Haplotype Structure and Genotype-Phenotype Correlations of the Sulfonylurea Receptor and the Islet ATP-Sensitive Potassium Channel Gene Region

Jose C. Florez,1,2,3,4 Noël Burtt,3 Paul I.W. de Bakker,1,3,5 Peter Almgren,6 Tinamaija Tuomi,7 Johan Holmkvist,6 Daniel Gaudet,8 Thomas J. Hudson,9 Steve F. Schaffner,3 Mark J. Daly,3 Joel N. Hirschhorn,3,5,10 Leif Groop,6 and David Altshuler1,2,3,4,5

The genes for the sulfonylurea receptor (SUR1; encoded by ABCC8) and its associated islet ATP-sensitive potassium channel (Kir6.2; encoded by KCNJ11) are adjacent to one another on human chromosome 11. Multiple studies have reported association of the E23K variant of Kir6.2 with risk of type 2 diabetes. Whether and how E23K itself—or other variant(s) in either of these two closely linked genes—influences type 2 diabetes remains to be fully determined. To better understand genotype-phenotype correlation at this important candidate gene locus, we 1) characterized haplotype structures across the gene region by typing 77 working, high-frequency markers spanning 207 kb and both genes; 2) performed association studies of E23K and nearby markers in >3,400 patients (type 2 diabetes and control) not previously reported in the literature; and 3) analyzed the resulting data for measures of insulin secretion. These data independently replicate the association of E23K with type 2 diabetes with an odds ratio (OR) in the new data of 1.17 (P = 0.003) as compared with an OR of 1.14 provided by meta-analysis of previously published, nonoverlapping data (P = 0.0002). We also found that the E23K variant in Kir6.2 demonstrates very strong allelic association with a coding variant (A1369S) in the neighboring SUR1 gene (r² > 0.9) across a range of population samples, making it difficult to distinguish which gene and polymorphism in this region are most likely responsible for the reported association. We show that E23K is also associated with decreased insulin secretion in glucose-tolerant control subjects, supporting a mechanism whereby β-cell dysfunction contributes to the common form of type 2 diabetes. Like peroxisome proliferator–activated receptor γ, the SUR1/Kir6.2 gene region both contributes to the inherited risk of type 2 diabetes and encodes proteins that are targets for hypoglycemic medications, providing an intriguing link between the underlying mechanism of disease and validated targets for pharmacological treatment. Diabetes 53:1360–1368, 2004

The sulfonylurea receptor (SUR1; encoded by ABCC8) and its associated potassium channel (Kir6.2; encoded by KCNJ11) have been implicated in susceptibility to type 2 diabetes on the basis of the clinical efficacy of sulfonylurea medications, the genetics of the rare human disorder familial hyperinsulinemic hypoglycemia of infancy (reviewed in 1), and the putative association of common variation in one or both genes with the common form of type 2 diabetes. ABCC8 and KCNJ11 reside adjacent to one another on human chromosome 11p15.1, and their gene products form a heterodimer that regulates the transmembrane potential in the pancreatic β-cell, thus providing a key intracellular signal resulting in vesicular fusion and insulin secretion (1).

Several studies have assessed the possible association of single-nucleotide polymorphisms (SNPs) in ABCC8 and KCNJ11 with type 2 diabetes. The E23K polymorphism was not significantly associated in initial reports (2–5), but studies with larger sample sizes (6–9) and subsequent meta-analyses (6,8,10,11) indicate that this variant in KCNJ11 is likely to be associated with type 2 diabetes. For example, of the three recently published large studies (8–10), Glyn et al. (8) focused on this gene and found a nominally significant association of E23K with type 2 diabetes, whereas Barroso et al. (9) examined 152 SNPs across many genes and achieved nominal statistical significance for E23K only under a recessive model and without correction for the multiple hypotheses examined. Nielsen et al. (10) failed to find significant association of E23K to type 2 diabetes but observed that E23K was associated with decreased insulin secretion in glucose-tolerant subjects.

