

## Brief Report

# Analysis of Genetic Variation in Akt2/PKB- $\beta$ in Severe Insulin Resistance, Lipodystrophy, Type 2 Diabetes, and Related Metabolic Phenotypes

Karen Tan,<sup>1,2</sup> Wendy A. Kimber,<sup>1</sup> Jian'an Luan,<sup>3</sup> Maria A. Soos,<sup>1</sup> Robert K. Semple,<sup>1</sup> Nicholas J. Wareham,<sup>3</sup> Stephen O'Rahilly,<sup>1</sup> and Inês Barroso<sup>2</sup>

We previously reported a family in which a heterozygous missense mutation in Akt2 led to a dominantly inherited syndrome of insulin-resistant diabetes and partial lipodystrophy. To determine whether genetic variation in *AKT2* plays a broader role in human metabolic disease, we sequenced the entire coding region and splice junctions of *AKT2* in 94 unrelated patients with severe insulin resistance, 35 of whom had partial lipodystrophy. Two rare missense mutations (R208K and R467W) were identified in single individuals. However, insulin-stimulated kinase activities of these variants were indistinguishable from wild type. In two large case-control studies (total number of participants 2,200), 0 of 11 common single nucleotide polymorphism (SNPs) in *AKT2* showed significant association with type 2 diabetes. In a quantitative trait study of 1,721 extensively phenotyped individuals from the U.K., no association was found with any relevant intermediate metabolic trait. In summary, although heterozygous loss-of-function mutations in *AKT2* can cause a syndrome of severe insulin resistance and lipodystrophy in humans, such mutations are uncommon causes of these syndromes. Furthermore, genetic variation in and around the *AKT2* locus is unlikely to contribute significantly to the risk of type 2 diabetes or related intermediate metabolic traits in U.K. populations. *Diabetes* 56:714–719, 2007

**T**he serine/threonine-protein kinase Akt/protein kinase B (PKB) plays a critical role in insulin receptor–coupled phosphatidylinositol 3-kinase–mediated signaling (1). There are three Akt mammalian isoforms (Akt1–3), of which Akt2 is the most important in glucose metabolism (1). Mice deficient in

Akt2 exhibit fed and fasting hyperglycemia, hyperinsulinemia, glucose intolerance, and impaired muscle glucose uptake (2,3). We identified a missense mutation in the kinase domain of Akt2 (R274H) in a single family with autosomal dominantly inherited severe insulin resistance and diabetes (4). The proband of this family had partial lipodystrophy (4), suggesting that Akt2 may play a role in adipogenesis. To date, detailed genetic association studies of *AKT2* have not been reported.

We undertook studies to determine 1) whether other missense/nonsense mutations in *AKT2* might result in human syndromes of severe insulin resistance with or without accompanying lipodystrophy and 2) whether common genetic variants in *AKT2* might be associated with metabolic phenotypes related to insulin resistance. The entire coding sequence and splice junctions of *AKT2* were screened in 94 probands with severe insulin resistance, 35 of which had partial lipodystrophy. The results of this screen are shown in online appendix Table 1 (available at <http://dx.doi.org/10.2337/db06-0921>). We identified two novel missense mutations (Fig. 1A). R467W was found in a white female patient with type 2 diabetes and partial lipodystrophy. This variant was present in neither 47 ethnically matched control subjects nor in 2 unaffected sons of the carrier. R208K was identified in a white female patient with severe insulin resistance and acanthosis nigricans. This variant was not present in her affected son but was present in 1 of 47 white control subjects. Unfortunately, parental DNA was not available to determine whether these mutations were inherited or spontaneous.

We investigated whether these mutations might cause functional impairment of the Akt2 kinase in vitro. CHO-T cells overexpressing the insulin receptor were transfected with either wild-type HA-Akt2 or mutant HA-Akt2. The ability of Akt2 to phosphorylate an artificial peptide substrate based on glycogen synthase kinase-3 was measured in an in vitro kinase assay using anti-HA immunoprecipitates from the transiently transfected cells, which were serum starved and then stimulated with insulin or left untreated. The known kinase-dead R274H mutant (4) was used as a negative control. Neither R208K nor R467W significantly altered either the basal or the insulin-stimulated kinase activity of Akt2 on the peptide substrate (Fig. 1B). The mutations did not affect expression levels (Fig. 1C). Upon stimulation with insulin, both Akt2 mutants were phosphorylated on T309 and S474 to a similar extent as wild-type Akt2 (Fig. 1C).

