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ABCC8 R1420H Loss-of-Function Variant in a Southwest American Indian Community: Association With Increased Birth Weight and Doubled Risk of Type 2 Diabetes

Missense variants in KCNJ11 and ABCC8, which encode the Kir6.2 and SUR1 subunits of the β-cell K<sub>ATP</sub> channel, have previously been implicated in type 2 diabetes, neonatal diabetes, and hyperinsulinemic hypoglycemia of infancy (HHI). To determine whether variation in these genes affects risk for type 2 diabetes or increased birth weight as a consequence of fetal hyperinsulinemia in Pima Indians, missense and common noncoding variants were analyzed in individuals living in the Gila River Indian Community. A R1420H variant in SUR1 (ABCC8) was identified in 3.3% of the population (N = 7,710). R1420H carriers had higher mean birth weights and a twofold increased risk for type 2 diabetes with a 7-year earlier onset age despite being leaner than noncarriers. One individual homozygous for R1420H was identified; retrospective review of his medical records was consistent with HHI and a diagnosis of diabetes at age 3.5 years. In vitro studies showed that the R1420H substitution decreases K<sub>ATP</sub> channel activity. Identification of this loss-of-function variant in ABCC8 with a carrier frequency of 3.3% affects clinical care as homozygous inheritance and potential HHI will occur in 1/3,600 births in this American Indian population.

The Pima Indians of Arizona have a high prevalence of type 2 diabetes and obesity (1). As part of our ongoing work to identify genetic risk factors for these diseases in this ethnic group, we have pursued both genome-wide and targeted gene studies in American Indians living in the Gila River Indian Community (Sacaton, AZ) (2–7). In the current study, we performed a targeted analysis of two adjacent diabetes candidate genes, KCNJ11 and ABCC8. KCNJ11 and ABCC8 encode the subunits Kir6.2 and SUR1, respectively, of the heteroctomer K<sub>ATP</sub> channel (8). K<sub>ATP</sub> channels regulate membrane K<sup+</sup> flux for various cell types including pancreatic β-cells, where increased glucose metabolism results in the closure of the K<sub>ATP</sub> channels leading to calcium influx and subsequent insulin secretion (9). A common E23K missense variant in KCNJ11 has been widely associated with polygenic type 2 diabetes in adulthood (10,11). The E23K variant is also common in Pima Indians, but no association with diabetes was detected in this group (12). Various rare inactivating missense mutations in ABCC8 are the most frequent cause of hyperinsulinemic hypoglycemia of infancy (HHI) (13–15). Individuals with HHI who do not undergo pancreatectomy for treatment of uncontrollable hypoglycemia have been found to be at increased risk for developing diabetes even at very young ages (16). The hyperinsulinemia that occurs with ABCC8 inactivating mutations often results in increased birth weight, consistent with the “fetal insulin hypothesis” of Hattersley and Tooke (17). Increased birth weight has also been observed in offspring with HNF4α mutations that cause maturity-onset diabetes of the young (MODY), which may be due to previously unrecognized fetal hyperinsulinemia (18–20).

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See accompanying article, p. 3993.
In the current study, we used previously analyzed whole-genome sequence data from 335 Pima Indians to identify variation in the genomic region spanning KCNJ11 and ABCC8. All missense variants, as well as tag single nucleotide polymorphisms (SNPs) that capture common (minor allele frequency [mAF] ≥0.05) noncoding variants, were initially genotyped and analyzed for association with type 2 diabetes, birth weight, and BMI during adulthood in 3,468 full-heritage Pima Indians from the Gila River Indian Community. As prior studies of KCNJ11 and ABCC8 almost exclusively implicate missense variants in risk for disease and several of the coding variants identified in the current study are rare, the missense variants were additionally genotyped in all Gila River Indian Community members for whom DNA and phenotypic data are currently available to maximize the sample size for analysis of type 2 diabetes (N = 7,710), birth weight (N = 2,377), and maximum BMI measured at a nondiabetic exam during adulthood (N = 5,918).

