Genome-Wide Association Study Identifies a Novel Locus Contributing to Type 2 Diabetes Susceptibility in Sikhs of Punjabi Origin From India

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

Citation

Published Version
doi:10.2337/db12-1077

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:12407045

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
We performed a genome-wide association study (GWAS) and a multistage meta-analysis of type 2 diabetes (T2D) in Punjabi Sikhs from India. Our discovery GWAS in 1,616 individuals (842 case subjects) was followed by in silico replication of the top 513 independent single nucleotide polymorphisms (SNPs) ($P < 10^{-5}$) in Punjabi Sikhs ($n = 2,819; 801$ case subjects). We further replicated 66 SNPs ($P < 10^{-4}$) through genotyping in a Punjabi Sikh sample ($n = 2,894; 1,711$ case subjects). On combined meta-analysis in Sikh populations ($n = 7,329; 3,354$ case subjects), we identified a novel locus in association with T2D at 13q12 represented by a directly genotyped intronic SNP (rs9552911, $P = 1.82 \times 10^{-7}$) in the SGCG gene. Next, we undertook in silico replication (stage 2b) of the top 513 signals ($P < 10^{-5}$) in 29,157 non-Sikh South Asians (10,971 case subjects) and de novo genotyping of up to 31 top signals ($P < 10^{-4}$) in 10,817 South Asians (5,157 case subjects) (stage 3b). In combined South Asian meta-analysis, we observed six suggestive associations ($P < 10^{-4}$ to $< 10^{-3}$), including SNPs at HMG1L1/CTCFL, PLXNA4, SCAP, and chr9p11. Further evaluation of 31 top SNPs in 33,707 East Asians (16,746 case subjects) (stage 3c) and 47,117 Europeans (8,130 case subjects) (stage 3d), and joint meta-analysis of 128,127 individuals (44,358 case subjects) from 27 multiethnic studies, did not reveal any additional loci nor was there any evidence of replication for the new variant. Our findings provide new evidence on the presence of a population-specific signal in relation to T2D, which may provide additional insights into T2D pathogenesis. Diabetes 62:1746–1755, 2013

S
outh Asians (people originating from the Indian subcontinent) comprise more than a quarter of the global population and contribute the highest number of patients with type 2 diabetes (T2D) (1). According to latest estimates, ~61 million people in India alone are currently afflicted with T2D, and their number is projected to increase to ~101 million by 2030 (2). Consequently, ~60% of the world’s coronary artery disease

From the 1Center for Human Genetic Research and Department of Anesthesiology, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; the 2Center for Non-Communicable Diseases, Karachi, Pakistan; the 3Department of Public Health and Primary Care, University of Cambridge, Cambridge, U.K.; the 4Department of Biostatistics and Department of Epidemiology and Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; the 5Department of Pediatrics, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; the 6Center for Statistical Genetics and Department of Statistics, University of Michigan, Ann Arbor, Michigan; the 7Center for Molecular Epidemiology, National University of Singapore, Singapore; the 8Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, U.K.; the 9Madras Diabetes Research Foundation, Chennai, India; the 10Medical College of Dental Sciences, University of Birmingham, Birmingham, U.K.; the 11Diabetes Centre, Heart of England National Health Service Trust, Birmingham, U.K.; the 12Saw Swee Hock School of Public Health, National University of Singapore, Singapore; the 13Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; the 14Department of Ophthalmology, National University of Singapore, Singapore; the 15Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia; the 16Department of Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; the 17Department of Integrated Molecular Science on Metabolic Diseases, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo, Japan; the 18Department of Internal Medicine, Division of Metabolism and Endocrinology, St. Mariana University School of Medicine, Kagawa, Japan; the 19Health Center, Keio University School of Medicine, Tokyo, Japan; the 20Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K.; the 21Oxford Institute for Health Research Biomedical Research Centre, Churchill Hospital, Oxford, U.K.; the 22Baqai Institute of Diabetology and Endocrinology, Karachi, Pakistan; the 23Diabetes Research Unit, Department of Clinical Medicine, University of Colombo, Colombo, Sri Lanka; the 24Dr. Mohan’s Diabetes Specialities Centre, Chennai, India; the 25Hero Dayanand Medical College and Heart Institute, Ludhiana, Punjab, India; the 26Central University of Punjab, Bathinda, Punjab, India; the 27All India Institute of Medical Sciences and Research, New Delhi, India; the 28Department of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania; the 29Department of Medicine, Shiga University of Medical Science, Shiga, Japan; the 30First Department of Internal Medicine, University of Toyama, Toyama, Japan; the 31Laboratory for Endocrinology and Metabolism,RIKEN Center for Genomic Medicine, Kanagawa, Japan; the 32Department of Biomedical Science, Hallym University, Chuncheon, Gangwon-do 200-702, Republic of Korea; the 33Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; the 34Duke-NUS Graduate Medical School Singapore, Singapore; the 35Eating Hospital National Health Service Trust, Middlesex, U.K.; the 36Imperial College Healthcare National Health Service Trust, London, U.K.; the 37Imperial College Healthcare National Health Service Trust, London, U.K.;38Epidemiology and Biostatistics, Imperial College London, London, U.K.; the 39National Heart and Lung Institute, Imperial College London, Hamme-smith Hospital National Health Service Trust, London, U.K.; the 40Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Corresponding author: Dharambir K. Sanghera, dharambir-sanghera@ouhs.c