Although it is possible that these in vitro assays may fail to detect subtle changes in function of the Akt2 kinase,

From the <sup>1</sup>Department of Clinical Biochemistry, University of Cambridge, Cambridge, U.K.; the <sup>2</sup>Metabolic Disease Group, The Wellcome Trust Sanger Institute, Cambridge, U.K.; and the <sup>3</sup>Medical Research Council Epidemiology Unit, Cambridge, U.K.

Address correspondence and reprint requests to Dr. Inês Barroso, Metabolic Disease Group, Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, U.K. E-mail: [ib1@sanger.ac.uk](mailto:ib1@sanger.ac.uk).

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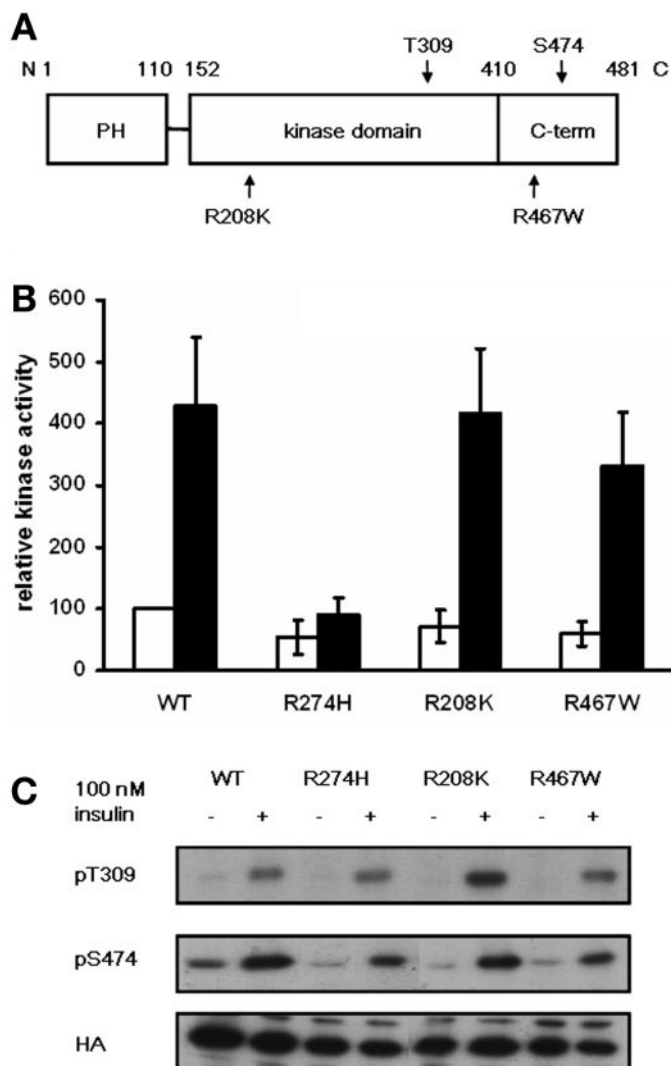
Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db06-0921>.

CCC, Cambridgeshire Case Control; EPIC, European Prospective Investigation into Cancer and Nutrition; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; MRC, Medical Research Council; SIR, severe insulin resistant; SNP, single nucleotide polymorphism.

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**FIG. 1.** Rare mutations in Akt2 in human individuals. **A:** Location of the identified mutations *R208K* and *R467W* in relation to functional domains and known phosphorylation sites. **B:** In vitro kinase assay of Akt2 mutants. HA-Akt2 and HA-Akt2 mutants were immunoprecipitated from lysates of appropriately transfected CHO-T cells treated with (■) or without (□) 100 nmol/l insulin 10 min before lysis. Kinase assays were performed as described in RESEARCH DESIGN AND METHODS. Kinase activities are adjusted relative to that of unstimulated wild-type control subjects (100%). Data measured are means  $\pm$  SD of five independent experiments. **C:** Equal amounts of lysates were immunoblotted with anti-phospho-Thr<sup>308</sup> Akt (upper panel [pT309]) and anti-phospho-Ser<sup>473</sup> Akt (middle panel [pS474]) antibodies to demonstrate increased phosphorylation in response to insulin. Immunoprecipitates were also immunoblotted with anti-HA antibody (lower panel [HA]) to demonstrate similar levels of immunoprecipitates used in the kinase assays.

including, for example, selective impairments of activity at specific substrates, the normality of responses of the mutant kinases in two different assay systems suggests that their function is likely to be unimpaired. Taken together, these data suggest these mutations are unlikely to be directly implicated in the severe insulin resistance of the probands.

