P = 0.44$ ; rs757110: OR 1.06 [0.69–1.62],  $P = 0.78$ ).

In the tumor necrosis factor- $\alpha$  (*TNF*) gene in the HLA region, we observed significant association with an intron 1 A/G SNP (rs1800610) ( $P = 0.0073$ ) (Table 3). Tumor necrosis factor- $\alpha$  is involved in many biological functions including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. SNPs in *TNF* have been shown to be associated with obesity or obesity-related

phenotypes in several studies (available at <http://obesity.gene.pbr.c.edu>). In our combined stage 1 and 2 samples, the OR for the risk A allele under the multiplicative model was 1.42 (95% CI 1.10–1.82). The risk allele had frequencies of 0.069 in case subjects and 0.049 in control subjects and was strongly correlated ( $r^2 = 0.994$ ) with the T allele of the -857 SNP rs1799724. The *TNF* intron 1 A/G SNP rs1800610 also showed strong association with BMI and WHR ( $P_{\text{corrected}} = 0.002$ ). Individuals with one or two copies of the type 2 diabetes risk A allele at this locus had a mean BMI of 30.3 kg/m<sup>2</sup> (SD = 5.6,  $n = 42$ ) compared with individuals with the CC genotype who had a mean BMI of 27.4 (SD = 4.1,  $n = 372$ ).

The *TNF*-308 G/A (rs1800629) SNP in this gene has previously been associated with obesity, insulin resistance and hypertension (34), type 2 diabetes (35–37), insulin area under the curve (38), fasting insulin (39), and fasting glucose-to-insulin ratios (40). However, we observed no association in our sample between the -308 SNP and type 2 diabetes ( $P = 0.47$ ) nor with BMI ( $P = 0.60$ ), fasting insulin ( $P = 0.97$ ), or 2-h insulin levels ( $P = 0.43$ ). The -308 SNP was in weak LD ( $D' = 0.999$ ,  $r^2 = 0.01$ ) with SNPs rs1800610 and rs1799724. *TNF* is in the major histocompatibility complex region, and HLA haplotypes that confer the major genetic susceptibility to type 1 diabetes may also confer susceptibility to common type 2 diabetes (41).

We observed significant association with the nonsynonymous variant Ile110Thr polymorphism in the *SLC2A2* gene ( $P = 0.014$ ) (Table 3), which is in perfect LD ( $r^2 = 1$ ) with SNPs rs5406 and rs6785803 in our sample. Association to at least one of these SNPs, although with different risk alleles, has been reported in three studies (4,42,43). *SLC2A2* encodes the glucose transporter (GLUT2) expressed in liver, kidney, intestine, and pancreatic  $\beta$ -cells and is a key regulator of insulin secretion. We obtained an OR of 2.98 [95% CI 1.31–6.79],  $P = 0.014$  in our combined stage 1 and 2 samples, similar to the that (2.08 [1.06–4.1])

observed in a previous Finnish study (43), and both were under the dominant model with the same risk allele.

We observed modest evidence for association to SNPs in *IL6*, *PCK1*, and *ENPP1* (Table 3). Interleukin-6 is a cytokine with many biological functions, including immune response, and variants in this gene have been associated with type 2 diabetes in two independent studies (44,45). We genotyped two promoter SNPs ( $-598 = rs1800797$  and  $-174 = rs1800795$ ) in strong LD with one another ( $r^2 = 0.98$ ) and observed modest evidence of association (OR 1.25 [95% CI 1.02–1.55],  $P = 0.038$ ) with the  $-174$  SNP in the combined stage 1 and 2 samples. We also observed association between the *IL6*  $-174$  C/G polymorphism and the combined quantitative traits ( $P_{corrected} = 0.022$ ). The type 2 diabetes-associated G allele was associated with decreased fasting free fatty acids ( $P_{uncorrected} = 0.0010$ ), increased disposition index ( $P_{uncorrected} = 0.0078$ ), decreased HDL ( $P_{uncorrected} = 0.0092$ ), and increased glucose effectiveness ( $P_{uncorrected} = 0.014$ ).