Received 13 August 2012 and accepted 22 November 2012. DOI: 10.2337/db12-1077

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db12-1077/-/DC1.

See accompanying commentary, p. 1369.
incidence of T2D and CAD is about three to five times higher in immigrant South Asians compared with Europeans (5–7), and the age of onset of T2D is roughly a decade earlier in South Asians than in Europeans (5–7). The higher prevalence of T2D among South Asians settled in developed countries compared with the host population reflects the genetic and ethnic predisposition to cardiometabolic disease under an adverse environment and the joint effects of genes and environment in the predisposition to T2D (8). For these reasons, we conducted ethnic-specific genetic studies in a Sikh population to dissect genetic pathways that may contribute to T2D etiology in different ethnic groups.

The vast majority of genome-wide association studies (GWAS) on T2D so far have been performed on Europeans. Studies on non-European populations, especially those with unique demographic and cultural histories, are important for identifying population-specific linkage disequilibrium (LD) patterns and environmental factors that may modulate disease risk or protection (9). Interestingly, many, but not all, of the common loci originally identified in Europeans have been replicated in non-European groups (10–18). Recent GWAS in non-European populations have yielded intriguing new variants (19–21), including six novel signals in South Asians represented by single nucleotide polymorphisms (SNPs) near GRB14, ST6GAL1, VPS26A, HMG20A, AP3S2, and HNF4A in our recent meta-analysis of GWAS (22). Given the existence of marked genetic variability among South Asian communities, in addition to diversity in culture, language, caste system, physical appearance, and diet, they do not constitute a single homogeneous community (23). Therefore, screening populations with a different genetic and racial background or environmental exposures may improve insights about the disease and genetic risk factors (24).

People from India have a complex racial history complicated by the presence of a caste system that has prohibited interbreeding to a great extent and consequently separated people into numerous endogamous groups (25). The Sikhs, a relatively young, inbred population of ~26 million (2% of the Indian population), are from the northwestern province of India and follow a distinct and unique religion born ~500 years ago in Punjab. They have an interesting background for “nontraditional” disease enrichment in the absence of conventional risk factors such as smoking, obesity, and a diet rich in meats (26). Sikhs do not smoke or chew tobacco because of religious and cultural compulsions, and ~50% of them are lifelong vegetarians. Despite the absence of these lifestyle-related risk factors, T2D and CAD have reached epidemic proportions in Sikhs. Our initial genetic studies in a Sikh cohort as part of the Asian Indian Diabetic Heart Study (AIDHS) or the Sikh Diabetes Study (SDS) revealed an association of FTO and MTNR1B, ADIPOQ, and PPARG polymorphisms with T2D and risk factors in the absence of obesity (11,27,28). In this investigation, we conducted a GWAS in a relatively homogenous Punjabi Sikh population of 1,850 individuals and performed multistage replication in up to 27 case-control studies of Punjabi, other South Asian, East Asian, and Caucasian ancestries (total n = 128,127; 44,358 T2D case and 83,769 control subjects) (Supplementary Tables 1 and 2). Study design of the discovery, replication, and meta-analysis phases was optimized to detect new population-specific and multiethnic T2D loci (Fig. 1). One important difference in the current study from our previous South Asian GWAS (22) is that in the previous study, the SNPs that were common between South Asians and Europeans were selected for replication based on the European Diabetes Genetics Replication and Meta-analysis (DIAGRAM) sample. However, in this study, the SNP selection was prioritized based on the top signals (P < 10−5) from our discovery Sikh cohort.