To explore whether common single nucleotide polymorphisms (SNPs) in *AKT2* are associated with type 2 diabetes, we studied two U.K. case-control studies: the Cambridgeshire Case-Control (CCC) Study and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. Based on the criteria described (see

RESEARCH DESIGN AND METHODS), we obtained results for 11 polymorphic SNPs (Table 1). Average call rates were 93.3 and 97.7% for the CCC and EPIC-Norfolk studies, respectively. All SNPs were in Hardy-Weinberg equilibrium (HWE) ( $P > 0.01$ ) in these populations. To increase power, we performed a joint analysis of the two case-control studies, with a term for study, and tested for heterogeneity between the studies (Table 1). When the genotype frequencies were compared between the combined case and control subjects, no statistically significant associations were detected (Table 1). Additional genotyping of other loci in these populations did not show evidence for heterogeneity; thus, the nominally significant associations in the separate case-control studies are likely to be due to chance. However, the heterogeneity could also be due to subtle differences in population substructure between study populations.

We next evaluated the degree of linkage disequilibrium among the 11 SNPs genotyped (Fig. 2). As shown, all but two SNPs (rs4273150 and rs3730256) fall into two linkage disequilibrium blocks (5,6). Haplotype analysis in each of these blocks did not provide evidence of statistically significant associations with disease risk (data not shown). Furthermore, to estimate coverage of the genetic variation captured, we compared our selected SNPs with those in Centre d'Etude du Polymorphisme Humain samples in HapMap II (release no. 20). There are 21 SNPs with minor allele frequency (MAF)  $>5\%$ , spanning from 22 kb upstream of the start of the transcript to the 3' untranslated region of the gene. We have tagged all SNPs present in HapMap (pairwise tagging with  $r^2 = 0.8$  and MAF  $>5\%$ ) except for SNPs rs10426842 and rs12460555 (each of these just tag themselves). Taken together, our data suggest that common variants in *AKT2* do not significantly contribute to the type 2 diabetes status in the U.K. population.

To examine whether the common variants in *AKT2* are associated with metabolic parameters related to type 2 diabetes, we further genotyped 1,721 unrelated U.K. white participants in the Medical Research Council (MRC) Ely Study, a prospective population-based study of the etiology of type 2 diabetes (7). Eleven SNPs were genotyped with an average call rate of 96.6%, although SNP8 and SNP11 were not in HWE ( $P < 0.01$ ) (Table 2). We tested for association of these SNPs with fasting and 2-h post-challenge plasma glucose levels, fasting plasma insulin levels, and 30-min insulin incremental response, a measure of insulin secretion (8) (Table 2). None of the SNPs showed any association ( $P < 0.01$ ) with glucose or insulin levels. Therefore, these data suggest that common polymorphisms in *AKT2* do not significantly contribute to plasma glucose or insulin levels.

In summary, we screened *AKT2* as a candidate gene for human insulin resistance and partial lipodystrophy and detected two novel missense mutations, which did not affect function, at least in the assays used. We conducted the first association study of common variants in the *AKT2* gene with human type 2 diabetes and related metabolic phenotypes. No statistically significant associations were found, suggesting that common variants in *AKT2* are not associated with type 2 diabetes, in the populations studied. For a SNP with a MAF of 0.2, the power to detect an effect with an odds ratio  $>1.2$  (or  $<0.82$ ) is 80%,  $\alpha = 0.05$  (960 case and 1,386 control subjects). Therefore, small to moderate effects, if they exist, would be detected by our study. Further studies will be required to completely rule out small effects of *AKT2* on type 2 diabetes risk.

