Brief Report

Type 2 Diabetes–Associated Missense Polymorphisms *KCNJ11* E23K and *ABCC8* A1369S Influence Progression to Diabetes and Response to Interventions in the Diabetes Prevention Program

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The common polymorphisms *KCNJ11* E23K and *ABCC8* A1369S have been consistently associated with type 2 diabetes. We examined whether these variants are also associated with progression from impaired glucose tolerance (IGT) to diabetes and responses to preventive interventions in the Diabetes Prevention Program. We genotyped both variants in 3,534 participants and performed Cox regression analysis using genotype, intervention, and their interactions as predictors of diabetes incidence over ~3 years. We also assessed the effect of genotype on insulin secretion and insulin sensitivity at 1 year. As previously shown in other studies, lysine carriers at *KCNJ11* E23K had reduced insulin secretion at baseline; however, they were less likely to develop diabetes than E/E homozygotes. Lysine carriers were less protected by 1-year metformin treatment than E/E homozygotes ($P < 0.02$). Results for *ABCC8* A1369S were essentially identical to those for *KCNJ11* E23K. We conclude that the lysine variant in *KCNJ11* E23K leads to diminished insulin secretion in individuals with IGT. Given our contrasting results compared with case-control analyses, we hypothesize that its effect on diabetes risk may occur before the IGT-to-diabetes transition. We further hypothesize that the diabetes-preventive effect of metformin may interact with the impact of these variants on insulin regulation. *Diabetes* 56: 531–536, 2007

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DPP, Diabetes Prevention Program; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

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The *KCNJ11* gene encodes the islet ATP-sensitive potassium channel Kir6.2. Severe activating mutations in *KCNJ11* cause a novel form of monogenic neonatal diabetes (1). A common glutamate (E) → lysine (K) change at position 23 (E23K) has been consistently associated with type 2 diabetes, with an overall allelic odds ratio (OR) near 1.15 (2–9) for the comparison of diabetic individuals with nondiabetic control subjects. Moreover, we and others (5,8,10) have shown that normoglycemic lysine carriers consistently display a defect in insulin secretion. In vitro, the lysine risk allele seems to affect potassium channel properties (11,12). Interestingly, this variant is in strong linkage disequilibrium with the upstream missense single nucleotide polymorphism (SNP) A1369S in the adjacent gene *ABCC8*, which encodes the functionally related sulfonylurea receptor SUR1 (7,8,13); thus, in all examined populations, lysine carriers at *KCNJ11* E23K almost invariably carry the alanine allele at *ABCC8* A1369S, and it remains possible that either or both variants are actually required to mediate these effects.

The risk of type 2 diabetes conferred by *KCNJ11* E23K has been evaluated prospectively. The Finnish Diabetes Prevention Study (14) randomized 522 subjects with impaired glucose tolerance (IGT) to either placebo or a lifestyle intervention and found that lysine carriers at *KCNJ11* E23K appeared more likely to develop diabetes over time than E/E homozygotes, although the difference was not statistically significant. In contrast, the larger Botnia Prospective Study (10) suggested that the lysine allele was protective, although, again, this effect was not statistically significant. Whether statistical fluctuations or differences in ascertainment schemes (IGT vs. a population sample) and analytical methods (logistic regression vs. Cox proportional hazards analysis) explain these discrepancies is not yet clear.

Two studies have examined the effect of the *KCNJ11* E23K variant on response to sulfonylurea therapy. Gloyn

et al. (3) studied 364 subjects randomized to sulfonylurea therapy in the UK Prospective Diabetes Study (UKPDS) and determined that the presence of the lysine allele did not predict failure to treatment with sulfonylureas at 1 year. Recently, Sesti et al. (15) reported a higher proportion of lysine carriers among 208 subjects who failed sulfonylurea-metformin combination therapy (defined as a rise in fasting plasma glucose >300 mg/dl); interestingly, islets isolated from lysine carriers showed a diminished insulin response to glyburide.