The evidence for association of SUR1 polymorphisms is
well as the number of genetic and phenotypic models
genes does, in fact, in genetic variation somewhere in the region of these two
disequilibrium (LD) with the E23K polymorphism.

meta-analysis of all published data concerning the exon 16
mutation (R1273R) (18) in type 2 diabetes. A subsequent
silent mutation (T759T) (12,16,17), and an exon 31 silent

silent mutation in the region and examine the predictive power of these selected markers for the
remaining “hidden” SNPs. These “hidden” markers are meant to represent the
undiscovered common variants in the region (with the caveat that the
distribution of these markers is weighted on the basis of heterozy-
gosity). This sampling procedure was iterated 100 times and performed both
with a set of tag SNPs selected randomly (from among the preselected SNPs
that we genotyped in this region) and with markers chosen on the basis of
their ability to tag specific haplotypes observed across each haplotype block
(as defined above).

Clinical samples. The characteristics of three of our diabetic subsamples
have been described elsewhere (5). Briefly, they comprise 1,189 siblings
discordant for type 2 diabetes: a Scandinavian case-control sample totalling
942 subjects who were individually matched for age, BMI, and geographic
region and another individually matched case-control sample totalling 254
subjects from the Saguenay Lac-St. Jean region in Quebec. The current study
also includes analysis of an additional case-control sample from Sweden
totalling 1,028 subjects who were individually matched for sex, age, and BMI.
The phenotypic characteristics of these four patient subsamples are presented in
Table 1.

Clinical analysis. Plasma glucose (fasting and during an oral glucose
tolerance test [OGTT]) was measured by a glucose oxidase method on a
Beckman Glucose analyzer (Beckman Instruments, Fullerton, CA). Insulin
was measured by radioimmunoassay. The insulinogenic index was calculated
from the OGTT data as [(insulin at 30 min) – (insulin at 0 min)]/[(glucose at 30
min) (23). An estimate of insulin resistance was derived by homeostasis model
assessment as [(fasting serum insulin × fasting plasma glucose)/22.5] (24).
The insulin sensitivity index (ISI) was calculated as in Matsuda and DeFronzo
(25). The insulin disposition index was calculated as (insulinogenic index ×
ISI)/100.

Genotyping. Genotyping was performed as previously described (22) by
primer extension of multiplex products with detection by matrix-assisted
laser desorption ionization-time of flight mass spectroscopy (26) using a
Sequenom platform. The average completeness of genotypes for working
markers was 99.1%. Using both genotypes performed in replicate (25).

Statistical analysis. To examine the association of each particular SNP with
type 2 diabetes, we used simple χ² analysis in the case-control samples
and the discordant allele test (27) in the sibling pairs. Results were combined
by Mantel-Haenszel meta-analysis of the odds ratios (ORs). For haplotype
analysis, the frequency of haplotypes was estimated in the combined case-

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Fasting plasma glucose (mmol/l)</th>
<th>Plasma glucose at 2 h OGTT (mmol/l)</th>
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<td>Sibships</td>
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<td>Diabetes/severe IGT sib</td>
<td>280/329</td>
<td>65 ± 10</td>
<td>29 ± 5</td>
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</tr>
<tr>
<td>Diabetes/severe IGT</td>
<td>252/219</td>
<td>60 ± 10</td>
<td>28 ± 5</td>
<td>9.8 ± 3.4</td>
<td>15.0 ± 5.3</td>
</tr>
<tr>
<td>NGT</td>
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<td>27 ± 4</td>
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<td>6.8 ± 2.8</td>
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<tr>
<td>Diabetes</td>
<td>70/57</td>
<td>53 ± 8</td>
<td>29 ± 5</td>
<td>6.4 ± 1.8</td>
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</tr>
<tr>
<td>Sweden C/C</td>
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<td></td>
</tr>
<tr>
<td>Diabetes/severe IGT</td>
<td>267/247</td>
<td>66 ± 12</td>
<td>28 ± 4</td>
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<td>66 ± 12</td>
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<td>4.8 ± 0.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Plasma glucose was measured at baseline (fasting) and 2 h after an OGTT. IGT, impaired glucose
tolerance; IFG, impaired fasting glucose; NGT, normal glucose tolerance; C/C, case/control; ND, not done.