**RESEARCH DESIGN AND METHODS**

**Subjects**

Individuals living in the Gila River Indian Community are predominantly of Pima Indian heritage, and many participated in a longitudinal study of type 2 diabetes (1). Among the participants, 3,625 are full-heritage Pima Indian (defined as 8/8th Pima Indian heritage), and the remaining 4,085 are, on average, 6/8th American Indian (typically 4/8th Pima Indian and an additional 2/8th from other tribes). Participants underwent biennial research examinations beginning at age ≥5 years that included measurements of height and weight by medically trained personnel to calculate BMI and administration of a 75-g oral glucose tolerance test (OGTT). BMI used in this study (with the exception of that depicted in Fig. 3) was the maximum BMI recorded at a medical exam when the subject did not have diabetes and was an age ≥15 years. Subjects who had diabetes at the time of their first exam were excluded from the BMI analysis. Type 2 diabetes was determined according to the criteria of the American Diabetes Association (21) at that exam or based on medical record review. Homeostasis model assessment–estimated insulin resistance (HOMA-IR) (22) and the corrected insulin response (CIR) (23) were calculated as previously described using OGTT data from an individual’s last nondiabetic exam. Birth weight was obtained from birth records, which were available on a subset of the subjects. The birth weight analysis was restricted to singletons and was adjusted for gestational age (required to be ≥33 weeks). As the presence of maternal diabetes influences birth weight (24) and birth weight has increased over time (25), adjustments for maternal diabetes and birth year were made as previously described (25).

A subset of adults from the longitudinal study was also metabolically phenotyped as inpatients in our Clinical Research Center. Those who were full-heritage Pima Indian and had normal glucose tolerance were analyzed in the current study (N = 298). Body composition was estimated by underwater weighing until 1994 and by dual energy X-ray absorptiometry (DPX-L; Lunar Radiation Corp.) thereafter. The acute insulin response to a 25-g intravenous glucose tolerance test (IVGTT) was assessed as the mean increment in plasma insulin concentrations from 3 to 5 min (26). Insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp to measure rates of insulin-stimulated glucose clearance (26). Informed consent was obtained from all subjects and ethics approval was received from the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board.

**Genotyping**

Genotyping of the tag SNPs was done as part of a larger genotyping project that utilized a custom Pima Indian Axiom genome-wide array (Affymetrix, Santa Clara, CA) in 3,468 full-heritage Pima Indians and 92 blind duplicate samples. The tag SNPs had a call rate >96%, a discrepant rate of <1% among the duplicates, and a lack of deviation from Hardy-Weinberg equilibrium (P > 1.0 × 10⁻³). The KCNJ11 and ABCC8 missense variants were further genotyped in all 7,710 (3,625 full-heritage and 4,085 non–full-heritage Pima Indians) individuals from the Gila River Indian Community who had DNA and phenotypic data. Individual genotyping of the missense variants was done by allelic discrimination using TaqMan genotyping assays (Applied Biosystems, Foster City, CA) in 384-well plates, and all variants had a call rate of >95%, a lack of deviation from Hardy-Weinberg equilibrium (P > 1.0 × 10⁻³), and a discrepancy rate of <0.5% for 430 blind duplicates. For the six rare ABCC8 missense variants, samples that clustered as heterozygous or homozygous for the variant allele or as undetermined using TaqMan assays (clustering for the R1420H variant is shown in Supplementary Fig. 1) were directly sequenced to confirm the genotypes. Sequencing was carried out using a BigDye Terminator Sequencing Kit (Applied Biosystems) on an automated DNA capillary sequencer (model 3730xl; Applied Biosystems). After sequencing confirmation, the six rare ABCC8 variants had a call rate of >97% and no discrepancies for 430 blind duplicates and no genotypes were incompatible with known pedigree information.

**Statistical Analyses**

Statistical analyses were performed using SAS Institute (Cary, NC) software. Logistic regression analysis was used to assess the association of genotypes with type 2 diabetes with covariates of age, sex, birth year, and ancestry (American Indian/European admixture based on genetic markers and self-reported fraction of Pima Indian heritage). The individual estimate of the proportion of European ancestry was derived by the method of Hanis et al. (27) from 45 informative markers with large differences in allele frequency between populations (28). The model was fitted with the generalized estimating equations to account for dependence among siblings.
Genotype was analyzed as a numeric variable representing 0, 1, or 2 copies of a given allele. The association of quantitative traits with genotypes was analyzed by linear regression using the generalized estimating equation procedure to account for correlation among siblings, and results were adjusted for covariates as indicated in table footnotes and figure legends. To analyze the association with age of diabetes onset, a survival analysis approach was used. This was implemented as a proportional hazards (Weibull) model that was fit using a nonlinear mixed model that included genotype and other covariates as fixed effects and family membership as a random effect.