*PCK1* encodes an enzyme that plays an important role in gluconeogenesis by catalyzing the production of phosphoenolpyruvate. An association was reported with a  $-232$  promoter SNP (46) in a Caucasian sample (OR 2.8 [95% CI 1.7–4.7],  $P = 0.0003$ ). We identified a modest association in our sample with the same allele of *rs2071023* (1.27 [1.02–1.57],  $P = 0.031$ ). Mutations in the *ENPP1* gene, which encodes a membrane protein that cleaves nucleotides, have been shown to be associated with insulin resistance (47–49) and type 2 diabetes (50,51). We also observed modest association to the *ENPP1* Lys121Gln polymorphism (*rs1044498*, 1.24 [1.01–1.51],  $P = 0.038$ ).

We genotyped nine SNPs in the MODY gene *HNF1A/TCF1* that were previously reported to be associated with type 2 diabetes in a large set of stage 1 samples (8,9). Although we observed association with two SNPs, *rs2701175* and *GE117884\_349* ( $P = 0.015$  and  $P = 0.050$ ), the level of significance was attenuated after correcting for the 9 SNPs tested here ( $P = 0.056$ ) and became nonsignificant after correcting for the 20 SNPs ( $P = 0.21$ ) selected as tag SNPs to assess the entire gene for association in a different study from our group (18). After analysis of the 20 tag SNPs, we did observe significant association to SNP *rs2071190* in *HNF1A/TCF1*. Similarly, we observed modest evidence for association to the previously reported Thr45Ala SNP in the MODY gene *NEUROD1* (OR 1.15 [95% CI 1.01–1.30],  $P = 0.033$ ), but we obtained much stronger association evidence to the tag SNP *rs3916026* 5.4 kb upstream of the gene (18).

## DISCUSSION

We have carried out an unbiased replication of SNPs reported to be associated with type 2 diabetes before May 2005 based on our review of the literature. We assessed 114 SNPs in a sample of up to 2,155 Finnish individuals, and we observed 12 significantly associated SNPs in 9 genes, which is in excess of expectation (one-sided  $P = 0.012$ ). The two SNPs that showed the strongest evidence for association in our sample were those most commonly found to be associated with type 2 diabetes thus far: the *PPARG* Pro12Ala variant and the *KCNJ11* Glu23Lys variant. We also found an intron 1 SNP in *TNF* to be associated with type 2 diabetes, as well as with BMI and WHR, providing evidence that polymorphisms other than the commonly studied  $-308$  polymorphism in this gene may

influence risk of type 2 diabetes and obesity-related traits. We identified association to a *SLC2A2* Thr110Ile variant. The *SLC2A2* gene encodes the GLUT2 high  $K_m$  glucose transporter protein primarily responsible for glucose sensing in the pancreatic  $\beta$ -cells. We also observed modest evidence for association with the *ENPP1* Lys121Gln polymorphism, *PCK1*  $-232$  SNP, and the *IL6*  $-598$  SNP. We identified association with SNPs in the MODY genes *NEUROD1* and *HNF1A/TCF1*; these genes are assessed in a more comprehensive manner in another report by our group (18).

We did not replicate most of the previously reported associations between SNPs and type 2 diabetes. This is consistent with findings from attempted replications in other complex diseases (52,53) such as rheumatoid arthritis (54). Some of the previous reports of association may have been false-positive because of low stringency thresholds for declaring significance or mismatching of ancestry between case subjects and control subjects (55). Furthermore, when a polymorphism in moderate LD with the actual causal variant is being tested, variable LD patterns in different ancestral populations may result in increased or decreased power to detect the association. Other reasons for discordance in replication include differences among samples because of ancestral heterogeneity, disease heterogeneity, and ascertainment (52).