In sum, these data strongly support the hypothesis that
genetic variation somewhere in the region of these two
genes does, in fact, influence risk of type 2 diabetes and
perhaps quantitative metabolic traits. Given the varied
results in different studies of this gene region, however, as
well as the number of genetic and phenotypic models
explored, it is clear that further replication and study of
genomic variation in the region is necessary to illuminate a
consistent pattern of correlation of these polymorphisms
with disease (19,20). We therefore set out to characterize the
haplotype structure of the SUR1/Kir6.2 gene region,
test the association of Kir6.2 E23K and nearby polymor-
phisms in a new patient population, refine our understand-
ing of the genetic model of transmission, and investigate the
relationship of different genotype combinations to type 2
diabetes and insulin secretion.

RESEARCH DESIGN AND METHODS

Haplotype structure. To evaluate the haplotype structure of the SUR1/Kir6.2
gene region, we genotyped 129 publicly available SNPs in a multigenerational
panel of 12 Centre d’Étude du Polymorphisme Humain (CEPH) pedigrees
totalling 96 chromosomes from Utah white subjects. We also studied two
additional panels of individuals of Asian and African-American self-reported
ethnicity to define the haplotype structure of KCNJ11 in a more diverse panel.
Selected SNPs span 207 kb, from -23 kb upstream of the ABCG8 transcription
start site to -92 kb downstream of the KCNJ11 termination codon. Where
possible, we preferentially selected SNPs that had been validated by more than
one submitter (i.e., double-hit SNPs [21]). SNPs were initially selected on the basis
of an evenly spaced grid across the region, and then additional markers were
added in segments displaying low levels of LD. In total, 31 of the 129 SNPs
attempted (24%) were technical failures, and 21 of the 98 working SNPs (21%)
were monomorphic in the CEPH panel, resulting in a final set of 77 working,
polyorphic SNPs (see Table 5 in online appendix [available at http://diabetes.
diabetesjournals.org]). The mean interval between these 77 markers is 2.7 kb,
with the largest interval spanning the coding regions measuring 9 kb (larger
intervals were chosen downstream of the KCNJ11 termination codon to help
characterize the extent of the last haplotype block). Complete information on all
SNPs selected for study (including details of primer sequences, failures, and the
raw genotype data) are posted on our web site (http://genetics.mgh.harvard.edu/
AltshulerWeb/publicationData/Florez_E23K.html).

Haplotype blocks were determined by a modification of the criteria
outlined in Gabriel et al. (22). In our dataset, we choose to merge nearby,
adjacent blocks displaying a multiallelic D’ > 0.9, because recent analysis has
shown that this can be done with little or no loss in power (J. Drake, M.

To examine how comprehensively our selected SNPs captured variation
across this region, we performed a resampling procedure of the data. We
randomly selected subsets of the 77 markers (from 1 marker to 76 markers) and
examined the predictive power of these selected markers for the
remaining “hidden” SNPs. These “hidden” markers are meant to represent the
undiscovered common variants in the region (with the caveat that the
distribution of these markers is weighted on the basis of heterozy-
gosity). This sampling procedure was iterated 100 times and performed both
with a set of tag SNPs selected randomly (from among the preselected SNPs
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Table 1 presents the characteristics of these four patient subsamples.

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control sample using an expectation maximization algorithm (Patterson et al., unpublished observations), and the frequency and estimated counts of each haplotype were assessed against all others by the above tests.