TABLE 1  
Relationship between genotype in *AKT2* and type 2 diabetes status in a U.K. population

Study	Cases*			Controls*			OR (95% CI)	<i>P</i> (trend)	<i>P</i> (heterogeneity)
	11	12	22	11	12	22			
EPIC-Norfolk									
SNP1	325	28	0	676	60	1	0.88 (0.55–1.43)	0.627	
SNP2	265	78	8	535	176	23	0.87 (0.67–1.13)	0.312	
SNP3	161	143	40	382	279	57	1.25 (1.02–1.53)	0.031	
SNP4	270	73	7	595	130	10	1.21 (0.91–1.61)	0.190	
SNP5	297	54	2	622	110	7	0.95 (0.68–1.33)	0.783	
SNP6	145	142	36	332	256	50	1.24 (1.01–1.53)	0.045	
SNP7	183	138	30	433	260	43	1.25 (1.01–1.54)	0.037	
SNP8	159	146	37	370	284	54	1.22 (0.99–1.49)	0.061	
SNP9	165	151	37	386	294	57	1.19 (0.97–1.45)	0.091	
SNP10	265	81	8	540	174	23	0.91 (0.70–1.18)	0.462	
SNP11	102	167	80	182	370	176	0.92 (0.76–1.11)	0.390	
CCC									
SNP1	439	45	2	458	37	2	1.19 (0.77–1.83)	0.433	
SNP2	338	123	12	344	118	9	1.09 (0.84–1.42)	0.505	
SNP3	242	173	33	226	199	44	0.81 (0.65–1.00)	0.047	
SNP4	405	75	1	391	102	2	0.69 (0.49–0.96)	0.028	
SNP5	421	58	2	419	76	1	0.78 (0.54–1.12)	0.182	
SNP6	288	205	45	243	227	51	0.78 (0.64–0.95)	0.012 <sup>†</sup>	
SNP7	286	170	19	263	194	35	0.74 (0.59–0.92)	0.007 <sup>†</sup>	
SNP8	253	166	36	220	181	45	0.80 (0.65–0.99)	0.042	
SNP9	254	189	38	235	215	47	0.82 (0.67–1.00)	0.050	
SNP10	338	132	10	358	128	10	1.09 (0.84–1.41)	0.518	
SNP11	123	229	124	149	231	114	1.17 (0.98–1.41)	0.082	
EPIC-Norfolk and CCC									
SNP1	764	73	2	1,134	97	3	1.04 (0.76–1.43)	0.789	0.373
SNP2	603	201	20	879	294	32	0.98 (0.81–1.17)	0.794	0.237
SNP3	403	316	73	608	478	101	1.01 (0.66–1.54)	0.981	0.003 <sup>‡</sup>
SNP4	675	148	8	986	232	12	0.92 (0.53–1.60)	0.764	0.012
SNP5	718	112	4	1,041	186	8	0.87 (0.68–1.11)	0.269	0.415
SNP6	433	347	81	575	483	101	0.98 (0.62–1.55)	0.938	0.001 <sup>‡</sup>
SNP7	469	308	49	696	454	78	0.96 (0.58–1.61)	0.882	0.001 <sup>‡</sup>
SNP8	412	312	73	590	465	99	0.99 (0.66–1.48)	0.959	0.006 <sup>‡</sup>
SNP9	419	340	75	621	509	104	0.99 (0.68–1.43)	0.938	0.010
SNP10	603	213	18	898	302	33	0.99 (0.83–1.19)	0.941	0.336
SNP11	225	396	204	331	601	290	1.04 (0.92–1.19)	0.528	0.068

\*Odds ratio (OR) calculated per allele 2 from an additive logistic model on the genotypes, adjusted for age, sex, and BMI. <sup>†</sup>*P* < 0.05 for linear trend model. <sup>‡</sup>*P* < 0.01 for heterogeneity between studies. Results were adjusted for heterogeneity whenever significant heterogeneity between studies was present. Because there were six SNPs with significant heterogeneity (*P* < 0.05), we reported the results for each case-control study and the combined data. 1, major allele, 2, minor allele.

Evidence from recent years has suggested that studying rare monogenic forms of diabetes not only contributes to our understanding of the mechanisms of glucose homeostasis but also might constitute an effective strategy to identify genes involved in more common and complex forms of diabetes. Indeed, while rare highly penetrant mutations in *HNF4A*, *PPARG*, and *KIR6.2* lead to monogenic forms of diabetes, common variants in each of these genes have been shown to increase disease risk in type 2 diabetes (9). This study suggests that this is not the case for *AKT2*.