RESEARCH DESIGN AND METHODS

Participants. Participants were part of the Punjabi Sikh GWAS.

Study design and characteristics. Our primary Sikh GWAS (discovery) cohort used in this investigation is comprised of 1,616 individuals from the Punjabi Sikh population that was a part of the AIDHS (also named the SDS). The AIDHS/SDS has unique characteristics that are ideal for genetic studies. Sikhs are strictly a nonsmoking population, and ~50% of participants are teetotalers and lifelong vegetarians. All individuals for the GWAS discovery cohort were recruited from one geographical location. Diagnosis of T2D was confirmed by medical records, clinical examination, and testing for measuring fasting glucose levels according to the guidelines of the American Diabetes Association (29), as described previously (11). Data on lipids, insulin, glucose, anthropometric measurements, education, socioeconomic status, job grade, diet, and physical activity were available on >95% of the AIDHS/SDS individuals selected for this study. Dietary questions involving alcohol consumption were scored using a scale from 0 to 5; details are described elsewhere (26). T2D is often asymptomatic and remains undiagnosed for many years, especially in people from the developing world due to poor healthcare provisions. Therefore, it is reasonable to assume that the actual age of onset of T2D in Sikhs may range from 30 to 42 years of age compared with the observed age at diagnosis (46 years). This age is in sharp contrast to the mean age at onset of 60 years or above in developed countries (26,30). A medical record indicating either 1) a fasting plasma glucose level ≥7.0 mmol/L (126 mg/dL) after a minimum 12-h fast or 2) a 2-h postglucose level of ≥11.1 mmol/L (≥200 mg/dL) estimated during a 2-h oral glucose tolerance test on more than one occasion, combined with symptoms of diabetes, confirmed the diagnosis. Impaired fasting glucose is defined as a fasting blood glucose level ≥5.6 mmol/L (≥100 mg/dL) but <7.0 mmol/L (<126 mg/dL). Impaired glucose tolerance is defined as a 2-h OGTT >7.5 mmol/L (>140 mg/dL) but <11.1 mmol/L (<200 mg/dL). The 2-h OGTTs were performed according to the criteria of the World Health Organization (75-g oral load of glucose). BMI was calculated as weight (kg)/height (m)2, and waist-to-hip ratio was calculated as the ratio of abdomen or waist circumference to hip circumference. Subjects with type 1 diabetes, or those with a family member with type 1 diabetes, or rare forms of T2D subtypes (maturity-onset diabetes of the young) or secondary diabetes (from, e.g., hemochromatosis or monoclonal gammopathy) were excluded from the study. The selection of control subjects was based on a fasting glucose <100.8 mg/dL or a 2-h glucose <141.0 mg/dL. Subjects with impaired fasting glucose or impaired glucose tolerance were excluded when data were analyzed for association of the variants with T2D. All blood samples were obtained at the baseline visits. All participants signed a written informed consent for the investigations. The study was reviewed and approved by the University of Oklahoma Health Sciences Center Institutional Review Board, as well as the Human Subject Protection Committee at the participating hospitals and institutes in India.

South Asian cohorts. For stage 2a replication, the Sikh component of the London Life Sciences Population (LOLPOP) study (22) comprised 2,919 individuals (801 T2D case and 2,018 control subjects). For stage 2b, the non-Sikh South Asian components of the LOLPOP and the Pakistan Risk of Myocardial Infarction (PRoMIS and the Risk Assessment for Events [RACE]) study GWAS (22) comprised 29,157 individuals (10,971 case and 18,186 control subjects) (22). Stage 3a Punjabi-specific replication was carried out on 2,894 individuals (1,711 case and 1,183 control subjects) of Punjabi ancestry from India as part of AIDHS/SDS, and replication testing among South Asians for stage 3b was carried out among 10,817 participants (5,157 case and 5,660 control subjects), which were part of the following studies: Asian Indians from the Singapore Indian Eye (SINDI) study (31), the Chennai Urban Rural Epidemiology Study (CURES) (32), the Diabetes Genetics in Pakistan (DGP) study, the UK Asian Diabetes Study (UKADS) (33), and the Sri Lankan Diabetes Study (SLDS) (34). Details of the contributing cohorts are provided in the Supplementary Data.