**Functional Analysis of the ABCC8 R1420H and R1420C Missense Mutations**

Site-directed mutagenesis (QuikChange; Stratagene, La Jolla, CA) of hamster ABCC8 cDNA cloned into pCMV6B was used to generate the R1420H and R1420C missense mutations. COS7 cells maintained in DMEM plus 10 mmol/L glucose supplemented with 10% FCS were transfected with ABCC8 (0.5 μg), KCNJ11 (0.3 μg), and green fluorescent protein (gfp, marker for transfection efficiency, 0.3 μg) plasmids using FuGENE 6 (Roche Diagnostics, Indianapolis, IN). Forty-eight hours posttransfection, the cells were incubated overnight in medium containing 45 mmol/L NaCl, 2.5 mmol/L CaCl2, 1.2 mmol/L KH2PO4, 4.7 mmol/L KCl, 25 mmol/L NaHCO3, 1.2 mmol/L MgSO4, 10 mmol/L HEPES; pH 7.4) with or without metabolic inhibition (1 mmol/L 2-deoxy-D-glucose and 2.5 μg/mL oligomycin) or with 100 μmol/L diazoxide. At selected time points, the Ringer's solution was removed for subsequent 86RbCl (1 μCi/mL) and then washed twice with Ringer's solution. At the end of the assay, the cells were lysed with 1% SDS, and 86Rb+ levels in the saved samples and cell lysates were measured in a scintillation solution. The relative 86Rb+ efflux ratio at each time point was calculated as the cumulative 86Rb+ counts in the saved samples divided by the total 86Rb+ counts from the solutions and cell lysates.

**RESULTS**

Previously analyzed whole-genome sequence data (~40× coverage) from 335 Pima Indians (291 were 8/8th [full-heritage] Pima Indian and 44 were 6/8th Pima Indian) across a 91.7 kb genomic region that spans KCNJ11 and ABCC8 (chromosome 11:17,406,795–17,498,449) identified 2 missense variants in KCNJ11, 1 synonymous variant and 7 missense variants in ABCC8, and 451 non-coding variants. Three of the missense variants (2 in KCNJ11 that encode V337I and E23K and 1 in ABCC8 that encodes A1369S) are common with mAFs of 0.38–0.39 and are in high linkage disequilibrium (φ2 ≥ 0.98) (Supplementary Fig. 2). The other 6 missense variants (all in ABCC8: K156S, R1420H, G1316Q, M801I, D691E, and S165L) are novel with mAFs of ≤0.03. Only the R1420H variant in ABCC8 is predicted to be damaging based on SIFT (29) and PolyPhen-2 (30) protein sequence analysis. Among the 451 noncoding variants, 232 had mAFs ≥0.05, and these common variants could be captured (φ2 > 0.85) by genotyping 35 tag SNPs. The 9 missense variants and 35 tag SNPs were initially genotyped in a sample of 3,468 full-heritage Pima Indians and analyzed for association with type 2 diabetes (N = 3,468), birth weight (N = 936), and adult BMI (N = 2,762) (characteristics of subjects and association data with adjustments are provided in Supplementary Tables 1 and 2, respectively). After correction for multiple comparisons by the Bonferroni method across the 44 variants and three traits (significance threshold P ≤ 0.0004), only the ABCC8 R1420H variant showed a significant association with birth weight (P = 0.0004) and adult BMI (P = 0.0001), where the H allele was associated with higher birth weight but lower BMI in adulthood. The type 2 diabetes association for R1420H in this sample was P = 0.04 and the odds ratio (OR) was 1.57 (95% CI 1.03–2.40) per copy of the H allele.

As most of the novel missense variants were rare, all 9 missense variants were further individually genotyped and analyzed for association in all available DNA samples from the Gila River Indian Community (characteristics for 7,710 subjects, which include the 3,468 subjects described above, are given in Table 1) to improve our ability to discern valid associations by maximizing the sample size. The ABCC8 G1316Q variant may be a private mutation as it was only detected in 10 individuals who were all from a single non–full-heritage Pima Indian pedigree. All other variants were observed in several pedigrees. When analyzed in all available DNA samples, the 3 common missense variants, V337I, E23K, and A1369S, again showed no association with type 2 diabetes. R1420H carriers (52.1 years), and the cumulative incidence of type 2 diabetes association (hazard ratio 2.05, 95% CI 1.45–2.82, P = 3.6 × 10−5) (Table 2). The increased diabetes risk for R1420H carriers (3.3%–4.2%) per copy of the H allele.