## RESEARCH DESIGN AND METHODS

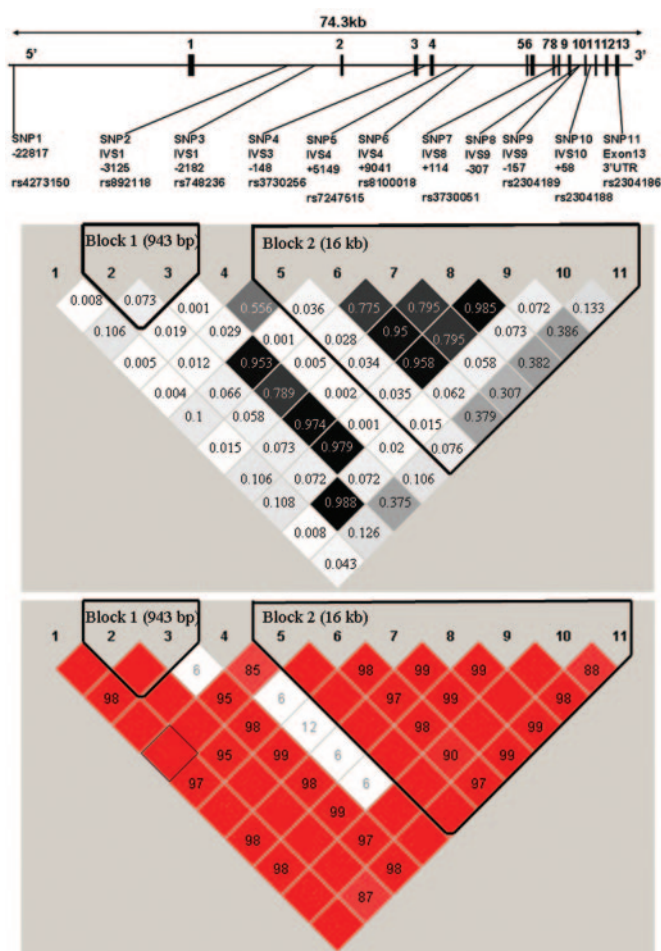
**Genetic screening.** Genomic DNA was preamplified in a GenomiPhi reaction (Amersham Biosciences). Twelve primer pairs were designed to cover all coding exons and intron-exon boundaries using Primer3 software (online appendix Table 2). PCR was performed using Taq polymerase (Abgene), and PCR products were sequenced using Big Dye Terminator 3.1 DNA sequencing kit (Applied Biosystems).

**Plasmid cloning and mutagenesis.** Akt2 cDNA was provided by D. Alessi (10). Mutagenesis was performed by QuickChange Site Directed Mutagenesis Kit (Stratagene).

**Transient transfection and protein blotting.** CHO-T cells overexpressing the insulin receptor were transfected with pCDNA3.1-Akt2 or pCDNA3.1-Akt2 mutants using polyethylenimine (11). Twenty-four hours after transfection, cells were serum starved for 8 h; 100 nmol/l insulin (human Actrapid; Abbott Laboratories) was added for 10 min, and the cells were lysed. Total protein was quantified using Bio-Rad D<sub>c</sub> Protein Assay, and equal amounts of protein were run on 10% SDS-PAGE followed by Western blotting with anti-phospho-Akt (T308), anti-phospho-Akt (S473), and total Akt antibodies (Cell Signaling).

**Immunoprecipitation and in vitro kinase assay.** Immunoprecipitation was carried out using 10  $\mu$ g anti-HA (F-7) agarose conjugate (Santa Cruz) for 4 h at 4°C. Immunoprecipitates containing HA-Akt2 and HA-Akt2 mutants were assayed for in vitro kinase activity as previously described (10). Crosstide (Sigma) was used as the substrate in a reaction containing 50 mmol/l Tris, pH 7.5, 0.1 mmol/l EGTA, 0.1% (wt/vol)  $\beta$ -mercaptoethanol, 10 mmol/l magnesium acetate, 100  $\mu$ mol/l 32P $\gamma$ -ATP, and 30  $\mu$ mol/l Crosstide. The reaction was stopped after incubation at 30°C for 30 min, and the incorporated radioactivity was counted.