Given this divergent (and contradictory) literature, we set out to investigate the effect of this variant on glycemic parameters in obese individuals at higher risk for type 2 diabetes (i.e., IGT or elevated fasting glucose), to prospectively examine its impact on the development of diabetes, and to assess whether it influences the efficacy of the lifestyle or pharmacological interventions used in the Diabetes Prevention Program (DPP) (16).

RESEARCH DESIGN AND METHODS

The details of the DPP study design have been described elsewhere (16–18). The DPP was a multicenter randomized clinical trial that hypothesized that modifying risk factors for type 2 diabetes (elevated fasting and postload plasma glucose concentrations, overweight, and sedentary lifestyle) with lifestyle intervention or treatment with metformin would prevent or delay the development of diabetes. The clinical trial was conducted at 27 centers, each of which obtained institutional review board approval. The DPP enrolled 3,234 nondiabetic individuals with IGT and elevated fasting glucose and randomized them to placebo, 850 mg metformin twice daily, or a lifestyle intervention program aimed at ≥7% weight loss and ≥150 min of physical activity per week; a fourth arm of 585 subjects assigned to 400 mg troglitazone daily was stopped 2 years after the trial commenced because of hepatotoxicity (17). The principal end point was the development of diabetes by confirmed oral glucose tolerance tests (OGTTs). Over an average of 3 years, the lifestyle and metformin interventions reduced the incidence of diabetes in high-risk individuals by 58% (95% CI 48–66) and 31% (17–43), respectively, versus placebo; diabetes incidence rates were 11.0, 7.8, and 4.8 per 100 person-years in the placebo, metformin, and lifestyle groups, respectively (16).

The 3,548 participants included in this study (92.9% of all DPP participants: 2,994 who completed the trial in their originally assigned treatment groups, plus 554 originally randomized to troglitazone) provided informed consent specific to genetic investigation. Of the participants in this genetic study, 56.4% were Caucasian, 20.2% were African American, 16.8% were Hispanic, 4.3% were Asian American, and 2.4% were American Indian by self-report. Similar to the entire DPP cohort, the participants' mean age was 51 years and mean BMI was 34.0 kg/m^2. Treatment effects were consistent across sex and self-reported ethnicity.

Genotyping. DNA was extracted from peripheral blood leukocytes through conventional procedures and quantitated by picogreen analysis. Genotyping was performed by allele-specific primer extension of single-plex amplified products, with detection by matrix-assisted laser desorption ionization–time-of-flight mass spectroscopy on a Sequenom platform (19,20). Genotyping success rate was 99.3%. The allele frequencies of both SNPs in each of the five ethnic groups were in Hardy-Weinberg equilibrium ($P > 0.05$).

Quantitative glycemic measures. Data from the baseline and 1-year OGTTs were used to calculate one measure of insulin secretion and two measures of insulin sensitivity. The insulinogenic index (21) was calculated as [(insulin at 30 min) − (insulin at 0 min)]/[(glucose at 30 min) − (glucose at 0 min)]. The insulin sensitivity index (reciprocal of insulin resistance by the homeostasis model assessment [22]) was calculated as 22.5/[fasting insulin × (fasting glucose/18.01)]. We have previously shown, in the same cohort, that these measures correlate strongly with the corrected insulin response (insulin secretion) and the inverse of fasting insulin (insulin sensitivity), respectively (23).

We again elected to analyze follow-up quantitative traits at 1 rather than 3 years (23). While a very high proportion of DPP participants were diabetes free and had an OGTT at 1 year, this percentage was much lower at 3 years (many participants had developed diabetes by this time, and those who enrolled in the latter half of the recruitment period did not have to undergo the 3-year exam). Since these quantitative traits were calculated in nondiabetic subjects, so as to avoid confounding by treatment, power is greater at 1 year. In addition, weight loss was maximal at 6–12 months (16); therefore, one might expect to see the greatest effect of the lifestyle intervention in the 1-year data.