When analyzed in all available DNA samples, the ABCC8 R1420H variant showed the strongest evidence for association with type 2 diabetes. R1420H carriers (i.e., heterozygotes) had twice the risk for type 2 diabetes as compared with individuals homozygous for R1420R (OR 2.02 [95% CI 1.45–2.82], P = 3.6 × 10−5) (Table 2). The increased diabetes risk for R1420H carriers (3.3% of the population) was observed at all ages (Fig. 1A). The mean age of diabetes onset was, on average, earlier for R1420H carriers (45.0 years) compared with non-carriers (52.1 years), and the cumulative incidence of diabetes by age differed significantly between the two groups (hazard ratio 2.05, P = 1.1 × 10−5) (Fig. 1B). Among the 7,528 individuals successfully genotyped for the ABCC8 R1420H variant, only one was homozygous (i.e., H1420H). The clinical course of this individual (based on review of medical records from the 1960s) included hypoglycemia with seizures at 4 months old due to hyperinsulinemia. The medical records for this individual are missing treatment details, but at 3.5 years of age an OGTT was diagnostic of diabetes.
Despite the increased diabetes risk, R1420H carriers had, on average, lower BMIs in adulthood prior to their development of type 2 diabetes ($P = 2.5 \times 10^{-3}$) (Table 2). Analysis of the association with type 2 diabetes among subjects stratified by their maximum adult BMI at a non-diabetic exam showed an increase in the prevalence of type 2 diabetes among R1420H carriers at every level of BMI (Fig. 2). However, the younger age of type 2 diabetes onset observed in R1420H carriers could affect this BMI analysis (i.e., the last non-diabetic exam is more likely to occur at a younger age and, consequently, a lower BMI among R1420H carriers). Therefore, to obtain a more complete longitudinal profile of BMI in 2,458 subjects who developed type 2 diabetes, BMI from all examinations ($N = 9,089$ exams) was analyzed (Fig. 3A). In both the R1420H carriers and noncarriers, BMI was the highest around the time of diabetes diagnosis with lower values prior to diagnosis and at longer diabetes duration, which is a pattern that has been previously described in this population (31). However, the mean BMI was consistently lower in R1420H carriers at all times before and after diabetes diagnosis ($P = 7 \times 10^{-5}$) (Fig. 3A). For comparison, analysis of maximum BMI stratified by age at maximum BMI from 4,210 subjects who did not have diabetes as of their last exam showed that R1420H carriers without diabetes also had lower BMIs as compared with similarly aged noncarriers without diabetes ($P = 0.01$) (Fig. 3B).

The ABC8 R1420H variant also had an association with birth weight ($P = 1.5 \times 10^{-3}$) (Table 2), where newborns who carry R1420H were, on average, 170 g heavier than noncarriers. In a within-family analysis, the mean birth weight for offspring carrying R1420H was also higher than that of their siblings who do not carry the variant, adjusted for sex, gestational age, and maternal diabetes status during the pregnancy ($N = 23$ sibships, $P = 0.01$ for within-sibship difference in birth weight) (Fig. 4).

In a sample of 298 full-heritage Pima Indian adults with normal glucose tolerance (8 carry R1420H) who had undergone detailed metabolic phenotyping in our Clinical Research Center, there was no association between R1420H and the acute insulin response to a 25-g IVGTT ($P = 0.83$), while there was a nominal association with rate of insulin-stimulated glucose uptake such that the R1420H carriers were more insulin sensitive ($P = 0.03$) (Supplementary Table 3). However, these data are potentially unreliable due to the small number of R1420H carriers. Therefore, surrogate measures of insulin sensitivity (HOMA-IR, $N = 5,257$) and secretion (CIR derived from 2-h postload insulin and glucose measures, $N = 3,961$) were also analyzed. In these larger samples, there was no difference in HOMA-IR between R1420H carriers and noncarriers ($P = 0.90$, data not shown), but CIR was lower in the 118 R1420H carriers by 0.12 SD ($P = 0.01$). A lower CIR among R1420H carriers was observed across all age-groups (Fig. 5).