The possibility of false-negative results in our sample cannot be excluded. However, our Finnish sample had  $\geq 78\%$  power to detect variants with ORs  $\geq 1.3$  and MAFs  $\geq 10\%$ . The estimated OR in the original study was  $> 1.3$  for 84% of SNPs (81 of 96) for which ORs were available. Samples that were only moderately powered to detect smaller risk effect sizes probably resulted in inflated OR estimates in the original reports (53). Our moderate sample size is not well powered to detect common variants with ORs  $< 1.2$ . Our primary Finnish sample was derived from a sample of affected sibling pair families, and the enrichment of case subjects with a positive family history may be associated with a different set of susceptibility genes compared with other type 2 diabetic individuals. The case subjects and control subjects in our study were matched for age and sex and, whenever possible, birth province in Finland. We expect little, if any, influence of population substructure on our findings.

In five of the nine genes that showed association to type 2 diabetes in our analysis, *PPARG*, *KCNJ11*, *SLC2A2*, *NEUROD1*, and *ENPP1*, the associated variants were nonsynonymous coding SNPs with the strong possibility of having functional consequences. However, it is possible that other SNPs in LD with the associated SNPs are the true functional variants. Further work exploring other variants in these genes in this sample and others and experiments addressing genetic changes and their relationship to expression levels or protein function will help identify the true causal variants.

We examined whether the likelihood of replicating type 2 diabetes association of a previously associated SNP in our sample was related to a priori significance levels in the original report. We considered a “replication” as any SNP with a type 2 diabetes association  $P < 0.05$  and included the four monomorphic SNPs as not showing association in our sample. There were 43 SNPs from 13 genes in which we identified  $\geq 3$  reported associations for any SNP in that gene and replicated 19% of these findings (8 of 43). This was significantly greater than the replication rate of 5.6% (4 of 71) for SNPs in genes with only one or two previous

reports ( $P = 0.032$ ). We also compared the cumulative number of times that a report of association for a particular gene was referenced in the literature and segregated genes into those with association reports that had been cited  $\geq 100$  times or  $< 100$  times. We found no difference in the rate of replication using this criterion (4 of 27 vs. 8 of 87,  $P = 0.31$ ). Finally, when attempting to replicate SNPs that encode putative nonsynonymous changes, we observed no difference in the rate of replication compared with that of other SNPs (5 of 32 vs. 7 of 82,  $P = 0.22$ ).

Among the 99 SNPs in which the previous reports unambiguously suggested a type 2 diabetes-associated risk allele, we found 13 SNPs for which the same allele was associated with increased risk of type 2 diabetes in our sample. In contrast, we found only two SNPs for which the nonrisk allele would have shown significant association ( $P < 0.05$ ). Confirmation of the same risk allele in our sample as in the original report was significantly more likely than association to the opposite allele ( $P = 0.003$ ). These results are based on the stage 1 sample only to ensure that no bias resulted from our selection of SNPs for stage 2 genotyping.

Although we did not replicate many of the previously reported associations with type 2 diabetes in our Finnish sample, we did find an excess of significantly associated SNPs in our sample compared with expectations under the null hypothesis of no association. If we assume type I and II error rates of 0.05 and 0.22 for our study, respectively, and assume arbitrarily that 5% (or 1%) of the previously reported associations are true positives, then the false-positive report probability (26) is estimated to be 54.9% (or 86.3%). This suggests that less than half of the significant associations in our sample are true positives, and additional study will be required to determine their true role in type 2 diabetes susceptibility.

In summary, we observed strong evidence in our sample for replication of the most widely replicated associations in type 2 diabetes, *PPARG* Pro12Ala and *KCNJ11* Glu23Lys. We replicated association to variants in *TNF*, a gene in which extensive association with obesity and diabetes-related phenotypes has been shown (34). We also observed significant evidence for association to *SLC2A2*, recently reported in another Finnish sample (43). We found modest evidence for type 2 diabetes association with SNPs in *PCK1*, *ENPP1*, and *IL6*. Given the excess of significant type 2 diabetes-SNP associations in our samples, we expect some of these SNPs to be true type 2 diabetes susceptibility variants or in strong LD with such variants.