Statistical analysis. Time to onset of diabetes was the primary end point. We examined Cox regression models with genotype, intervention, and genotype-by-intervention interactions as the independent variables predicting time to diabetes. These models were also examined with BMI at randomization, age at randomization, sex, and self-reported ethnicity as covariates. When allele frequencies differed significantly across self-reported ethnic groups, the analyses were repeated only in those populations that had comparable allele frequencies; no significant effect of self-reported ethnicity was detected in any of our analyses.

In this study, we addressed five distinct hypotheses, limiting subsequent analyses to their further refinement: *1*) genotype at *KCNJ11* E23K (or at *ABCC8* A1369S, as both are highly correlated) influences diabetes incidence, *2*) this genetic effect is modified by a lifestyle intervention or *3*) by metformin treatment, *4*) genotype at *KCNJ11* E23K affects insulin secretion, and *5*) genotype at *KCNJ11* E23K does not affect insulin resistance. Thus, in order to account for the multiple hypotheses tested and to obtain a conservative estimate of true statistical significance, we applied a Bonferroni correction factor of 5 to the nominal two-sided *P* values.

For the quantitative trait analyses, we first compared baseline measures in the entire cohort according to genotype at each of the two SNPs. General linear models were used to test for mean differences between the quantitative traits. Means were compared, and the *P* values were further adjusted for additional comparisons across three genotypic groups (within each trait) using the Holm procedure (24). This modification of the Bonferroni adjustment ranks P_i values, and each P_i is compared with $\alpha/(n - i + 1)$ for rejection. Starting with the smallest *P* value, one continues applying these comparisons (from $i = 1$ and proceeding in order) until the first nonrejection; thus, the rejected hypotheses H_i (at $\alpha = 0.05$) are those for which $P_j \leq \alpha/(n - j + 1)$ for all $j \leq i$. The SAS analysis system version 9.1 was used for all analyses (SAS Institute, Cary, NC).

RESULTS

Allele frequency distribution. The frequency of the minor lysine allele at *KCNJ11* E23K in DPP U.S. Caucasians (0.37) was comparable with that previously reported in other Caucasian populations. We found significant differences in minor allele frequencies only in African Americans (0.08) when compared with Caucasians; therefore, analyses for incidence of diabetes were performed with and without this ethnic group. We found strong linkage disequilibrium between *ABCC8* A1369S and the downstream variant *KCNJ11* E23K in the five ethnic groups: Lewontin's D′ (25) and r^2 were 0.97/0.93, 0.98/0.93, 0.99/0.95, 0.99/0.88, and 0.97/0.95 in U.S. Caucasians, African Americans, Hispanic Americans, Asian Americans, and American Indians, respectively. Thus, not surprisingly, our findings in carriers of the alanine allele at *ABCC8* A1369S were essentially the same as those for lysine carriers at *KCNJ11* E23K in all of the analyses reported below. For simplicity of presentation, given the relative focus on *KCNJ11* E23K in the literature and the functional data on this allele, we will highlight results on that SNP in this report.

At *KCNJ11* E23K, genotypic frequencies were equally distributed among the four treatment arms and two sex groups. We found no significant differences in baseline age, BMI, or waist circumference by genotype (Table 1).

Baseline quantitative glycemic traits. We examined whether individuals with IGT also have a detectable defect in insulin release. As expected, lysine carriers at *KCNJ11* E23K had reduced insulin release at baseline, compared with E/E homozgyotes, in proportion to the number of lysine alleles (Bonferroni-corrected $P = 0.015$ for the pairwise comparison between both homozygous genotypes). Insulin sensitivity at baseline did not differ among genotypic groups (Table 2).