East Asian cohorts. Replication testing for stage 3c was carried out on a total of 30,707 East Asians, comprising 14,800 Japanese from RIKEN (n = 7,480...
Weinberg equilibrium (HWE) as were SNPs with call rate computation using the Impute 2 program (38). The multiethnic HapMap3 reference panel of estimated distribution of missing genotypes based on a set of known haplotypes and an imputation factor (A and B) was analyzed. We selected all independent association signals in the discovery sample as well as those SNPs with an information score $\geq 0.5$ in the discovery sample as well as those SNPs with an information score $\geq 0.5$ in the discovery sample.

**Stage 2 replication.** We selected all independent association signals ($P < 10^{-8}$ for lookup in GWAS of $F$) the Sikh component of the Stage 3 replication. Significant association results with $P < 10^{-8}$ based on meta-analysis of stages 1, 2a, and 2b were selected for de novo or in silico replication in Sikh, South Asian, other Asian, and European populations. In addition, we selected SNPs from a Sikh-only meta-analysis of stages 1a and 2a for genotyping in an in-house Punjabi Sikh T2D case-control population. In our previous South Asian GWAS by Kooper et al. (22), 300 of the 3,200 samples of the GWAS set (n = 1,616) SNPs were genotyped in the remaining replication set. Signals with $P < 10^{-8}$ after meta-analysis of stages 1, 2a, and 3a were also genotyped in the South Asian, other Asian, and European populations to test if they were specific to the Sikh ethnic group or spanned ethnicities. All meta-analyses were performed using a fixed-effects, inverse-variance meta-analysis (as implemented in METAL) (42) was used to combine the results for individual studies.

**Stage 3 replication.** Significant association results with $P < 10^{-8}$ based on meta-analysis of stages 1, 2a, and 2b were selected for de novo or in silico replication in Sikh, South Asian, other Asian, and European populations. In addition, we selected SNPs from a Sikh-only meta-analysis of stages 1a and 2a for genotyping in an in-house Punjabi Sikh T2D case-control population. In our previous South Asian GWAS by Kooper et al. (22), 300 of the 3,200 samples of the AIDHS/SDS (used in replication) were genotyped using Illumina 660 Quad arrays, and the remaining samples (from 1,187 case and 1,632 control subjects) were available for association testing.

**Genotyping and quality control**. Genomic DNA was extracted from buffy coats using QiaAmp blood kits (Qiagen, Chatsworth, CA) or by the salting-out method (40). Genomic DNA was extracted from buffy coats using QiaAmp blood kits (Qiagen, Chatsworth, CA) or by the salting-out method. Individuals with pi-hat $<0.5$ and samples with $<30\%$ call rate were excluded, as were SNPs with Hardy-Weinberg equilibrium (HWE) $P < 10^{-4}$ or minor allele frequency (MAF) $<1\%$. After quality control, 524,216 directly genotyped SNPs in 1,616 subjects (842 cases and 774 control subjects) were available for association testing.

**Stage 1** AIDHS/SDS Sikhs (842 cases; 774 controls)
524,216 genotyped SNPs and 707,792 imputed SNPs

**Stage 2a** LOLIPOP Sikhs GWAS
(801 cases; 2,018 controls)
66 SNPs $P < 10^{-4}$

**Stage 3a** Sikh replication
(1,711 cases; 1,183 controls)

**Stage 3b** South Asian replication
(5,157 cases; 5,660 controls)

**Stage 2b** LOLIPOP, PROMIS, RACE
South Asians GWAS
(10,971 cases; 18,186 controls)

**Stage 3d** DIAGRAM+ European GWAS
(8,130 cases; 38,987 controls)

**Stage 3c** East Asian (AGEN+)
replication/evaluation
(16,746 cases; 16,961 controls)

**Sikh Meta-analysis**
Novel locus
SGCG ($P = 1.82 \times 10^{-4}$)

**SA Meta-analysis**
Suggestive Locis
HMG1L1 ($P = 1.55 \times 10^{-10}$)
PLXNA4 ($P = 9.70 \times 10^{-9}$)
SCAP ($P = 7.40 \times 10^{-9}$)
Chr. 5p11 ($P = 7.74 \times 10^{-9}$)