As we move toward even larger-scale testing of type 2 diabetes-SNP associations, soon on a genome-wide scale, these results highlight the importance of several methodological principles. For individual studies to have acceptable power to detect association and reduce the reporting of false-positive association, large sample sizes and suitable significance thresholds are critical. Furthermore, genome-wide association studies are likely to identify new variants with unknown functional consequences in novel genes that may be less well characterized for their potential role in type 2 diabetes. Replication in multiple independently ascertained datasets and functional follow-up studies will be critical for determining which variants identified by genome-wide association studies are true susceptibility variants.

## ACKNOWLEDGMENTS

The FUSION study was funded by National Institutes of Health (NIH) Grants DK62370 to M.B. and DK72193 to K.L.M. and intramural funds from the National Human Genome Research Institute (Project 1Z01HG000024-11). J.T. was partially supported by the Academy of Finland (38387 and 46558). K.L.M. is supported by a Burroughs Wellcome Career Award in the Biomedical Sciences. R.N.B. is supported by NIH Grants DK27619 and DK29867. R.M.W. is supported by the American Diabetes Association.

We are thankful to the Finnish citizens who generously participated in the FUSION study. We also thank our colleagues at the Center for Inherited Disease Research, particularly Craig Bark and Ya-Yu Tsai for their assistance with genotyping, Peggy White for assistance with the literature search, Terry Gliedt for computer systems management, and other FUSION collaborators for their advice and technical assistance.

## REFERENCES

- Hirschhorn JN, Altshuler D: Once and again—issues surrounding replication in genetic association studies. *J Clin Endocrinol Metab* 87:4438–4441, 2002
- Hirschhorn JN, Daly MJ: Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 6:95–108, 2005
- Barroso I: Genetics of type 2 diabetes. *Diabet Med* 22:517–535, 2005
- Barroso I, Luan J, Middelberg RP, Harding AH, Franks PW, Jakes RW, Clayton D, Schafer AJ, O'Rahilly S, Wareham NJ: Candidate Gene Association Study in type 2 diabetes indicates a role for genes involved in  $\beta$ -cell function as well as insulin action. *PLoS Biol* 1:E20, 2003
- Evans JC, Frayling TM, Cassell PG, Saker PJ, Hitman GA, Walker M, Levy JC, O'Rahilly S, Rao PV, Bennett AJ, Jones EC, Menzel S, Prestwich P, Simecek N, Wishart M, Dhillon R, Fletcher C, Millward A, Demaine A, Wilkin T, Horikawa Y, Cox NJ, Bell GI, Ellard S, McCarthy MI, Hattersley AT: Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. *Am J Hum Genet* 69:544–552, 2001
- Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS: Genetic variation near the hepatocyte nuclear factor-4  $\alpha$  gene predicts susceptibility to type 2 diabetes. *Diabetes* 53:1141–1149, 2004
- Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA: A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4  $\alpha$  gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140, 2004
- Winckler W, Burt NP, Holmkvist J, Cervin C, de Bakker PI, Sun M, Almgren P, Tuomi T, Gaudet D, Hudson TJ, Ardlie KG, Daly MJ, Hirschhorn JN, Altshuler D, Groop L: Association of common variation in the HNF1 $\alpha$  gene region with risk of type 2 diabetes. *Diabetes* 54:2336–2342, 2005
- Weedon MN, Owen KR, Shields B, Hitman G, Walker M, McCarthy MI, Hattersley AT, Frayling TM: A large-scale association analysis of common variation of the HNF1 $\alpha$  gene with type 2 diabetes in the U.K. Caucasian population. *Diabetes* 54:2487–2491, 2005
- Minton JA, Hattersley AT, Owen K, McCarthy MI, Walker M, Latif F, Barrett T, Frayling TM: Association studies of genetic variation in the WFS1 gene and type 2 diabetes in U.K. populations. *Diabetes* 51:1287–1290, 2002
- Skol AD, Scott LJ, Abecasis GR, Boehnke M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 38:209–213, 2006
- Valle T, Tuomilehto J, Bergman RN, Ghosh S, Hauser ER, Eriksson J, Nylund SJ, Kohtamaki K, Toivanen L, Vidgren G, Tuomilehto-Wolf E, Ehnholm C, Blaschak J, Langefeld CD, Watanabe RM, Magnuson V, Ally DS, Hagopian WA, Ross E, Buchanan TA, Collins F, Boehnke M: Mapping genes for NIDDM: design of the Finland-United States Investigation of NIDDM Genetics (FUSION) study. *Diabetes Care* 21:949–958, 1998
- Silander K, Scott LJ, Valle TT, Mohlke KL, Stringham HM, Wiles KR, Duren WL, Doheny KF, Pugh EW, Chines P, Narisu N, White PP, Fingerlin TE, Jackson AU, Li C, Ghosh S, Magnuson VL, Colby K, Erdos MR, Hill JE, Hollstein P, Humphreys KM, Kasad RA, Lambert J, Lazaridis KN, Lin G,

