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

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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 find that the E23K variant in Kir6.2 demonstrates very strong allelic association with a coding variant (A1369S) in the neighboring SUR1 gene ($r^2 > 0.9$) 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), Gloyn 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

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L.G. and D.A. jointly supervised the project.

CEPH, Centre d'Etude du Polymorphisme Humain; ISI, insulin sensitivity index; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; SNP, single-nucleotide polymorphism; SUR1, sulfonylurea receptor; TDT, transmission disequilibrium test.

TABLE 1
Clinical characteristics of patient samples

Sample	Sex (M/F)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	Plasma glucose at 2 h OGTT (mmol/l)
Sibships					
Diabetes/severe IGT sib	280/329	65 ± 10	29 ± 5	9.3 ± 3.3	14.3 ± 5.6
NGT sib	275/305	62 ± 10	26 ± 3	5.4 ± 0.4	6.0 ± 1.1
Scandinavia C/C					
Diabetes/severe IGT	252/219	60 ± 10	28 ± 5	9.8 ± 3.4	15.0 ± 5.3
NGT	254/217	60 ± 10	27 ± 4	6.2 ± 1.8	6.8 ± 2.8
Canada C/C					
Diabetes	70/57	53 ± 8	29 ± 5	6.4 ± 1.8	12.8 ± 2.1
NGT	70/57	52 ± 8	29 ± 4	5.1 ± 0.6	6.1 ± 1.1
Sweden C/C					
Diabetes/severe IGT	267/247	66 ± 12	28 ± 4	8.5 ± 2.5	15.5 ± 4.0
NGT	267/247	66 ± 12	28 ± 4	4.8 ± 0.6	ND

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.

less clear, with a variety of results implicating an exon 16 polymorphism at the −3 position (12–15), an exon 18 silent mutation (T759T) (12,16,17), and an exon 31 silent mutation (R1273R) (18) in type 2 diabetes. A subsequent meta-analysis of all published data concerning the exon 16 and exon 18 variants, however, yielded negative results (8). In the report by Barroso et al. (9), a nominal association of five SUR1 SNPs was seen with type 2 diabetes, although some or all of this effect might be due to linkage disequilibrium (LD) with the E23K polymorphism.

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 polymorphisms in a new patient population, refine our understanding 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'Etude du Polymorphisme Humain (CEPH) pedigrees totaling 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 *ABCC8* 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, polymorphic 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. Loomer, J. Hirschhorn, S.F.S., M.J.D., D.A., unpublished observations).

