The Krüppel-Like Factor 11 (*KLF11*) Q62R Polymorphism Is Not Associated With Type 2 Diabetes in 8,676 People

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Krüppel-like factor 11 is a pancreatic transcription factor whose activity induces the insulin gene. A common glutamine-to-arginine change at codon 62 (Q62R) in its gene *KLF11* has been recently associated with type 2 diabetes in two independent samples. Q62R and two other rare missense variants (A347S and T220M) were also shown to affect the function of KLF11 in vitro, and insulin levels were lower in carriers of the minor allele at Q62R. We therefore examined their impact on common type 2 diabetes in several family-based and case-control samples of northern-European ancestry, totaling 8,676 individuals. We did not detect the rare A347S and T220M variants in our samples. With respect to Q62R, despite >99% power to detect an association of the previously published magnitude, Q62R was not associated with type 2 diabetes (pooled odds ratio 0.97 [95% CI 0.88–1.08], $P = 0.63$). In a subset of normoglycemic individuals, we did not observe significant differences in various insulin traits according to genotype at *KLF11* Q62R. We conclude that the *KLF11* A347S and T220M mutations do not contribute to increased risk of diabetes in European-derived populations and that the Q62R polymorphism has, at best, a minor effect on diabetes risk. *Diabetes* 55:3620–3624, 2006

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AUC, area under the curve; GCI, Genomics Collaborative; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

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Krüppel-like factor 11 (*KLF11*) encodes an SP1-like transcription factor that is induced by transforming growth factor-β and regulates cell growth in the exocrine pancreas (1). Because mutations in other transcription factors that act during pancreatic β-cell development can cause monogenic forms of diabetes (2) and the transforming growth factor-β signaling pathway also determines endocrine cell fate, Neve et al. (3) recently studied this gene for its impact on insulin secretion and putative association with type 2 diabetes. They first documented expression of *KLF11* in pancreatic β-cells and showed that activated KLF11 bound and induced the insulin promoter under high-glucose conditions. Sequencing of *KLF11* in families enriched for early onset of type 2 diabetes uncovered two novel missense mutations (A347S and T220M), which segregated with diabetes in three pedigrees but were absent in other samples. Other sequencing efforts led to the identification of 19 common polymorphisms (minor allele frequency >5%), several of which were associated with type 2 diabetes in an initial case-control sample totaling 626 French individuals. Further genotyping in an additional case-control sample of 2,846 northern-European subjects replicated the association of the Q62R polymorphism (rs35927125) with type 2 diabetes (combined odds ratio [OR] 1.29 [95% CI 1.12–1.49], $P = 0.0003$, under an additive model, and 1.32 [1.13–1.54], $P = 0.0005$, under a dominant model). The authors went on to show that these missense variants impaired transcriptional activity of KLF11 and that the presence of the R-allele correlated with lower levels of insulin expression in vitro and insulin secretion in vivo (3).

Replication is essential in genetic association studies (4). In type 2 diabetes, the associations of the *PPARG* P12A and *KCNJ11* E23K polymorphisms have been widely reproduced (5) and a similar level of robust statistical evidence has emerged for variants in the *TCF7L2* gene (6–13). Determining which of the many variants in the genome are reproducibly associated with type 2 diabetes is essential in understanding the physiology and genetic architecture of this complex phenotype and pursuing viable prognostic and therapeutic options. The availability of well-characterized large diabetes samples and high-throughput genotyping technologies has allowed investigators to systematically attempt to confirm such reports of association. We therefore examined the three aforemen-

TABLE 1
Characteristics of patient samples

Population	n (male/female)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	HbA$_{1c}$ (%)* or plasma glucose at 2-h OGTT (mmol/l)†
Scandinavia trios					
Probands	168/153	39 ± 9	27 ± 5	7.2 ± 2.6	8.5 ± 2.9†
Parents	236/236				
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 case/control					
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†
Sweden case/control					
Diabetes/severe IGT	267/247	66 ± 12	28 ± 4	9.6 ± 2.9	6.5 ± 1.5*
NGT	267/247	66 ± 12	28 ± 4	5.5 ± 0.7	ND
Canada case/control					
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†
U.S. case/control					
Diabetes	644/582	63 ± 11	33 ± 7	9.8 ± 3.0	8.0 ± 3.1*
NGT	644/582	61 ± 10	27 ± 5	5.1 ± 0.9	ND
Poland case/control					
Diabetes	422/587	62 ± 10	30 ± 5	8.9 ± 4.0	7.9 ± 1.3*
NGT	422/587	59 ± 7	26 ± 4	4.8 ± 1.2	ND

Data are means ± SD. Plasma glucose was measured at baseline (fasting) and 2 h after an OGTT. Severe impaired glucose tolerance (IGT) was defined as an OGTT 2-h blood glucose ≥8.5 mmol/l but <10.0 mmol/l. ND, not determined; NGT, normal glucose tolerance.

tioned missense variants in *KLF11* for association with type 2 diabetes and/or insulin-related phenotypes in several well-powered case-control and family-based samples.