**Meta-analysis.** We compiled all published studies that have examined the association of the Kir6.2 E23K polymorphism with type 2 diabetes (2-11). We did not include the Utah samples from Inoue et al. (3) because E23K was not in Hardy-Weinberg equilibrium in the control group (6). We did not include Sakura et al. (2) because it was not clear whether the U.K. Prospective Diabetes Study samples overlapped with those analyzed in Gloyn et al. (8). However, the U.K. Prospective Diabetes Study samples from Inoue et al. (3) were included in our meta-analysis because they did not overlap with those examined in Gloyn et al. (Anna Gloyn, personal communication). Finally, we did not include the samples from Hansen et al. (4) because these were included in Nielsen et al. (10), and we did not include the Ashkenazi Jewish sample reported in Love-Gregory et al. (11) because detailed genotype counts were not available in the publication.

**Tests of genetic models.** To test possible genetic models of E23K transmission, we combined our new data with all available nonoverlapping, published case-control samples (3,6–10). We first estimated the OR for the KK and EE genotypes under both recessive (KK versus KE/EE) and multiplicative (KK versus KE) models (giving the modest estimate of the OR, multiplicative and additive models are similar). On the basis of the overall (allele count) OR of 1.15 in favor of the K allele, we generated the genotypes expected in each sample under each genetic model. To guard against stratification in the analysis (as a result of different allele frequencies in different populations), we analyzed each sample separately. Expected genotype counts from all samples were tested against the observed genotypes by $\chi^2$ analysis. As an additional test, we performed a sample of diabetic trios (5) according to the parental transmission of the K allele from the heterozygote parent in EE versus KK parental pairs. In a recessive model, in contrast, transmission from parents with the EE versus KE genotypes should not deviate from 50:50. Similarly, in both recessive and multiplicative models, one would expect overtransmission of the K allele from the heterozygote parent in KE versus KK parental pairs. We combined both partial transmission disequilibrium test (TDT) results by meta-analysis.

**Phenotype comparisons.** We compared pairs of nondiabetic Scandinavian siblings who are discordant for the E23K genotype, and analyzed data from OGTTs to obtain the insulinogenic index. Because the relationship between the insulinogenic index and insulin resistance estimated by homeostasis model assessment (24) was not linear, we used the ISI of Matsuda and DeFronzo (25) to calculate the insulin disposition index. Within each pair, the insulinogenic index and insulin disposition index were compared with the corresponding variable in the respective sibling by paired $t$ test, depending on the number of excess K alleles (EE versus KK pairs were counted twice, whereas EE versus KE or KK versus KK pairs were counted once). In cases in which there were multiple siblings from which to choose, the two discordant siblings who were closest in age were selected. We also performed a test of the recessive model by restricting our analysis to sibling pairs in which one of the siblings had the KK genotype (EE or KE versus KK). As an independent test, we compared the insulinogenic index and insulin disposition index in the control subjects from the case-control studies by $t$ test. No significant differences were observed.

**RESULTS**

**Haplotype structure across the SUR1/Kir6.2 gene region.** To characterize haplotype structure across the SUR1/Kir6.2 gene region, we typed 77 polymorphic markers in a multigenerational panel of 12 CEPH pedigrees totaling 96 Utah white individuals (see RESEARCH DESIGN AND METHODS for details of SNP selection and genotyping). These markers were selected from the public SNP map and included variants previously associated with type 2 diabetes (6–8,10–17). Analysis of allelic associations revealed substantial LD across the region, with the two genes spanning at least five regions of consistent and strong LD ("haplotype blocks") as defined by a minor modification of the methods of Gabriel et al. (22) (see RESEARCH DESIGN AND METHODS). As expected, the strength and the extent of LD are heterogeneous across even this small region: one of the blocks of strong LD (defined by five SNPs) spans <5 kb, whereas another (defined by 15 common SNPs) spans >75 kb (Fig. 1). The highly variable extent of allelic association illustrates the value of empirically evaluating patterns of LD across regions, rather than relying on an evenly spaced marker map.