- Morales-Mena A, Patzkowski K, Pfahl C, Porter R, Rha D, Segal L, Suh YD, Tovar J, Unni A, Welch C, Douglas JA, Epstein MP, Hauser ER, Hagopian W, Buchanan TA, Watanabe RM, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A large set of Finnish affected sibling pair families with type 2 diabetes suggests susceptibility loci on chromosomes 6, 11, and 14. *Diabetes* 53:821–829, 2004
14. *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Organization, 1985
  15. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation*. Geneva, World Health Organization, 1999
  16. Saaristo T, Peltonen M, Lindstrom J, Saarikoski L, Eriksson J, Tuomilehto J: Cross-sectional evaluation of the Finnish Diabetes Risk Score: a tool to identify undetected type 2 diabetes, abnormal glucose tolerance and metabolic syndrome. *Diab Vasc Dis Res* 2:67–72, 2005
  17. Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, Hansen M, Steemers F, Butler SL, Deloukas P, Galver L, Hunt S, McBride C, Bibikova M, Rubano T, Chen J, Wickham E, Doucet D, Chang W, Campbell D, Zhang B, Kruglyak S, Bentley D, Haas J, Rigault P, Zhou L, Stuelpnagel J, Chee MS: Highly parallel SNP genotyping. *Cold Spring Harb Symp Quant Biol* 68:69–78, 2003
  18. Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS: A genome-wide scalable SNP genotyping assay using microarray technology. *Nat Genet* 37:549–554, 2005
  19. Conneely KN, Silander K, Scott LJ, Mohlke KL, Lazaridis KN, Valle TT, Tuomilehto J, Bergman RN, Watanabe RM, Buchanan TA, Collins FS, Boehnke M: Variation in the resistin gene is associated with obesity and insulin-related phenotypes in Finnish subjects. *Diabetologia* 47:1782–1788, 2004
  20. Bonnycastle LL, Willer CJ, Conneely KN, Jackson AU, Burrill CP, Watanabe RM, Chines PS, Narisu N, Scott LJ, Enloe ST, Swift AJ, Duren WL, Stringham HM, Erdos MR, Riebow NL, Buchanan TA, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Boehnke M, Collins FS: Common variants in maturity-onset diabetes of the young genes contribute to risk of type 2 diabetes in Finns. *Diabetes* 55:2534–2540, 2006
  21. Ghosh S, Langefeld CD, Ally D, Watanabe RM, Hauser ER, Magnuson VL, Nylund SJ, Valle T, Eriksson J, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: The W64R variant of the  $\beta_3$ -adrenergic receptor is not associated with type II diabetes or obesity in a large Finnish sample. *Diabetologia* 42:238–244, 1999
  22. Fingerlin TE, Erdos MR, Watanabe RM, Wiles KR, Stringham HM, Mohlke KL, Silander K, Valle TT, Buchanan TA, Tuomilehto J, Bergman RN, Boehnke M, Collins FS: Variation in three single nucleotide polymorphisms in the calpain-10 gene not associated with type 2 diabetes in a large Finnish cohort. *Diabetes* 51:1644–1648, 2002
  23. Douglas JA, Erdos MR, Watanabe RM, Braun A, Johnston CL, Oeth P, Mohlke KL, Valle TT, Ehnholm C, Buchanan TA, Bergman RN, Collins FS, Boehnke M, Tuomilehto J: The peroxisome proliferator-activated receptor- $\gamma$ 2 Pro12A1a variant: association with type 2 diabetes and trait differences. *Diabetes* 50:886–890, 2001