**FIG. 1.** Summary of study design and outcome of key findings.
healthy female twins from the Twins UK adult registry. Whole-genome expression profiling of the samples, each with either two or three technical replicates, was performed using the Illumina HumanHT-12 v3 BeadChips according to the protocol supplied by the manufacturer. Log-transformed expression signals were normalized separately per tissue as follows. Quantile normalization was performed across technical replicates of each individual followed by quantile normalization across all individuals. Genotyping was performed with a combination of Illumina arrays HumanHap300, HumanHap610Q, 1M-Duo, and 1.2M-Duo 1M. Untyped Hapmap2 SNPs were imputed using the IMPUTE software package (v2). The number of adipose samples with genotypes and expression values is 776. Association between all SNPs (MAF >5%; IMPUTE info >0.8) within a gene or within 1 Mb of the gene transcription start or end site and normalized expression values was performed with the GenABEL/ProbABEL packages using the polygenic linear model incorporating a kinship matrix in GenABEL followed by the ProbABEL nmsscore test with imputed genotypes. Age and experimental batch were included as cofactors.

RESULTS

Punjabi Sikh discovery GWAS. Clinical characteristics of the stage 1 Punjabi Sikh T2D GWAS cohort and stage 2a and 2b (replication) cohorts are described in Supplementary Table 3. Principal components analysis revealed little population structure (Supplementary Fig. 1). After quality control, 524,216 directly genotyped SNPs in 1,616 subjects (842 case and 774 control subjects) from 1,850 total participants. We undertook a two-stage replication in T2D case-control samples of Punjabi Sikh ancestry (stages 2a and 3a in Fig. 1). Lead SNPs representing 513 novel, independent ($r^2 <0.25$) association signals with $P < 10^{-8}$ in the discovery GWAS (including only two previously known GWAS SNPs from TCF7L2 and IGF2BP2 and excluding 62 SNPs with $P < 10^{-8}$ from other known T2D loci) were tested for in silico replication in the Punjabi Sikh subcomponent of the Lollipop GWAS comprising 801 T2D case and 2,018 control subjects (Supplementary Table 1). Top SNPs representing 66 putatively novel signals with $P < 10^{-8}$ after stage 1 and 2a meta-analysis using a fixed effects, inverse-variance approach were directly genotyped in the stage 3a sample of 2,884 Punjabi Sikh individuals (1,711 T2D case and 1,183 control subjects) (Fig. 1 and Supplementary Table 2). In a combined meta-analysis of the three Punjabi studies (n = 7,329), we identified one new locus reaching genome-wide significance ($P < 5 \times 10^{-8}$) along with robust replication of the established SNP rs7903146 in TCF7L2 ($P = 3.32 \times 10^{-10}$) in Sikhs (Figs. 2, 3, and 4). This novel association signal lies in a 164-kb region of strong LD at 13q12 (harboring genes gamma-sarcoglycan [SGCG] and sacsin [SACS]) and is represented by a directly genotyped intronic SNP, rs9552911 in SGCG (odds ratio [OR] 0.67 [95% CI 0.58–0.77], $P = 1.82 \times 10^{-8}$ for the minor “A” allele) (Table 1, Fig. 4, and Supplementary Table 5). Excluding BMI from the logistic regression model did not affect the association (Supplementary Table 6). Furthermore, including five additional principal components in the model did not attenuate the signal; indeed, the effect and significance were slightly improved (Supplementary Table 6). The genetic variance ($R^2$) explained by this variant for the T2D phenotype in Punjabi Sikh discovery and replication sets was 1.57 and 1.34%, respectively. There were 15 additional independent loci with suggestive evidence ($P < 10^{-5}$ to $< 10^{-7}$) of association, including six unknown regions along with IGF2BP2, originally identified in Caucasians (43) (Supplementary Table 5). Meta-analysis results including non-Sikh Punjabis from PROMIS (Pakistan) revealed suggestive association ($P < 10^{-5}$ to $< 10^{-7}$) at SNPs from three new regions: chromosome 18q21 ZBTB7C (rs1893835), 20q13, near HMG1L1/CTCFL/RBM38/PCK1 (rs9328506), and 5p33 (rs17053082) (Supplementary Table 7). Association results for 42 previously reported T2D loci in the Punjabi cohort are summarized in Supplementary Table 14. Most loci showed consistent effect in the same direction and 33 out of 42 were associated with T2D at $P < 0.05$ in Sikhs.