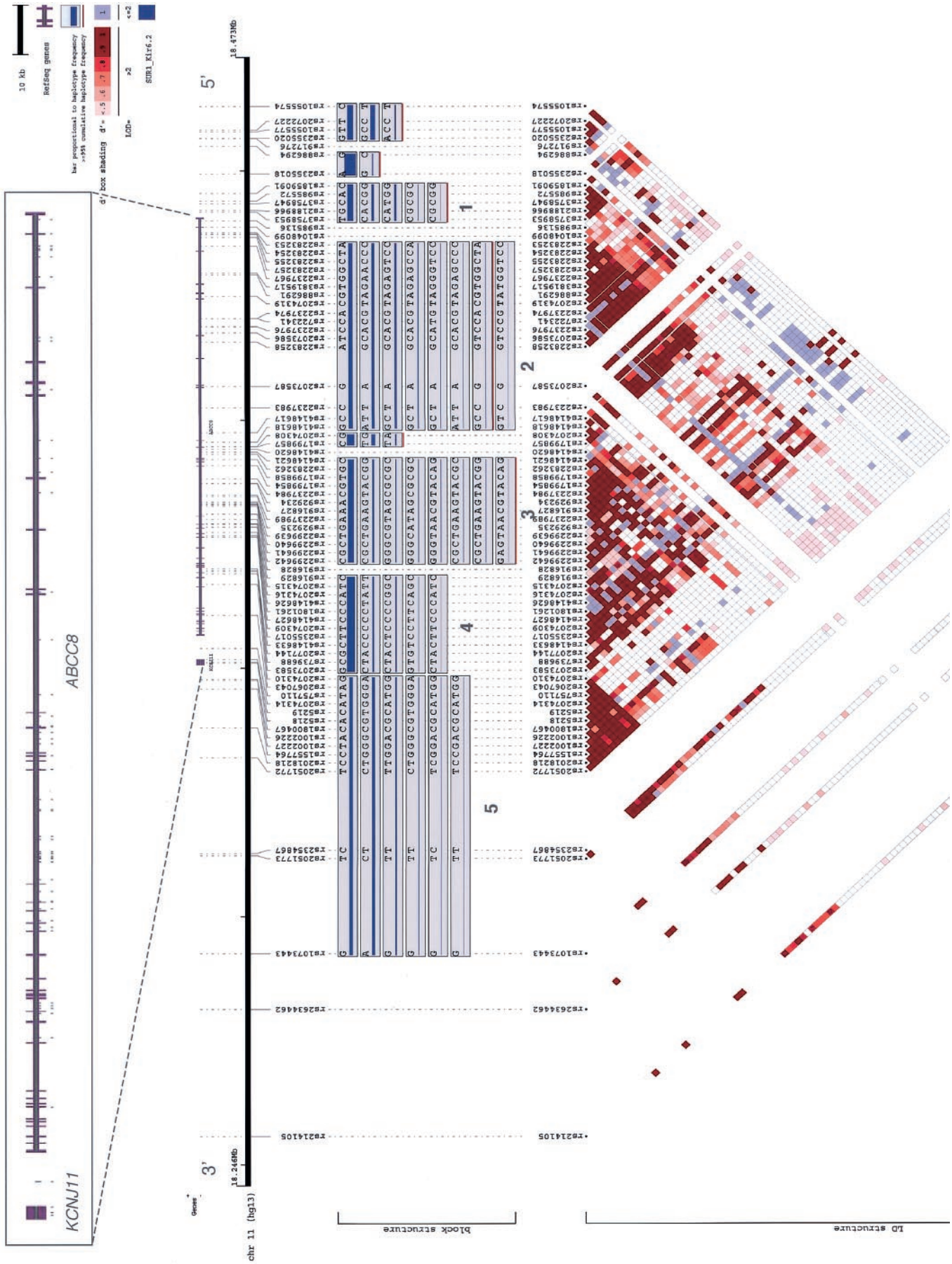
To examine how comprehensively our selected SNPs captured variation across this region, we performed a resampling procedure of the data. We randomly selected subsets of the 77 markers (from 1 marker to 76 markers) and examined the predictive power of these selected markers for the remaining "hidden" SNPs. These "hidden" markers are meant to represent the undiscovered common variants in the region (with the caveat that the frequency distribution of these markers is weighed on the basis of heterozygosity). 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 (n = 54,808) and errors of Mendelian inheritance, we documented a consensus error rate of 0.2% in the analyzed data.

Statistical analysis. To examine the association of each particular SNP with type 2 diabetes, we used simple χ^2 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-


10 kb
RefSeq genes
bar proportional to haplotype frequency
>.95% cumulative haplotype frequency
d' box shading d'= <.5 .6 .7 .8 .9 1
LOD= >2 <=2
SUR1_Kir6.2
18.473Mb
5'
3'
16.246Mb
chr 11 (hg13)
ABCC8
KCNJ11
Genes
Block structure
LD structure
1
2
3
4
5

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 EK genotypes under both recessive (KK versus EK/EE) and multiplicative (EK versus EE) models (given 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 χ^2 analysis. As an additional test, we partitioned a sample of diabetic trios (5) according to the parental homozygote/heterozygote genotype combinations (EE $\times$ EK and KK $\times$ EK). Under both dominant and multiplicative models, one would expect overtransmission of the K allele from the heterozygote parent in EE $\times$ EK parental pairs. In a recessive model, in contrast, transmission from parents with the EE $\times$ EK 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 KK $\times$ EK 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 EK or EK 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 EK 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.