To evaluate how thoroughly the 77 typed SNPs capture common genetic variation across the SUR1/Kir6.2 gene region, we performed a resampling procedure on the data. Specifically, we randomly sampled subsets of these markers and evaluated (as a function of marker density) how much of the total variation (as defined by the remaining set of 77 markers) was successfully captured (see RESEARCH DESIGN AND METHODS for details). As shown in Fig. 2, the tested markers demonstrate substantial redundancy: as few as 20 randomly selected markers provide an average maximal $r^2$ of 0.5 for all untested markers, and a subset of approximately half of the tested SNPs provides an average $r^2 > 0.8$ for the remaining untested markers (Fig. 2). These data suggest that although a complete description of the structure of common variation will require resequencing of the contiguous 200 kb in hundreds of individuals, this initial haplotype map allows us to select tag SNPs that capture much of the common genetic variation across the region.

One characteristic of regional LD is the existence of sets of adjacent markers that show little evidence for historical recombination and limited haplotype diversity, known as haplotype blocks (22,28,29). We find that the single exon of KCNJ11 and part of the SUR1 gene are contained in a 75-kb block of strong LD, estimated from these data as spanning from SNP 61 (rs2074310) in the coding region of ABCG8 to SNP 75 (rs1073443), 58 kb downstream from the KCNJ11 stop codon. These 15 polymorphic markers display limited haplotype diversity: eight haplotypes that display a frequency >2% explain 92% of all haplotypes observed (Fig. 3).

We noted that in this initial haplotype characterization, SNP 63 (marking an Ser$\rightarrow$Ala change at the 1,369 position in exon 33 of SUR1) is in near-perfect LD with SNP 65 marking Kir6.2 E23K ($r^2 = 0.98$), such that in our CEPH panel, virtually every chromosome containing the K allele in E23K also contains the A allele in A1308G. Such strong LD can make it challenging to differentiate the effect of different polymorphisms (see, e.g., 30). One approach to discriminate the effect of such polymorphisms is cross-population studies that make use of the presence of different recombinant haplotypes in different populations. We genotyped both of these markers in an African-American and an Asian panel of individuals, and in both samples these two missense changes again show a perfect corre-
Gene expression in our combined meta-analysis is negative. We note that a formal test for heterogeneity is weak and the family-based samples are relatively small, respectively. However, because the estimated effect is 1.17 (one-tailed P = 0.003, 95% CI 1.05–1.32), which is in close agreement with a meta-analysis of all previously published, nonoverlapping data (OR = 1.14, two-tailed P = 0.0002, 95% CI 1.06–1.22). When the results from the present study are included in the meta-analysis (including 75-kb block encompassing KCNJ11 in a large patient sample. We first examined the genotype at E23K in a total of 3,413 subjects in whom this locus had not previously been genotyped (Table 1). These results provide an independent replication of the association of E23K with type 2 diabetes: we obtain an OR of 1.17 (one-tailed P = 0.003, 95% CI 1.05–1.32), which is in close agreement with a meta-analysis of all previously published, nonoverlapping data (OR = 1.14, two-tailed P = 0.0002, 95% CI 1.06–1.22). When the results from the present study are included in the meta-analysis (totaling 5,083 patients and 4,747 control subjects), the overall OR is 1.15 (two-tailed P = 0.003, 95% CI 1.08–1.22).

The use of family-based samples has been advocated as an approach to rule out population stratification as the cause of a positive association. The three family-based samples that have examined the association of E23K with type 2 diabetes (a set of trios previously published by our group [2], a set of trios published by Gloyn et al. [8], and the discordant siblings in this report) all have failed to individually replicate the association. When separate meta-analyses are performed for the case-control and family-based samples (Table 2), in the Kir6.2 region, five of seven SNPs (in addition to E23K) show a modest association with type 2 diabetes, with ORs ranging from 1.11 to 1.15 and nominal two-tailed P < 0.05. The other two coding variants in this region (SUR1 A1369S and Kir6.2 L270V) showed a signal for association that is statistically indistinguishable from that of E23K. LD between SUR1 A1369S and Kir6.2 E23K was very strong (r² > 0.9), and we again observed that <1% of haplotypes separate the two SNPs in this substantially larger sample.