Incidence of diabetes. Because we detected a nominally significant interaction between genotype and metformin

TABLE 1
Demographic characteristics of the DPP cohort, according to genotype at *KCNJ11* E23K

	E/E	E/K	K/K	*P**
n	1,690	1,476	374	
Treatment				
Placebo	487 (48.9)	400 (40.2)	108 (10.9)	0.82
Metformin	474 (47.9)	419 (42.4)	96 (9.7)	
Lifestyle	465 (46.4)	423 (42.2)	114 (11.4)	
Troglitazone	264 (47.7)	234 (42.2)	56 (10.1)	
Male	540 (46.2)	495 (42.3)	135 (11.5)	0.27
Age (years)	51.0 ± 10.3	50.6 ± 10.8	50.4 ± 10.9	0.49
BMI (kg/m^2)	34.2 ± 6.8	33.8 ± 6.5	33.9 ± 6.9	0.22
Waist circumference (cm)	105 ± 14.7	105 ± 14.3	105 ± 14.3	0.84

Data are means ± SD or *n* (%). *Based on ANOVA for continuous variables and χ^2 for categorical variables.

treatment (see below), we proceeded to analyses stratified by treatment arm. In previous cross-sectional studies, the lysine allele has been associated with type 2 diabetes; however, in the placebo arm of the DPP, E/K heterozygotes with IGT appeared 29% less likely to develop diabetes than E/E homozygotes (hazard ratio [HR] 0.71 [95% CI 0.55–0.92], nominal $P = 0.01$; Bonferroni-corrected $P = 0.053$). K/K homozygotes had a similar but nonsignificant extent of protection from diabetes (0.81 [0.54–1.22], $P = 0.31$). HRs in the lifestyle arm were not statistically significant (E/K vs. E/E: 1.09 [0.78–1.54], $P = 61$; K/K vs. E/E: 0.56 [0.28–1.09], $P = 0.08$). Adjustment for BMI at randomization, age at randomization, sex, and ethnicity did not alter the results.

Metformin effect. As stated above, we observed a nominally significant interaction of metformin and genotype at *KCNJ11* E23K (nominal $P = 0.017$; Bonferroni-corrected $P = 0.085$), such that lysine carriers did not seem to benefit from the preventive effect of metformin (HR 0.89 [95% CI 0.66–1.19] for E/K heterozygotes; 0.95 [0.54–1.67] for K/K homozygotes, both vs. placebo); E/E homozygotes had a greater preventive effect (0.55 [0.42–0.71], nominal $P < 0.0001$ vs. placebo) (Fig. 1). In contrast, there was no significant interaction between the lifestyle intervention and genotype. We observed no significant longitudinal changes in weight or in fasting glucose levels across genotypic groups in the metformin arm, which could explain these findings. Examination of quantitative glycemic measures suggested that the lack of protection by metformin in K/K homozygotes may have been due to a suppression of the beneficial effect of metformin on insulin sensitivity at 1 year (Table 3).

DISCUSSION

The documented and reproducible association of selected polymorphisms in genes that encode drug targets with type 2 diabetes highlights the possible application of human genomics to medicine (26). This avenue can be immediately tested in relevant clinical trials such as the DPP. In contrast to other diabetes prevention trials (27,28), the DPP includes both lifestyle and pharmacological interventions. In addition, its multiethnic design reflects the diversity of the U.S. population, and its large sample size makes it adequate for genetic studies where variants are thought to confer modest risk. Two important distinctions between the DPP and other large observational trials (10) are its interventional design and the exclusive enrollment of individuals with IGT, which suggests the presence of some degree of genetic risk at baseline and imposes constraints in ascertainment. On the other hand, because the DPP did not include a sulfonylurea arm, we could not directly evaluate the effect of these variants on sulfonylurea therapy.