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 *ABCC8* 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→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 A1369S. 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-

FIG. 1. LD plot across the SUR1/Kir6.2 locus. The horizontal black line depicts the 207-kb DNA segment of chromosome 11p15.1 analyzed in our CEPH sample. The *ABCC8* (SUR1) and *KCNJ11* (Kir6.2) genes are shown above in purple; because of their reverse orientation, 5′ to 3′ is read from right to left. The 77 working SNPs are indicated below the black line. An LD plot is depicted in the bottom part of the figure based on the measure D′ (37): each square represents the magnitude of LD for a single pair of markers, with red color indicating LD that is strong (D′ >0.8) and statistically significant (logarithm of odds >2.0). Analysis of this LD plot suggests that the segment that spans both genes can be described by five blocks of strong LD (numbered 1 to 5 in 5′ to 3′ orientation). *KCNJ11* is contained entirely in the fifth (leftmost) block. The haplotypes spanning each of these blocks are shown above the LD plot, with the thickness of the blue line indicating their frequency in the CEPH population. (Figure prepared using the program LocusView, T. Petryshen, A. Kirby, and P. Sklar, unpublished software.)

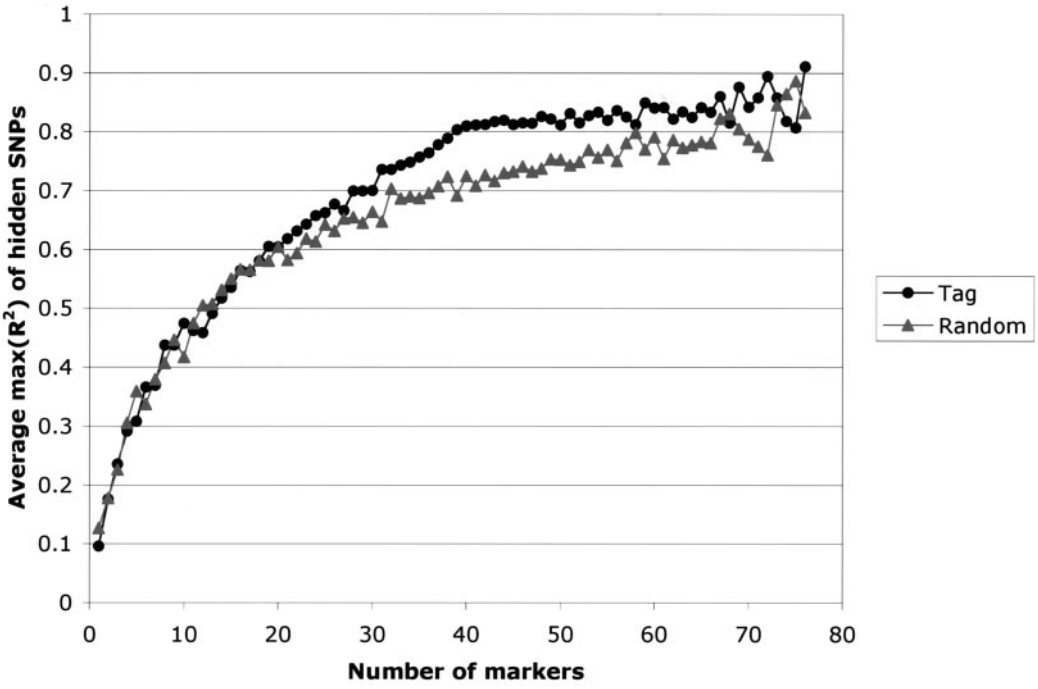


FIG. 2. Evaluation of SNP coverage across the SUR1/Kir6.2 gene region. We randomly selected subsets of the 77 working markers (ranging from 1 marker to 76 markers) and calculated the mean maximal r^2 value for those selected to the remaining "hidden" SNPs. Curves are displayed for a random set of tag markers (▲) and for the set of SNPs that tag the haplotype blocks as defined in this study (●; see RESEARCH DESIGN AND METHODS for details). The plateau of the curve at ~40 markers indicates that there is substantial redundancy among the markers typed and suggests that the marker density is adequate to guide well-powered association studies of the region.

lation (r^2 = 1.0), suggesting that in at least these three samples, the effects of E23K and A1369S are likely to be genetically indistinguishable.