Replication/evaluation and meta-analysis in other South Asians. In order to identify T2D association signals common to Punjabi and other South Asian populations, we tested the association of the 513 top independent signals ($P < 10^{-3}$) derived from the discovery cohort in GWAS from the Lollipop, PROMIS, and RACE studies as part of stage 2b replication (10,971 T2D case and 18,186 control subjects) (Fig. 1 and Supplementary Table 1). Thirty-one signals ($P < 10^{-4}$ from an interim analysis with stage 2b) were further genotyped in 10,817 South Asians (5,157 T2D and 5,660 control subjects) (Fig. 1) as part of stage 3b replication. Clinical characteristics of the stage 3 replication cohorts are described in Supplementary Table 4. Combined South Asian meta-analysis revealed nominally significant association in six SNPs with MAF >5% ($P < 10^{-4}$, but only the two previously known SNPs in TCF7L2 and IGF2BP2 reached genome-wide significance (Table 1 and Supplementary Table 8). Suggestive association signals included SNPs at chromosome 20q13, near HMG1L1/CTCFL/RBM38/PCK1 (rs9328506), and 7q32 near PLXNA4 (rs1593304), 3p21 in SCAF (rs4858889), and 5p11 (rs1315082) (Supplementary Table 8). Further studies and replication in a larger sample will be required to validate these results and identify causal variants at these loci.

Multiethnic replication and meta-analysis. To identify T2D signals spanning ethnicities, we extended the replication of 31 SNPs with $P < 10^{-4}$ in Punjabis and South Asians (stage 3b) to East Asians (AGEN+) and Europeans (DIAGRAM+) in stages 3c and 3d, respectively (Fig. 1). Upon meta-analysis of 31 loci in Asians (South Asians and AGEN+), genome-wide associations were only seen in South Asians (5,157 T2D and 5,660 control subjects) (Supplementary Table 14). Most loci showed consistent effect in the same direction and 15 out of 16 were associated with T2D at $P < 0.05$ in Sikhs.

Meta-analyses including the discovery and replication SNPs from the three Punjabi studies as part of stage 2 replication (10,971 T2D case and 18,186 control subjects) (Fig. 1) as part of stage 3b replication. Clinical characteristics of the stage 3 replication cohorts are described in Supplementary Table 4. Combined South Asian meta-analysis revealed nominally significant association in six SNPs with MAF >5% ($P < 10^{-4}$, but only the two previously known SNPs in TCF7L2 and IGF2BP2 reached genome-wide significance (Table 1 and Supplementary Table 8). Suggestive association signals included SNPs at chromosome 20q13, near HMG1L1/CTCFL/RBM38/PCK1 (rs9328506), and 7q32 near PLXNA4 (rs1593304), 3p21 in SCAF (rs4858889), and 5p11 (rs1315082) (Supplementary Table 8). Further studies and replication in a larger sample will be required to validate these results and identify causal variants at these loci. To identify T2D signals spanning ethnicities, we extended the replication of 31 SNPs with $P < 10^{-4}$ in Punjabis and South Asians (stage 3b) to East Asians (AGEN+) and Europeans (DIAGRAM+) in stages 3c and 3d, respectively (Fig. 1). Upon meta-analysis of 31 loci in Asians (South Asians and AGEN+), genome-wide associations were only seen in South Asians (5,157 T2D and 5,660 control subjects) (Supplementary Table 14). Most loci showed consistent effect in the same direction and 15 out of 16 were associated with T2D at $P < 0.05$ in Sikhs.
the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) study on glycemic trait GWAS (44,45) revealed several nominal associations of SNPs with fasting blood glucose and 2-h glucose levels (Supplementary Fig. 3). Some of these SNPs also showed an association with fasting blood glucose and waist or waist-to-hip ratio in Sikhs (Supplementary Table 11), but none of these were in LD ($r^2 > 0.20$) with our lead SNP.

**Gene expression studies.** We examined the expression of SGCG and neighboring genes (FLJ46358, MIPEP, SACS, and sTNFRSF19) within 1 Mb of the index SNP by cis expression quantitative trait locus (eQTL) analysis using adipose tissue, skin, and lymphoblastic cell line gene expression data from the MuTHER Consortium, comprising healthy female twins of European ancestry from Britain. Several SNPs in the SGCG region were associated with...
significantly elevated ($P_{eQTL} < 10^{-4}$ to $< 10^{-9}$) expression of SGCG mRNA in adipose tissues (Supplementary Table 12 and Supplementary Fig. 4). One adipose eQTL from MuTHER (rs572303, $P_{eQTL} = 5.47 \times 10^{-4}$) located within SGCG showed a nominally significant association with increased waist circumference in Sikhs ($\beta = 0.67, P = 5.2 \times 10^{-2}$) (Supplementary Table 11). As shown in Supplementary Fig. 4, the LD patterns in the region (~1.46 Mb) surrounding the SGCG variant (rs9552911) varied in East Asians (JPT), Africans (YRI), Caucasians (CEU), Gujarati Indians (GIH), and Sikhs. Interestingly, in Caucasians and Yorubians, this variant was monomorphic. However, several alternative SNPs from this region in Europeans were nominally associated with fasting blood glucose (MAGIC study, $r^2$ ranging from 0.10 to 0.20 with the index SNP [rs9552911]) and mRNA expression of adipose cells in Sikhs.