The protection from development of diabetes that we found in carriers of the lysine allele at *KCNJ11* E23K does not confirm previous reports and was unexpected: We cannot exclude that it may be a spurious result, given the marginal *P* value and multiple hypotheses examined. We note that the DPP has 73% power to detect the published

TABLE 2
Baseline measures of insulin secretion and sensitivity according to *KCNJ11* E23K and *ABCC8* A1369S genotypes in the DPP

KCNJ11 E23K	E/E	E/K	K/K	*P* value (E/E vs. E/K)*	*P* value (E/E vs. K/K)*
n	1,658	1,445	364		
Ins index [(μU/ml)/(mg/dl)]	1.07 (0.94)	1.04 (0.87)	0.97 (0.79)	0.046	0.003
ISI [(μU/ml) × (mmol/l)]$^{-1}$	0.161 (0.121)	0.162 (0.124)	0.169 (0.144)	0.98	0.76
Fasting insulin (μU/ml)	24 (16)	24 (17)	23 (18)	0.85	0.73
ABCC8 A1369S	S/S	A/S	A/A	*P* value (S/S vs. A/S)*	*P* value (S/S vs. A/A)*
n	1,635	1,424	378		
Ins index [(μU/ml)/(mg/dl)]	1.07 (0.95)	1.03 (0.86)	0.97 (0.81)	0.02	<0.01
ISI [(μU/ml) × (mmol/l)]$^{-1}$	0.162 (0.121)	0.162 (0.123)	0.172 (0.145)	0.87	0.67
Fasting insulin (μU/ml)	24 (17)	24 (17)	22 (17)	0.73	0.88

Data are median (interquartile range). The number of subjects for whom full OGTT data were available to calculate the insulinogenic index and insulin sensitivity index are displayed; a few additional subjects had fasting values only, which were used to calculate insulin sensitivity index. *Nominal two-sided *P* values are displayed; to correct for the number of hypotheses tested, a Bonferroni correction factor of ×5 can be applied (see text for details). Ins, insulinogenic; ISI, insulin sensitivity index.

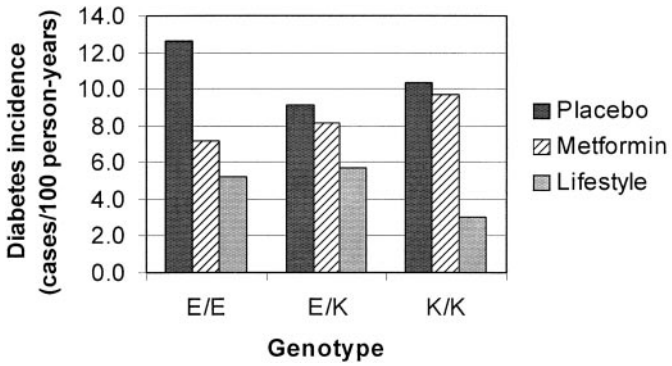

FIG. 1. Incidence rates of diabetes per genotypic group and treatment arm in the DPP (cases/100 person-years). Metformin does not seem to protect lysine carriers at *KCNJ11* E23K from developing diabetes.

HR of ~1.35 when comparing K/K with E/E homozygotes and only 51% power to detect the published HR of ~1.15 when comparing E/K heterozygotes with E/E homozygotes (29). As expected, our power drops further if the analysis is restricted to the placebo arm.

The smaller Finnish Diabetes Prevention Study (14), which also exclusively enrolled individuals with IGT, reported a nonsignificant unadjusted OR of 1.61 (95% CI 0.86–3.00), suggesting increased risk for lysine allele carriers. The Botnia Prospective Study (10), whose enrollment included 31% of subjects with either impaired fasting glucose or IGT, noted a protective but nonsignificant HR of 0.7 (95% CI 0.5–1.1) for lysine carriers versus E/E homozygotes. In a larger prospective Swedish cohort recently examined by the same investigators, the lysine allele conferred a modest but statistically significant risk of diabetes (V. Lyssenko, L. Groop, personal communication). The 95% CIs for this and the two published prospective studies overlap (albeit considering different genetic models and populations), suggesting that they may not be mutually exclusive. Since the totality of the published case/control data establishes the lysine allele as the risk variant influencing the general transition from normoglycemia to type 2 diabetes, although in this study lysine carriers in the placebo arm appeared to be protected against the development of diabetes (at a nominal $P = 0.01$) when starting from a baseline of IGT, our result raises the possibility that E23K may play its pathogenic role earlier in the course of the disease (i.e., from normoglycemia to IGT, rather than in progressing from IGT to overt diabetes), whereas other genetic and/or environmental factors may be necessary for diabetes to declare itself. This hypothesis could be tested in adequately powered prospective population cohorts. Moreover, these results illustrate the need for caution when comparing studies of different designs (cross-sectional vs. prospective), heterogeneous populations, and divergent subgroups (normoglycemia vs. IGT).