  24. Wei LJ, Lin DY, Weissfeld L: Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. *J Am Stat Assoc* 84:1065–1073, 1989
  25. Cheverud JM: A simple correction for multiple comparisons in interval mapping genome scans. *Heredity* 87:52–58, 2001
  26. Wacholder S, Chanock S, Garcia-Closas M, El Ghomli L, Rothman N: Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 96:434–442, 2004
  27. Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS: Truncated product method for combining P-values. *Genet Epidemiol* 22:170–185, 2002
  28. Abecasis GR, Cookson WO: GOLD—graphical overview of linkage disequilibrium. *Bioinformatics* 16:182–183, 2000
  29. Xu ZK, Chen NG, Ma CY, Meng ZX, Sun YJ, Han X: Role of peroxisome proliferator-activated receptor  $\gamma$  in glucose-induced insulin secretion. *Acta Biochim Biophys Sin (Shanghai)* 38:1–7, 2006
  30. Florez JC, Hirschhorn J, Altshuler D: The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. *Annu Rev Genomics Hum Genet* 4:257–291, 2003
  31. Gloyn AL, Siddiqui J, Ellard S: Mutations in the genes encoding the pancreatic  $\beta$ -cell K(ATP) channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat* 27:220–231, 2006
  32. Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Polymorphisms of the SUR1 (ABCC8) and Kir6.2 (KCNJ11) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *J Clin Endocrinol Metab* 89:6286–6290, 2004
  33. Parikh H, Groop L: Candidate genes for type 2 diabetes. *Rev Endocr Metab Disord* 5:151–176, 2004
  34. Sookoian SC, Gonzalez C, Pirola CJ: Meta-analysis on the G-308A tumor necrosis factor  $\alpha$  gene variant and phenotypes associated with the metabolic syndrome. *Obes Res* 13:2122–2131, 2005
  35. Heijmans BT, Westendorp RG, Droog S, Klufft C, Knook DL, Slagboom PE: Association of the tumour necrosis factor  $\alpha$ -308G/A polymorphism with the risk of diabetes in an elderly population-based cohort. *Genes Immun* 3:225–228, 2002
  36. Daimon M, Ji G, Saitoh T, Oizumi T, Tominaga M, Nakamura T, Ishii K, Matsuura T, Inageda K, Matsumine H, Kido T, Htay L, Kamatani N, Muramatsu M, Kato T: Large-scale search of SNPs for type 2 DM susceptibility genes in a Japanese population. *Biochem Biophys Res Commun* 302:751–758, 2003
  37. Zouari Bouassida K, Chouchane L, Jellouli K, Cherif S, Haddad S, Gabbouj S, Danguir J: Polymorphism of stress protein HSP70–2 gene in Tunisians: susceptibility implications in type 2 diabetes and obesity. *Diabete Metab* 30:175–180, 2004
  38. Nicaud V, Raoux S, Poirier O, Cambien F, O'Reilly DS, Tiret L: The TNF  $\alpha$ /G-308A polymorphism influences insulin sensitivity in offspring of patients with coronary heart disease: the European Atherosclerosis Research Study II. *Atherosclerosis* 161:317–325, 2002
  39. Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID: Association of the TNF- $\alpha$ -308 G/A promoter polymorphism with insulin resistance in obesity. *Obes Res* 10:401–407, 2002
  40. Jaquet D, Czernichow P: Born small for gestational age: increased risk of type 2 diabetes, hypertension and hyperlipidaemia in adulthood. *Horm Res* 59 (Suppl. 1):131–137, 2003