Association of E23K with type 2 diabetes. Because the E23K variant has shown the most consistent association with type 2 diabetes and the entire *KCNJ11* locus is contained within a single block of strong LD, this report focuses on typing of the missense and tag SNPs across the 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 (totalling 5,083 patients and 4,747 control subjects), the overall OR is 1.15 (two-tailed $P < 10^{-5}$, 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, the association is seen only for the case-control group (OR 1.19, $P < 10^{-7}$ vs. OR 0.91, P = 0.11, respectively). However, because the estimated effect is weak and the family-based samples are relatively small, this finding could represent a false-negative result rather than indicate that stratification is the cause for the observed association. We note that a formal test for heterogeneity in our combined meta-analysis is negative (P = 0.11), suggesting that all of the samples in our meta-analysis (case-control and family-based) are drawn from the same underlying distribution.

Association of other SNPs and haplotypes with type 2 diabetes. We next examined the association of each of the remaining seven tag SNPs in the complete set of 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 ($r^2 >$ 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 =

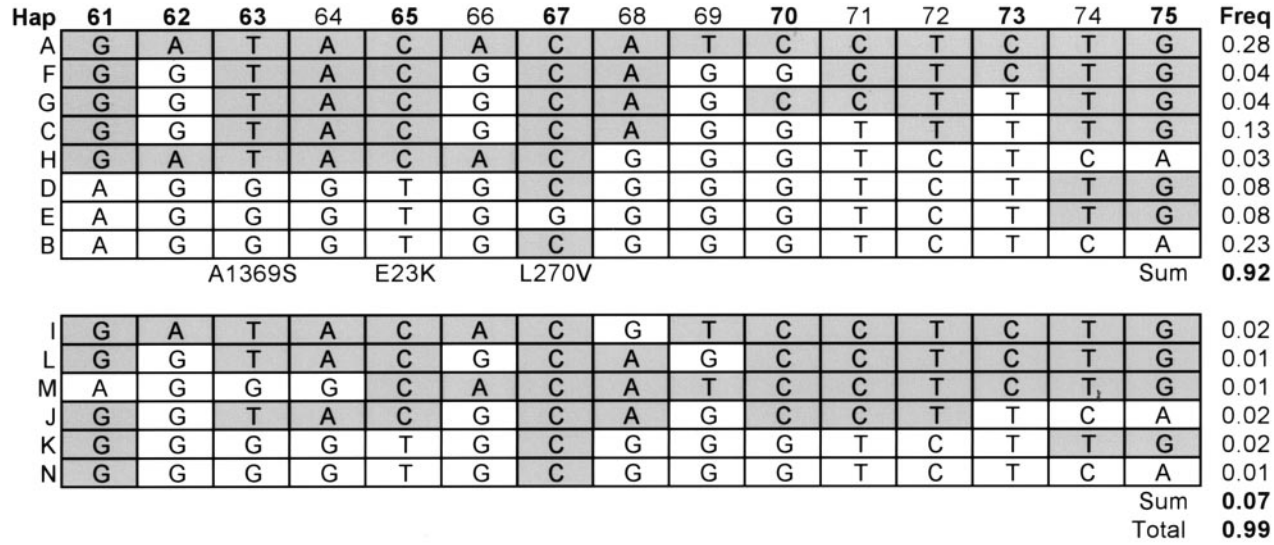


FIG. 3. Haplotypes of the 15 markers spanning the fifth block in the CEPH sample. This region measures 76 kb and spans part of the *ABCC8* gene and the entire *KCNJ11* gene. Each SNP is marked by a number in the top row (tag SNPs in bold), and each haplotype is depicted by a letter in the far left column; 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 >3%) 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 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.