We subsequently analyzed haplotype-specific risk to assess whether the signal for association with E23K might be attributable to another variant (observed or unobserved) in this region (Table 3). No haplotype showed a signal for association of stronger statistical significance than that observed with E23K. The lack of a stronger signal with other tag SNPs and haplotypes fails to support a model in which the association of E23K is due solely to one or more variants that are less frequent in the population but have a substantially larger OR. It is important to note, however, that even with 3,400 subjects, the current study lacks power to distinguish whether there are other variants in these 75 kb that might act, either singly or in combination with E23K, to alter risk in a modest manner. Moreover, not having completely resequenced each of our patients and control subjects, we cannot make any statement about the existence of rare variants that might influence risk in a manner independent of the observed association to E23K.

Haplotype E shows a marginally stronger (albeit statistically indistinguishable) OR for association with type 2 diabetes than does E23K (OR = 1.26 for HapE vs. OR =
1.17 for E23K). Haplotype E carries the K allele of E23K, but it is the only haplotype that carries the minor allele at another missense SNP, L270V (Fig. 3). This could suggest that the association of E23K might actually be attributable to L270V. To assess this possibility, we performed a meta-analysis of all nonoverlapping reports that have examined the E23K/L270V association. The order of the tag SNPs is identical to that shown in Table 2, and they are numbered as in Table 5 in the online appendix; 5’ to 3’ is left to right. LD between A1369S and E23K is nearly complete ($r^2 = 0.98$), making it impossible to distinguish (based on population-based data) which polymorphism may be the causal variant.

**Genetic models.** We considered a range of genetic models that might explain the association of E23K with type 2 diabetes. As previously suggested (10), our meta-analysis of a larger set of case-control samples (those in this report, and previously published data) shows a strong association of the KK genotype as compared with the combined EE/EK genotypes (OR = 1.12 (two-tailed $P = 0.006$ and 0.01, respectively)).

**TABLE 2**

<table>
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<th>SNP</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
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</tr>
<tr>
<td>rs2067043</td>
<td>1.14</td>
<td>0.04</td>
<td>1.01–1.28</td>
</tr>
<tr>
<td>rs757110 (A1369S)</td>
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<td>0.02</td>
<td>1.02–1.28</td>
</tr>
<tr>
<td>rs1800467 (L270V)</td>
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<td>0.05</td>
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<td>rs2354867</td>
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<td>rs1073443</td>
<td>0.88</td>
<td>0.03</td>
<td>0.78–0.99</td>
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</table>

In addition to E23K, seven SNPs tag the haplotype block that includes KCNJ11. These were examined for association with type 2 diabetes, combining the different subsamples in our study by Mantel-Haenszel meta-analysis. Coding variants are shown in parentheses. $P$ values are two-tailed.

**FIG. 3.** Haplotypes of the 15 markers spanning the fifth block in the CEPH sample. This region measures 76 kb and spans part of the ABCCS gene and the entire KCNJ11 gene. Each SNP is marked by a number in the top row (tag SNPs in bold), and each haplotype is depicted by a letter in the far left column; the frequency of each haplotype in the CEPH sample is shown in the far right column. Haplotypes are named in alphabetical order according to their frequency in the CEPH sample. Common haplotypes (frequency $>5\%$) are shown in the top panel, and together they compose $92\%$ of all haplotypes in this region; rare haplotypes are shown in the bottom panel. The order of the tag SNPs is identical to that shown in Table 2, and they are numbered as in Table 5 in the online appendix; 5’ to 3’ is left to right. LD between A1369S and E23K is nearly complete ($r^2 = 0.98$), making it impossible to distinguish (based on population-based data) which polymorphism may be the causal variant.
TABLE 4