**FIG. 3.** A: Regional association plot for a new T2D locus detected at 13q12 in the SGCG gene from the genome-wide meta-analysis in Sikhs. B: A strong confirmation of SNPs in the TCF7L2 gene in Sikh meta-analysis. In these plots, the SNPs showing the most strongly associated signal are depicted as a red diamond with blue border for the combined stage 1, 2a, and 3a results for meta-analysis, and the red diamond with black border shows evidence of association for the stage 1 results. Each square in color shows a SNP with the color scale relating the $r^2$ value for that SNP and the top SNP taken from the HapMap 3 GIH panel. We present LD using the GIH panel, the closest HapMap population to the Sikhs; however, we note that there could still be differential LD between the reference panel and the Sikh population. At the bottom of the plot, the locations of known genes in the region are shown.
the MuTHER study ($r^2$ ranging from 0.14 to 0.26 with the index SNP). These data suggest that population differences may underlie the weak LD. It is possible that a single causal variant may be responsible for these associations, but LD may differ between Sikhs, Europeans, and other populations.

**Comparative analysis of autozygosity.** We further looked to compare the distributions of inbreeding coefficients and autozygosity as described by Nalls et al. (41). As expected, the inbreeding coefficients in our sample were higher compared with two outbred populations of European Americans, Coriell, and Baltimore Longitudinal Study of Aging (BLSA) ($F = 0.041 \pm 0.018$ in Sikhs vs. $F = 0.007 \pm 0.019$ in Coriell and $F = -0.3 \pm 0.012$ in BLSA), as assessed by Nalls et al. (41). However, these results were similar to other Indian populations previously reported by Reich et al. (46). No significant difference in inbreeding was observed between case and control subjects ($P = 0.59$). Autozygosity analysis determined that there were 19 $\pm 7$ homozygous segments $>1$ Mb in length, with an average length of $2.0 \pm 0.95$ Mb. Hence, fewer but longer autozygous segments were found in our population than in outbred populations. No correlation of measures of autozygosity to age was observed ($P > 0.05$) across decades of age.

**DISCUSSION**

In this GWAS and multistage meta-analysis, a novel locus at 13q12 in the *SGCG* (rs9552911, $P = 1.82 \times 10^{-8}$) was identified as associated with T2D susceptibility in Punjabi Sikhs from Northern India. *SGCG* is a member of the sarcoglycan complex of transmembrane glycoproteins mutated in autosomal recessive muscular dystrophy, in particular limb-girdle muscular dystrophy type 2C (LGMD2C). *SGCG* is expressed in skeletal muscle, and its high expression is also seen in vascular smooth muscle cells as well as in breast cancer cell lines (47,48). Founder mutations in *SGCG* that cause LGMD2C predate migration of the Romani gypsies of Europe out of India around 1100 AD (49). Due to complete endogamy, this genetically isolated community had an increased incidence of autosomal recessive LGMD2C. *SGCG*-targeted knockout mice displayed a variety of phenotypes, including dystrophic cardiomyopathy and defects in skeletal muscle, metabolism, homeostasis, growth, apoptosis, aging, and behavior (50–53). Mice lacking the sarcoglycan
TABLE 1: GWAS, replication, and meta-analysis results of T2D loci identified in Punjabi Sikhs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome</th>
<th>Position (in UCSC genome build hg19)</th>
<th>Nearest gene</th>
<th>Effect/Other allele</th>
<th>EAF Punjabi Sikh Discovery GWAS (stage 1)</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9552911</td>
<td>13</td>
<td>23,864,657</td>
<td>SGCG</td>
<td>A/G</td>
<td>0.08</td>
<td>0.61 (0.47–0.80)</td>
<td>3.08×10⁻¹²</td>
<td>0.69 (0.59–0.82)</td>
<td>1.14×10⁻³</td>
<td>1.04 (1.00–1.07)</td>
<td>0.037</td>
<td>1.00 (0.98–1.03)</td>
<td>0.77</td>
<td>—</td>
<td>1.82×10⁻³</td>
</tr>
<tr>
<td>rs1470579</td>
<td>3</td>
<td>18,551,168</td>
<td>IGF2BP2</td>
<td>A/C</td>
<td>0.59</td>
<td>0.76 (0.66–0.88)</td>
<td>2.53×10⁻³</td>
<td>0.87 (0.80–0.95)</td>
<td>1.87×10⁻³</td>
<td>0.95 (0.93–0.97)</td>
<td>1.79×10⁻³</td>
<td>0.94 (0.92–0.97)</td>
<td>7.21×10⁻³</td>
<td>0.88 (0.92–0.94)</td>
<td>2.17×10⁻³</td>
</tr>
<tr>
<td>rs7903146</td>
<td>10</td>
<td>11,475,834</td>
<td>TCF7L2</td>
<td>T/C</td>
<td>0.35</td>
<td>1.31 (1.13–1.52)</td>
<td>3.23×10⁻³</td>
<td>1.50 (1.36–1.65)</td>
<td>7.83×10⁻³</td>
<td>1.13 (1.10–1.16)</td>
<td>6.12×10⁻³</td>
<td>1.12 (1.06–1.19)</td>
<td>1.06×10⁻³</td>
<td>1.40 (1.34–1.46)</td>
<td>2.21×10⁻³</td>
</tr>
</tbody>
</table>