**Severe insulin-resistant cohort.** The inclusion criteria for this cohort were 1) a fasting insulin >100 pmol/l or an insulin requirement >200 units/day, 2) acanthosis nigricans, and 3) BMI <33 kg/m<sup>2</sup>. From this cohort, 94 individuals who have not been previously screened for mutations in *AKT2* were screened for mutations in all coding sequence and splice junctions of *AKT2*. After confirmation of genetic variants in the severe insulin resistant (SIR) cohort



**FIG. 2.** Location and linkage disequilibrium map of *AKT2* SNPs genotyped. Thirteen exons of *AKT2* are represented by solid bars (numbered 1–13); intronic regions and 5' and 3' regions are represented by solid lines. The positions of SNPs 1–11 are indicated. The dbSNP reference numbers are indicated below each SNP. The pairwise linkage disequilibrium coefficient  $r^2$  (top) and  $D'$  (bottom) for the control subjects in the case-control studies were calculated for genotyped SNPs using Haploview. Haplotype blocks were identified using Haploview.

using unamplified genomic DNA, a control population, consisting of 47 white individuals, was screened for the presence of these variants. Lipodystrophic patients were a subset of the SIR patients in whom partial or complete lipodystrophy was diagnosed on the basis of clinical examination and in whom sequencing of the *LMNA* gene exons 8–12 and *PPARG* gene were normal.

#### Case-control studies

**CCC Study.** This population-based study has been previously described (12). Briefly, this study consists of 552 type 2 diabetic patients aged 47–75 years and individually age-, sex-, and geographical location-matched control subjects. Case subjects were defined by onset of diabetes after the age of 30 years without insulin treatment in the 1st year following diagnosis. Potential control subjects that had A1C levels  $>6\%$  were excluded.

**EPIC-Norfolk participants.** This is a nested case-control study within the EPIC-Norfolk prospective cohort study; both the case-control and full cohort study (13,14) have been previously described in detail. Briefly, the case-control study consists of 417 incident type 2 diabetic case and control subjects and two sets of 417 control subjects, matched by age, sex, time in study, and family physician with the second set additionally matched for BMI. A case was defined by a physician's diagnosis of type 2 diabetes, with no insulin prescribed within the 1st year after diagnosis and/or A1C  $>7\%$  at baseline or the follow-up health check. Control subjects were selected from those in the cohort who had not reported diabetes, cancer, stroke, or myocardial infarction at baseline and who had not developed diabetes by the time of selection. Potential control subjects with measured A1C levels  $>6\%$  were excluded. DNA was available for this analysis from 354 case and 741 control subjects.

**MRC Ely Study.** This is a population-based cohort study of the etiology and pathogenesis of type 2 diabetes and related metabolic disorders in the U.K.

(7). It is an ethnically homogeneous Europic population in which phenotypic data have been recorded at the outset and after 4.5 years. This analysis included 1,721 men and women aged 35–79 years without diagnosed diabetes who attended the study clinic for a health check between 2000 and 2004. Of these, 1,005 individuals were attending a follow-up health check, while the remaining 716 were newly recruited in 2000 from the original population sampling frame. Participants attending the health check underwent standard anthropometric measurements and a 75-g oral glucose tolerance test.

**Ethical permission.** Ethical permission for the three studies was granted by their respective local research ethics committee, and study participants provided informed consent.

**SNP selection.** SNP selection for this study was undertaken before HapMap I (15) data were available. SNPs were selected from the National Center for Biotechnology Information dbSNP database and direct sequencing of SIR samples. SNPs with a MAF  $\geq 5\%$  were selected. For dbSNPs with no frequency information, SNPs that had been validated by cluster and by submitter were preferentially selected. Where possible, it was attempted not to have gaps  $>2.5$ –3 kb between any consecutive SNPs selected. Nineteen different SNPs were chosen from the dbSNP database for genotyping. One additional SNP (SNP8) was identified through direct sequencing. Of these 20 SNPs, 5 failed assay design (rs4803320, rs892120, rs4803322, rs7409393, and rs6508935), 5 were monomorphic (rs2288917, rs3730260, rs1804324, rs1142298, and rs7247518), and 1 failed genotyping (rs892119) due to low call rate ( $<80\%$ ). After HapMap I data were available, two additional SNPs (rs3730051 and rs8100018) were selected to increase coverage of genetic variation in this gene. In total, data from 11 SNPs were available for analysis.