<table>
<thead>
<tr>
<th>Nondiabetic discordant siblings</th>
<th>General</th>
<th>Recessive</th>
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<td>Allele</td>
<td>E (39)</td>
<td>K (39)</td>
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<tr>
<td>Ins index</td>
<td>9.74 ± 5.8</td>
<td>6.89 ± 3.9</td>
</tr>
<tr>
<td>Disp index</td>
<td>7.76 ± 3.0</td>
<td>6.30 ± 3.5</td>
</tr>
</tbody>
</table>

| Scandinavian control subjects   |         |           |          |
| Genotype                        | EE (168) | EK (353)  | KK (153) |
| Ins index                       | 5.31 ± 4.0 | 5.59 ± 4.9 | 4.52 ± 3.3 |
| Disp index                      | 6.32 ± 3.8 | 5.91 ± 3.6 | 5.59 ± 3.7 |

Data are means ± SD. We calculated the insulinogenic index (Ins index) and insulin disposition index (Disp index) as described, and compared them across genotypes in nondiabetic siblings discordant for E23K and in all Scandinavian nondiabetic subjects for whom we had OGTT data (see text for details). Number of pairs or individuals is indicated in parentheses. NS, not significant. * Paired t test; † two-tailed; ‡ one-tailed.

Examination of the genotype counts suggests that the best fit would lie between a purely recessive model and a multiplicative model. This is most consistent with a model in which heterozygotes are at slightly increased risk and homozygotes have a degree of risk that exceeds double or the square of the heterozygote risk.

As an additional test of the recessive model, we analyzed data for E23K in a set of parent-offspring trios previously published by our group (5). In each case, we compared transmission of the K allele from a heterozygous parent, separately evaluating transmission ratios as a function of the genotype of the other parent. This allowed us to isolate cases in which the K allele can only be transmitted to create a heterozygous diabetic offspring (EE × EK) and those in which the K allele can be transmitted only to create a homozygous diabetic offspring (KK × EK). The transmission of the K allele to diabetic offspring was more frequent in cases that could create a KK homozygous offspring as compared with those that could not (KK × EK > EE × EK, one-tailed P < 0.04 in favor of recessive transmission). These data further support the model that the association of E23K with type 2 diabetes acts under a recessive model.

Genotype-phenotype correlations. It has been previously reported that E23K is associated with decreased levels of insulin secretion in nondiabetic subjects (10). We therefore compared the insulinogenic index in both family-based and population-based studies (we did not examine any other models of association to quantitative traits, because in this case there was a clear prior expectation on the basis of the known function of the encoded protein and previous human data). First, we studied pairs of nondiabetic siblings who were discordant for genotype at E23K. The sibling who inherited a larger number of K alleles had a significantly decreased insulinogenic index (P = 0.006) as compared with siblings with fewer K alleles (Table 4). The effect was substantial, with a reduction in β-cell function of 20–30% based on genotype at E23K. In a second sample of nondiabetic population-based control subjects, the insulinogenic index was again lower for individuals who carry K alleles, reaching statistical significance for the recessive model (P = 0.008). When the insulinogenic index was controlled for insulin resistance as estimated by the inverse of the ISI of Matsuda and DeFronzo (25), a similar trend was seen but did not reach statistical significance (Table 4). These two independent replications of the findings of Nielsen et al. (10) confirm that genotype at E23K influences insulin secretion in both nondiabetic siblings of diabetic patients and in population-based control subjects.