All P values are two sided. CEU, Euro-Caucasians; EAF, effect allele frequency; Het, heterozygous.
complex including SGCG in adipose and skeletal muscle were shown to be glucose intolerant and exhibited whole-body insulin resistance due to impaired insulin-stimulated glucose uptake in skeletal muscle (54).

The allelic distribution of the less common “A” (protective) allele of rs9552911 ranged from 0.06 to 0.15 in South Asians and differed between other South Asians (0.11) and Punjabi Sikhs (0.08) (see details in Supplementary Table 13). Further replication in large independent datasets of South Asians and Punjabi Sikhs would be needed to confirm the pattern of observed association. In view of the complex racial history complicated by a well-defined caste system, Indian populations display a great deal of genetic and cultural diversity (55). Studies suggest that genetic affinity among endogamous communities in India is inversely correlated with geographic distance between them (23). Therefore, it is possible that undetected causal variant(s) or multiple rare variants in LD with this marker arose on a haplotype tagged by rs9552911 in Punjabi Sikhs after divergence from other South and West Indian populations. This variation in the index SNP rs9552911 does not appear to be of recent origin, as suggested by comparative genomic analysis (Supplementary Fig. 5). Two important nuclear hormone receptors and transcription factors (peroxisome proliferator–activated receptor-γ [PPAR-γ] [1 and 2] and PPAR-α) bind to the promoter and intron 1 of the SGCG gene. Further, the maturity-onset diabetes of young 4 (MODY4) locus at chromosome 13q12, represented by insulin promoter factor 1 or PDX-1, lies next to the SGCG locus. Therefore, further in-depth examination by targeted resequencing in the extended region and functional studies may reveal putative causative variants in this extended region and provide insight into the physiological relevance of the observed association.

In summary, our study identified a novel locus associated with T2D in a population of Punjabi Sikh ancestry from Northern India. These findings not only provide new information on previously unknown regions associated with T2D but demonstrate a putative population-specific association that could lead to additional biological insights into T2D pathogenesis.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-082766 and a seed grant from the University of Oklahoma Health Sciences Center.

No potential conflicts of interest relevant to this article were reported.


The authors thank the research participants for their contribution and support for making this study possible. The authors also thank Bansari Mehta and Braden Juengel (University of Oklahoma Health Sciences Center) and Jackie Lane (Harvard Medical School) for their superb technical support in this study. The authors thank the collaborating groups of all the contributing sites including Dr. Nadereh Jafari and staff at the Northwestern University Genomics Core (Evaston, IL) for performing genome-wide genotyping and Dr. Andrew Brooks and staff at Rutgers University (New Brunswick, NJ) for replication genotyping. Study-specific acknowledgments are provided in the Supplementary Data.

REFERENCES


27. Chidambaram M, Radha V, Mohan V. Replication of recently described type 2 diabetes gene variants in a South Indian population. Metabolism 2010;59:1760–1766