The apparent failure of metformin to protect those individuals carrying the lysine allele at *KCNJ11* E23K from developing diabetes was also unexpected and not predictable from prior biological knowledge. The effect appears to be proportional to gene dosage, arguing against a mere statistical fluctuation. A possible mechanistic explanation for this phenomenon is illustrated by the failure of K/K homozygotes to improve their insulin sensitivity after 1 year of metformin treatment, in contrast to their robust response after a lifestyle intervention or troglitazone treatment (Table 3). Why the lysine variant in a β-cell channel would lead to a differential response to metformin in insulin sensitivity requires physiologic studies and validation in an independent cohort. Interestingly, Marchetti et al. (30) reported a beneficial effect of metformin on diabetic islets when exposed to high glucose, although the possible contribution of *KCNJ11* E23K genotype was not explored.

In conclusion, we have extended the finding of impaired insulin secretion among individuals carrying the lysine variant to a multiethnic population with IGT. Our results suggest that the lysine allele may manifest its deleterious effects at earlier stages in the evolution of type 2 diabetes (i.e., during the progression from normoglycemia to IGT). Moreover, lysine carriers seem to respond less well to the

TABLE 3
One-year measures of insulin secretion and sensitivity according to *KCNJ11* E23K genotypes by treatment arm in the DPP

	E/E	E/K	K/K	*P* value*
Ins index [(μU/ml)/(mg/dl)]				
n	1,658	1,445	364	
Placebo	1.26 (1.18–1.35)	1.15 (1.06–1.25)	1.20 (1.02–1.39)	0.24
Metformin	1.30 (1.21–1.39)	1.09 (1.00–1.18)	1.16 (0.95–1.36)	0.005
Lifestyle	1.17 (1.08–1.26)	1.26 (1.17–1.35)	1.09 (0.91–1.27)	0.18
Troglitazone	1.18 (1.02–1.34)	1.26 (1.10–1.41)	1.20 (0.87–1.53)	0.78
ISI $[(\mu U/ml) \times (mmol/l)]^{-1}$				
n	1,688	1,474	374	
Placebo	0.184 (0.175–0.194)	0.192 (0.181–0.202)	0.213 (0.193–0.232)	0.04
Metformin	0.235 (0.223–0.247)	0.241 (0.229–0.254)	0.194 (0.167–0.221)	0.007
Lifestyle	0.265 (0.250–0.281)	0.279 (0.263–0.295)	0.271 (0.240–0.302)	0.47
Troglitazone	0.274 (0.246–0.302)	0.275 (0.248–0.302)	0.256 (0.199–0.313)	0.83
Fasting insulin (μU/ml)				
n	1,688	1,474	374	
Placebo	28 (26–29)	28 (26–29)	26 (23–29)	0.53
Metformin	24 (23–25)	24 (22–25)	27 (24–29)	0.04
Lifestyle	22 (21–23)	21 (20–23)	20 (18–23)	0.61
Troglitazone	21 (19–22)	21 (20–23)	21 (17–24)	0.90

Data are least-squares means (95% CI), adjusted for baseline values. *Nominal two-sided *P* values for the effect of E23K genotype are displayed; to correct for the number of hypotheses tested, a Bonferroni correction factor of ×5 can be applied (see text for details). Ins, insulinogenic; ISI, insulin sensitivity index.

protective effect of metformin than E/E homozygotes. This result, as well as the impact of this polymorphism on sulfonylurea therapy, requires validation in specifically designed pharmacogenetic studies.

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