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 L270V (3,4,6). This yielded an OR of 0.88 (two-tailed P = 0.6, 95% CI 0.55–1.41). When combined with our results, which display an OR in the opposite direction, the overall OR is 1.17 (two-tailed P = 0.13, 95% CI 0.95–1.44). Because association with the L270V variant is less consistent and does not achieve nominally statistical significance, we believe that E23K rather than L270V is the more likely explanation for the observed association.

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 = 1.37, two-tailed $P < 10^{-6}$, 95% CI 1.21–1.54). This result is statistically indistinguishable from those produced by testing the KK genotype versus the EE genotype (OR = 1.47, two-tailed $P < 10^{-7}$, 95% CI 1.28–1.68) or the EK genotype (OR = 1.30, two-tailed $P < 10^{-4}$, 95% CI 1.14–1.47). In a test for the multiplicative model, when the EK genotype is tested against the EE genotype, we obtain an OR of 1.12 (two-tailed P = 0.017, 95% CI 1.02–1.24).

To assess these genetic models more rigorously, we tested the observed genotype counts against those predicted under dominant, multiplicative and recessive models, given the observed allele frequencies (Table 6 in online appendix). The observed genotype counts are strongly inconsistent with a dominant model ($P = 3.7 \times 10^{-10}$) and are mildly inconsistent with both the multiplicative and recessive models (P = 0.006 and P = 0.01, respectively).

TABLE 2
Association of other Kir6.2 tag SNPs with type 2 diabetes

SNP	OR	P	95% CI
rs2074310	1.14	0.02	1.02–1.28
rs2067043	1.14	0.04	1.01–1.28
rs757110 (A1369S)	1.14	0.02	1.02–1.28
rs1800467 (L270V)	1.25	0.05	1.00–1.58
rs1557764	1.11	0.09	0.98–1.25
rs2354867	1.15	0.02	1.03–1.30
rs1073443	0.88	0.03	0.78–0.99

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.

TABLE 3
Association of Kir6.2 haplotypes with type 2 diabetes

Haplotype	OR	P	95% CI	Frequency
A	0.87	0.02	0.77–0.99	0.27
B	1.09	0.07	0.97–1.23	0.26
C	0.91	0.11	0.78–1.06	0.16
D	1.04	0.65	0.84–1.30	0.06
E	1.26	0.03	0.99–1.60	0.05
F	0.96	0.37	0.74–1.23	0.05
G	1.23	0.09	0.91–1.66	0.03
H	1.10	0.33	0.73–1.64	0.02
M	1.00	0.50	0.52–1.93	0.01
B+D+E	1.15	0.01	1.03–1.29	0.38

Haplotypes of >2% frequency were tested for association with type 2 diabetes in our patient samples and analyzed by Mantel-Haenszel meta-analysis as above. Together, they compose 91% of the haplotypes in our Scandinavian samples. For comparison purposes, we show the pooled results for the three haplotypes (B, D, and E) that carry the K allele of E23K. The frequencies of each haplotype in these samples are strikingly similar to those in the CEPH samples (compare these data with Fig. 3).

TABLE 4
Genotype-phenotype correlation of measures of insulin secretion and E23K

Nondiabetic discordant siblings	General			Recessive		
Allele	E (39)	K (39)	*P**	EE or EK (22)	KK (22)	*P**
Ins index	9.74 ± 5.8	6.89 ± 3.9	0.006†	9.64 ± 6.1	7.45 ± 4.4	NS
Disp index	7.76 ± 3.0	6.30 ± 3.5	0.06†	7.69 ± 3.1	7.00 ± 4.0	NS

Scandinavian control subjects Genotype	EE (168)	EK (353)	KK (153)	*P*	EE/EK	KK	*P*
Ins index	5.31 ± 4.0	5.59 ± 4.9	4.52 ± 3.3	EE vs EK:NS EE vs KK: 0.028† EK vs KK: 0.008‡	5.50 ± 4.7	4.52 ± 3.3	0.008‡
Disp index	6.32 ± 3.8	5.91 ± 3.6	5.59 ± 3.7	EE vs EK: NS EE vs KK: 0.08† EK vs KK: NS	6.04 ± 3.6	5.47 ± 3.8	NS

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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