DISCUSSION

We have characterized genetic variation across the SUR1/Kir6.2 gene locus and replicated the published association of E23K with type 2 diabetes. Of the three coding SNPs and multimarker haplotypes in the immediate region of E23K (in both physical terms and those of LD), E23K shows the most significant and robust association. Both our meta-analysis and the partial TDT in our diabetic trios support a mostly recessive model of transmission in which heterozygotes have a slightly increased disease risk but homozygotes have a considerably greater risk than predicted by the heterozygote risk alone. Studies of insulin secretion in nondiabetic individuals (both in siblings of diabetic subjects and in a control population) provide support for the model that an impairment in β-cell function can be a primary defect even in patients with the common form of type 2 diabetes. Combined with data from other groups, these results indicate that genetic variation in this gene region is truly associated with risk of the common form of type 2 diabetes and decreased secretion of insulin in nondiabetic individuals drawn from the population.

Our analysis of haplotypes argues that it is unlikely that the signal for the association of E23K with type 2 diabetes originates solely from an as-yet-undiscovered common polymorphism in the 75-kb region studied herein. However, as noted by others (3,9), we have documented strong LD of the E23K variant in Kir6.2 with another coding variant in the adjacent SUR1 gene, A1369S in exon 33. Because in our samples virtually every chromosome containing the K allele in E23K also contains the A allele in A1369S, it is not yet possible to distinguish on genetic grounds which one of the two variants—or, for that matter,
genes—might be causal. One genetic approach to establish this difference involves genotyping both polymorphisms in a much larger sample to achieve enough power to test the risk attributable to the haplotypes on which E23K and A1369S are separated. Given the low frequency of the “M haplotype” in our study (~1%) and the current OR of 1.15, however, we estimate that 120,000 case/control pairs will be required to distinguish between the two. A second alternative is to genotype both polymorphisms in a population in which chromosomes recombinant for E23K and A1369S are present at a higher frequency. Preliminary analysis of the KCNJ11 haplotype structure in Asian and African-American samples, however, suggests that similarly high levels of LD are preserved across this region in these two samples, indicating that a cross-population approach (at least in these samples) may be less useful in this case.

In the absence of a genetic approach to distinguish which variant(s) plays a causal role, functional studies will be critical. In agreement with our phenotypic characterization, several in vitro studies have implicated the E23K polymorphism in increasing the open probability of the Kir6.2 channel, which should lead to diminished insulin secretion (31). This report does not specify which isoform of ABCC8 was used at the 1,369 amino acid position in the cell-based model; it is interesting that A1369S lies near the second nucleotide binding fold of SUR1, which may affect its binding affinity and influence the results observed. In addition, long-chain fatty acids are known to activate Kir6.2 (32,33); a recent study has shown that Kir6.2 channels that contain the E23K variant are more easily activated by palmitoyl-CoA than their wild-type counterparts (34). Although it is not clear which A1369S variant was used in the functional studies above, the authors do find that the increase in the E23K mutant channel activity persists in truncated constructs that lack the ability to dimerize with the SUR1 molecule, albeit at much higher palmitoyl-CoA concentrations. It is intriguing to hypothesize that both E23K and A1369S could interact in cis and that additional functional studies involving both variants would be valuable to clarify their possible respective contributions.

An extremely interesting aspect of these findings by our group and several others is that peroxisome proliferator–activated receptor γ (35) and Kir6.2 both are drug targets for medications used in the standard therapy for patients with type 2 diabetes, and both carry a common genetic variation that influences susceptibility to the disease. The correlation of pharmacological utility and inherited risk may not be coincidental: there are many examples of successful drug targets that also carry genetic variation influencing inherited risk of disease (36). More general, these anecdotes support the idea that genes that carry inherited variations that contribute modestly to individual risk of disease may represent “Achilles’ heels”: genes in which pharmacological alteration may be adequate to alter the course of disease. In addition, the knowledge that a drug target gene carries a common and functionally relevant SNP raises the hypothesis that drug response to the relevant agent might also track with genotype. It will be important to test this hypothesis in adequately powered clinical studies that measure clinical outcomes, in the hope that biological insight or a clinically useful test might emerge.

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