RESEARCH DESIGN AND METHODS

The diabetes samples are presented in Table 1 and have been described previously (14,15). Briefly, Scandinavian samples from the Botnia Study (16) include 321 trios, 1,189 siblings discordant for type 2 diabetes, and a case-control sample totaling 942 subjects individually matched for age, BMI, and region of origin. In the Botnia Study, case subjects included individuals with type 2 diabetes or severe impaired glucose tolerance, defined as a 2-h blood glucose ≥8.5 mmol/l but <10.0 mmol/l during an oral glucose tolerance test (OGTT). In addition, we studied a case-control sample from Sweden totaling 1,028 subjects who were individually matched for sex, age, and BMI; an individually matched case-control sample totaling 254 subjects from the Saguenay Lac-St. Jean region in Quebec (Canada); and two case-control Caucasian diabetes samples obtained from Genomics Collaborative (GCI): one comprised of 1,226 case and 1,226 control subjects from the U.S. and one comprised of 1,009 case and 1,009 control subjects from Poland, both matched for age, sex, and grandparental country of origin. These samples have been validated by the replication of the three most widely reproduced associations in type 2 diabetes, *PPARG* P12A (14), *KCNJ11* E23K (17,18), and *TCF7L2* (12).

Genotyping. Genotyping was performed by allele-specific primer extension of multiplex products with detection by MALDI-TOF on the Sequenom platform (19,20). Average genotyping success was 96.2%, and there were no discrepancies in comparisons of 1,873 duplicate genotypes, indicating a low error rate. Genotype counts for the various samples tested in this study are shown in Table 2.

Statistical analysis. Power calculations were performed using the program of Purcell et al. (21) (http://pngu.mgh.harvard.edu/~purcell/gpc/). To examine the association of single nucleotide polymorphisms (SNPs) and haplotypes with type 2 diabetes, we used simple χ^2 analysis in the case-control samples, the transmission disequilibrium test (22) in the diabetic trios, and the discordant allele test (23) in the sib pairs. Results from the various samples were combined by Mantel-Haenszel meta-analysis of the OR (24); all *P* values are two tailed. Homogeneity of ORs among study samples was tested using an asymptotic Breslow-Day statistic (25).

Quantitative trait comparisons. Plasma glucose was measured by a glucose oxidase method on a Beckman Glucose analyzer (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay. A 75-g OGTT was performed in a subset of the control Scandinavian subjects (n = 791, 382 female). The insulinogenic index was calculated as [(insulin at 30 min) − (insulin at 0 min)]/[(glucose at 30 min) − (glucose at 0 min)]; insulin area under the curve (AUC) was calculated by the trapezoidal method. BMI, the insulinogenic index, insulin AUC, and insulin levels at 0, 60, and 120 min were log transformed to improve normality and compared by ANOVA across the three genotypic groups and by t test between Q/Q homozygotes and Q/R heterozygotes.

RESULTS

The two rare variants (A347S and T220M) were monomorphic in our samples. Assuming a type 2 diabetes prevalence of 8%, a minor allele frequency for Q62R of 10%, and the published OR of 1.29, our power calculations demonstrated that our combined sample of 3,347 case-control pairs, 321 trios, and 1,189 discordant sibs had >99% power to detect an association with type 2 diabetes under additive or dominant models; this power would obviously be reduced if the initial association was an overestimate of the true effect due to the "winner's curse" (26).

Genotype counts, ORs, *P* values, and a meta-analysis of the association studies for the common Q62R polymorphism are presented in Table 2. No heterogeneity was detected among subsamples. We observed no significant association of Q62R with type 2 diabetes (pooled OR 0.97 [95% CI 0.88–1.08], P = 0.63).

Because our Scandinavian case-control samples were matched for BMI, it is possible that overmatching may have prevented us from detecting a true effect on risk of type 2 diabetes, if this effect was mediated through BMI. We therefore assessed whether BMI was associated with Q62R in the Scandinavian normoglycemic subjects; no significant differences in BMI were detected across genotypic groups at *KLF11* Q62R.

Since *KLF11* has been postulated to affect insulin gene expression in vitro and insulin levels in vivo (3), we attempted to replicate these findings in a subset of 791

TABLE 2
Genotype counts for the *KLF11* Q62R polymorphism in the various subsamples

Family based				MAF	OR (95% CI)	*P* value
	Transmitted		Untransmitted			
Trios	27		36	0.09	0.75 (0.46–1.23)	0.26
	Excess in affected		Excess in unaffected			
Discordant sibs	23		25	0.09	0.92 (0.52–1.62)	0.77
Case/control	G/G (R/R)	A/G (Q/R)	A/A (Q/Q)			
Scandinavia						
Case subjects	7	74	388	0.09		
Control subjects	8	66	394	0.09	1.08 (0.79–1.48)	0.64
Sweden						
Case subjects	7	88	409	0.10		
Control subjects	3	90	410	0.10	1.07 (0.80–1.43)	0.66
Canada						
Case subjects	2	23	86	0.12		
Control subjects	4	22	83	0.14	0.87 (0.50–1.51)	0.62
GCI U.S.						
Case subjects	16	253	938	0.12		
Control subjects	15	263	920	0.12	0.96 (0.81–1.14)	0.65
GCI Poland						
Case subjects	18	175	807	0.11		
Control subjects	11	193	793	0.11	0.98 (0.80–1.19)	0.81
Overall					0.97 (0.88–1.08)	0.63