  41. Tuomilehto-Wolf E, Tuomilehto J, Hitman GA, Nissinen A, Stengard J, Pekkanen J, Kivinen P, Kaarsalo E, Karvonen MJ: Genetic susceptibility to non-insulin dependent diabetes mellitus and glucose intolerance are located in HLA region. *BMJ* 307:155–159, 1993
  42. Alcolado JC, Baroni MG, Li SR: Association between a restriction fragment length polymorphism at the liver/islet cell (GluT 2) glucose transporter and familial type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 34:734–736, 1991
  43. Laukkanen O, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Polymorphisms in the SLC2A2 (GLUT2) gene are associated with the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes* 54:2256–2260, 2005
  44. Illig T, Bongardt F, Schopfer A, Muller-Scholz S, Rathmann W, Koenig W, Thorand B, Vollmert C, Holle R, Kolb H, Herder C: Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. *J Clin Endocrinol Metab* 89:5053–5058, 2004
  45. Vozarova B, Fernandez-Real JM, Knowler WC, Gallart L, Hanson RL, Gruber JD, Ricart W, Vendrell J, Richart C, Tataranni PA, Wolford JK: The interleukin-6 (-174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. *Hum Genet* 112: 409–413, 2003
  46. Cao H, van der Veer E, Ban MR, Hanley AJ, Zinman B, Harris SB, Young TK, Pickering JG, Hegele RA: Promoter polymorphism in PCK1 (phosphoenolpyruvate carboxykinase gene) associated with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 89:898–903, 2004
  47. Bacci S, Ludovico O, Prudente S, Zhang YY, Di Paola R, Mangiacotti D, Rauseo A, Nolan D, Duffy J, Fini G, Salvemini L, Amico C, Vigna C, Pellegrini F, Menzaghi C, Doria A, Trischitta V: The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. *Diabetes* 54:3021–3025, 2005
  48. Abate N, Carulli L, Cabo-Chan A Jr, Chandalia M, Snell PG, Grundy SM: Genetic polymorphism PC-1 K121Q and ethnic susceptibility to insulin resistance. *J Clin Endocrinol Metab* 88:5927–5934, 2003
  49. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V: A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. *Diabetes* 48:1881–1884, 1999
  50. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoer C, Vatin V, Ghossaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P: Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. *Nat Genet* 37:863–867, 2005
  51. Hamaguchi K, Terao H, Kusuda Y, Yamashita T, Hazoury Bahles JA, Cruz

- LM, Brugal VL, Jongchong WB, Yoshimatsu H, Sakata T: The PC-1 Q121 allele is exceptionally prevalent in the Dominican Republic and is associated with type 2 diabetes. *J Clin Endocrinol Metab* 89:1359–1364, 2004
52. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG: Replication validity of genetic association studies. *Nat Genet* 29:306–309, 2001
53. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177–182, 2003
54. Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, Wolfe F, Kastner DL, Alfredsson L, Altshuler D, Gregersen PK, Klareskog L, Rioux JD: Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet* 77:1044–1060, 2005
55. Lander ES, Schork NJ: Genetic dissection of complex traits. *Science* 265:2037–2048, 1994