**Methods for genotyping.** For the case-control populations, case and control samples were randomly distributed across each 96-well plate, with approximately the same number of case and control subjects per plate. Between 3 and 8.5% internal replicate samples were included in each population in all genotyping tests to assess genotyping accuracy. Genotyping of samples was performed in 384-well plates at the Wellcome Trust Sanger Institute, Cambridge, U.K., using an adaptation of the homogenous MassExtend protocol supplied by Sequenom for the MassArray system (Sequenom) (16). Call rates were  $\geq 90\%$ , and concordance rates between duplicate samples were  $\geq 99.8\%$  for all assays included in the analysis.

**Statistical analyses.** All analyses used Stata/SE 9.2 for Windows (Stata Corporation, College Station, TX). Genotype frequencies were tested for HWE using a  $\chi^2$  goodness-of-fit test in all samples in the Ely Study and in the control subjects of the case-control studies. For each SNP, an additive model (linear trend, which assumes an additive effect for the presence of zero, one, or two rare alleles) on 1 degree of freedom and a general model (compares the three genotypes as a categorical variable) on 2 degrees of freedom were performed to assess the association with diabetes and quantitative traits. Unconditional logistic regression was applied to the two combined case-control studies adjusted for age, sex, BMI, and study, and the matching variables were included as covariates. Heterogeneity between studies was tested, and, if significant, we used a random-effect meta-analysis, which incorporates an estimate of the between-study variation. Quantitative trait analysis was undertaken in the Ely Study population. Association between quantitative traits and genotype were tested in a linear regression model and adjusted for age, sex, and BMI. The likelihood-ratio test comparing statistical models did not show the general model to be better than the additive model in any of the SNPs; hence, we presented only the additive model.

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TABLE 2  
Relationship between genotype in *AKT2* and glucose and insulin levels in a U.K. population