The transmission disequilibrium test and the discordant allele test were performed in the Scandinavian trios and the discordant sibpairs, respectively. ORs were calculated for the minor allele (G, coding for arginine) versus the major allele (A, coding for glutamine) and pooled by Mantel-Haenszel analysis. MAF, minor allele frequency.

Scandinavian normoglycemic individuals for whom we had OGTT data. We found no significant differences across genotypic groups in the insulinogenic index, insulin AUC, or absolute insulin levels at 0, 60, or 120 min during an OGTT (Table 3).

To test our expectation that the haplotype structure of the French samples in the study by Neve et al. (3), and that of other European populations, is comparable at this locus, we also genotyped rs4444493 (SNP 17 in ref. 3, located 6.7 kb downstream of Q62R) in the HapMap CEU plate and in a subset of our samples. As previously observed in the French population, linkage disequilibrium between the two SNPs is strong ($D' = 1.0/r^2 = 1.0$ in the HapMap CEU sample, and 1.0/0.95, 0.98/0.94, and 0.99/0.97, respectively, in the Scandinavian, U.S., and Polish samples), reflecting their similar ancestral origin.

DISCUSSION

We set out to replicate the association of missense variants in the *KLF11* gene with type 2 diabetes and to confirm their role in influencing insulin secretion. In a sample of northern-European descent comprising both case-control and family-based panels totaling 8,676 individuals, we were unable to document a significant association of *KLF11* Q62R with type 2 diabetes or several related insulin traits.

Our negative results can have several explanations. First, the Q62R polymorphism may not contribute to risk of type 2 diabetes in the populations studied. While Neve et al. (3) reported a low *P* value in their smaller familial sample (OR 1.85 [95% CI 1.33–2.57], $P = 0.00023$), the effect was much more modest in their larger replication sample (1.18 [1.01–1.38], $P = 0.034$), raising the possibility that the familial sample may differ in some way from the general population. In addition, although they provided supporting functional data, they also performed a large number of statistical tests. In the context of the many association studies currently being performed by multiple research groups across the genome, the nominal statistical significance and estimated magnitude of effect for any original finding must be interpreted with caution.

TABLE 3
Quantitative traits in 791 control subjects according to genotype at Q62R

Trait	R/R	Q/R	Q/Q	*P* ANOVA	*P* (*t* test) Q/R vs. Q/Q
n	19	111	661		
BMI (kg/m^2)	25.8 ± 3.2	26.5 ± 3.5	26.4 ± 3.8	0.81	0.80
Insulin$_0$ (mU/l)	8.2 ± 5.5	8.6 ± 4.5	8.1 ± 5.0	0.56	0.30
Insulin$_{60}$ (mU/l)	50.4 ± 27.4	68.0 ± 51.4	65.7 ± 43.1	0.37	0.77
Insulin$_{120}$ (mU/l)	35.7 ± 25.6	49.7 ± 40.6	44.2 ± 35.2	0.36	0.28
Insulinogenic index	22.2 ± 20.9	18.3 ± 14.0	22.0 ± 36.0	0.83	0.78
Insulin AUC (units/l)	4.4 ± 2.6	5.4 ± 3.8	5.1 ± 3.2	0.55	0.59

Data are mean ± SD.

Second, our analysis may have yielded false-negative results. While the power of a meta-analysis of seven smaller subsamples is not equivalent to a single association study of the same size, we note that no heterogeneity was detected among our subsamples and that the present design takes advantage of two family-based panels that are robust to population stratification. In addition, these same samples have been adequate to detect the most commonly reproduced genetic associations with type 2 diabetes (12,14,17,18). Nevertheless, if the true genotypic risk ratio is lower than the original estimate, we may have been underpowered to detect it. We note that our 95% CI does not overlap with the one reported by Neve et al. (3) (see above), suggesting that it is highly unlikely that the OR in our population lies within the interval estimate of the original report. In either case, it appears that even if Q62R does increase the risk of type 2 diabetes, its influence is quite modest.

Finally, the initial association signal may have arisen from another *KLF11* variant that was in linkage disequilibrium with Q62R in the original samples but not in ours. The similar haplotype structure of populations that share northern-European ancestry (27,28), the strong linkage disequilibrium between Q62R and rs4444493 in the French, Scandinavian, U.S., and Polish samples, and our failure to detect such an association in three different Caucasian samples also makes this explanation less likely. Upcoming high-density whole-genome association scans in large samples should be able to answer whether other common variants in *KLF11* might contribute to diabetes risk.

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