Genotype frequencies	11	12	22	<i>P</i> (HWE)
SNP1	1,479	124	2	0.719
SNP2	1,203	430	38	0.954
SNP3	839	656	165	0.029
SNP4	1,362	297	15	0.788
SNP5	1,452	227	7	0.554
SNP6	834	664	166	0.047
SNP7	944	605	129	0.021
SNP8	847	637	166	0.005*
SNP9	849	664	161	0.063
SNP10	1,205	438	41	0.873
SNP11	472	768	409	0.006*
Parameter	11	12	22	<i>P</i> (trend)
PG0†				
SNP1	5.04 (5.01–5.07)	5.00 (4.89–5.12)	5.20 (4.35–6.22)	0.668
SNP2	5.00 (4.97–5.04)	5.10 (5.04–5.16)	5.11 (4.90–5.32)	0.034
SNP3	5.04 (5.00–5.09)	5.02 (4.97–5.08)	5.04 (4.94–5.14)	0.541
SNP4	5.04 (5.00–5.07)	5.04 (4.96–5.11)	5.01 (4.68–5.35)	0.459
SNP5	5.03 (5.00–5.07)	5.03 (4.94–5.12)	5.28 (4.79–5.82)	0.549
SNP6	5.04 (4.99–5.08)	5.02 (4.97–5.07)	5.05 (4.95–5.16)	0.701
SNP7	5.03 (4.98–5.07)	5.04 (4.99–5.09)	5.04 (4.93–5.15)	0.934
SNP8	5.05 (5.00–5.09)	5.02 (4.97–5.07)	5.04 (4.95–5.14)	0.366
SNP9	5.04 (5.00–5.09)	5.02 (4.97–5.07)	5.03 (4.93–5.13)	0.396
SNP10	5.01 (4.97–5.04)	5.11 (5.04–5.17)	5.11 (4.91–5.32)	0.034
SNP11	5.08 (5.02–5.14)	5.02 (4.97–5.06)	5.02 (4.95–5.08)	0.318
2-h plasma glucose‡				
SNP1	6.03 (5.93–6.14)	6.06 (5.71–6.44)	6.61 (4.18–10.45)	0.923
SNP2	6.02 (5.90–6.14)	6.02 (5.83–6.22)	6.28 (5.63–7.01)	0.745
SNP3	6.07 (5.93–6.21)	5.90 (5.75–6.06)	6.31 (5.99–6.65)	0.790
SNP4	5.99 (5.88–6.10)	6.19 (5.95–6.43)	6.32 (5.28–7.56)	0.070
SNP5	6.00 (5.90–6.11)	6.10 (5.83–6.38)	6.82 (5.11–9.10)	0.497
SNP6	6.06 (5.92–6.21)	5.91 (5.76–6.07)	6.26 (5.94–6.59)	0.804
SNP7	6.04 (5.90–6.17)	5.95 (5.79–6.11)	6.24 (5.89–6.61)	0.704
SNP8	6.07 (5.93–6.21)	5.87 (5.72–6.03)	6.29 (5.97–6.63)	0.908
SNP9	6.07 (5.93–6.21)	5.92 (5.77–6.08)	6.28 (5.95–6.62)	0.829
SNP10	6.01 (5.90–6.13)	6.03 (5.83–6.22)	6.36 (5.72–7.07)	0.839
SNP11	6.21 (6.02–6.41)	5.90 (5.76–6.04)	6.10 (5.90–6.30)	0.633
INS0§				
SNP1	49.3 (48.0–50.6)	50.9 (46.5–55.8)	51.9 (25.5–105.5)	0.246
SNP2	48.5 (47.1–49.9)	49.1 (46.8–51.6)	54.1 (46.0–63.6)	0.658
SNP3	49.6 (47.9–51.4)	47.8 (46.0–49.8)	51.3 (47.5–55.5)	0.830
SNP4	49.2 (47.8–50.5)	48.3 (45.6–51.2)	59.0 (45.5–76.4)	0.307
SNP5	48.6 (47.3–49.9)	50.8 (47.5–54.4)	71.1 (48.5–104.3)	0.025
SNP6	49.2 (47.5–51.0)	47.3 (45.5–49.3)	52.2 (48.3–56.5)	0.816
SNP7	49.5 (47.9–51.2)	47.9 (46.0–49.9)	50.8 (46.4–55.5)	0.482
SNP8	49.6 (47.9–51.4)	47.9 (46.0–49.9)	51.3 (47.5–55.5)	0.875
SNP9	49.5 (47.8–51.3)	47.5 (45.7–49.4)	51.4 (47.4–55.6)	0.770
SNP10	48.6 (47.2–50.0)	49.7 (47.4–52.2)	51.0 (43.5–59.8)	0.672
SNP11	50.3 (48.0–52.7)	48.3 (46.6–50.2)	48.5 (46.1–51.0)	0.348
INS incremental				
SNP1	30.4 (29.4–31.5)	30.5 (27.0–34.5)	22.9 (9.2–56.9)	0.874
SNP2	30.1 (29.0–31.1)	31.7 (29.7–33.9)	27.7 (22.2–34.7)	0.447
SNP3	30.7 (29.3–32.2)	30.3 (28.7–31.9)	28.6 (25.8–31.8)	0.329
SNP4	30.8 (29.7–31.9)	29.0 (26.8–31.4)	30.1 (20.8–43.6)	0.245
SNP5	30.6 (29.5–31.7)	30.0 (27.4–32.9)	26.3 (14.8–46.8)	0.715
SNP6	31.0 (29.5–32.5)	30.3 (28.7–31.9)	28.8 (26.0–32.0)	0.290
SNP7	30.9 (29.6–32.3)	30.4 (28.8–32.2)	27.9 (24.8–31.4)	0.183
SNP8	30.8 (29.4–32.3)	31.0 (29.3–32.7)	28.5 (25.6–31.6)	0.431
SNP9	30.8 (29.4–32.3)	30.4 (28.9–32.1)	28.6 (25.7–31.8)	0.305
SNP10	30.1 (29.0–31.3)	31.6 (29.6–33.7)	27.7 (22.3–34.4)	0.525
SNP11	29.1 (27.3–31.0)	31.2 (29.8–32.8)	30.5 (28.5–32.6)	0.404

Data are means (95% CI) adjusted for age, sex, and BMI. \**P* < 0.01 for HWE. †PG0 refers to fasting plasma glucose level (mmol/l). ‡2-h plasma glucose refers to plasma glucose level 2 h after glucose bolus (mmol/l). §INS0 refers to fasting plasma insulin level (pmol/l). ||INS incremental refers to a 30-min insulin incremental response (the difference between 30-min and fasting insulin concentrations divided by the 30-min glucose concentration in an oral glucose tolerance test) (pmol/mmol). 1, major allele, 2, minor allele.

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