The R1420H variant is a singleton and does not tag any of the other exonic variants detected in KCNJ11 or ABC8 (Table 2) or tag any noncoding variant identified in our whole-genome sequence data from 335 Pima Indians ($r^2 < 0.2$ with all other detected variants spanning chr11:17,406,795-17,498,449). Although the S165L variant also had a weak association with type 2 diabetes (OR 1.59 [95% CI 1.07–2.35], $P = 0.02$) (Table 2), conditional analysis showed that the signals from R1420H and S165L are likely to be independent (R1420H: conditional OR 2.04 [1.45–2.86], $P = 3.7 \times 10^{-5}$; S165L: conditional OR 1.56 [1.05–2.04], $P = 0.03$). Therefore, the in vitro effects of the R1420H amino acid change on $K_{ATP}$ channel activity were examined by measuring $^86$Rb$^{+}$ efflux across the plasma membrane of COSm6 cells cotransfected with KCNJ11 and either ABC8 R1420R or ABC8 R1420H. In addition to R1420H, a different substitution at the same amino acid (R1420C, rs28938469), which has previously been shown to decrease $K_{ATP}$ channel (32,33), was also
Table 2 — Association of ABCC8 and KCNJ11 variants with type 2 diabetes, birth weight, and adult maximum BMI in American Indians from the Gila River Indian Community.

| Gene     | Variant             | Frequency (Freq) | OR (95% CI) | OR (95% CI) minor allele (freq) | Birth Weight (N = 2,377) | Adult BMI (N = 5,918) |
|----------|---------------------|------------------|-------------|-------------------------------|--------------------------|-----------------------|
|          |                     |                  |             |                               | Birth year, family membership, and ancestry           | BP values were adjusted as follows:                                   |
|          |                     |                  |             |                               | sex, birth year, gestational age, maternal diabetes (25), and ancestry; and | adjusted for age, sex, birth year, family membership, and ancestry. ¶Heterozygous individuals with birth weight data were analyzed for comparison. Basal $^{86}$Rb$^+$ efflux was low in all cases, reflecting low $K_{ATP}$ channel activity under basal metabolic conditions (Fig. 6). However, when $K_{ATP}$-specific fluxes were activated by metabolic inhibition or diazoxide administration, $^{86}$Rb$^+$ effluxes were lower for both R1420H and R1420C $K_{ATP}$ channels (Fig. 6). Maximal $^{86}$Rb$^+$ effluxes in the combined presence of a metabolic inhibitor and diazoxide were not statistically different between the three $K_{ATP}$ channels (Fig. 6), implying a similar total number of active channels in each case (34,35). These data indicate that the R1420H amino acid change acts in a similar manner to the previously characterized R1420C substitution (32,33) to reduce $K_{ATP}$ channel activity in intact cells, predicting that in vivo insulin hypersecretion will result in individuals carrying the R1420H variant.

**DISCUSSION**

In our population-based study of American Indians, we detected a R1420H loss-of-function variant in ABCC8. R1420H carriers had increased birth weights, suggestive of insulin oversecretion in utero, and a twofold increase in diabetes risk with a 7-year younger age of onset. The increased risk for diabetes among heterozygous carriers was observed at all ages, suggesting that the variant may influence both early- and adult-onset diabetes. ABCC8 mutations have previously been identified in individuals diagnosed with MODY (36,37), while other groups reported that individuals with congenital hyperinsulinemia due to inactivating ABCC8 mutations eventually developed early- and adult-onset diabetes (16,38,39). The one individual homozygous for R1420H in our study had HHI and was diagnosed with diabetes at age 3.5 years, suggesting the potential for a profound phenotype when R1420H is inherited from both parents. Our observation of a 3.3% carrier rate among individuals living in the Gila River Indian Community, whose population is estimated to be about 14,000, suggests that 1/3,600 births in this community will be homozygous for R1420H. This carrier rate is exceptionally high compared with other known ABCC8 inactivating mutations that are typically very rare and are not observed on a population level (14,15). The only other exception is a splice site variant in ABCC8 associated with congenital hyperinsulinism that has a carrier rate of ~1.7% in an Ashkenazi Jewish population, which has prompted recommendations for prenatal parental genetic testing to identify carriers in this population (40).

As DNA is not available on a population basis for most American Indian communities, it is unknown whether the ABCC8 R1420H variant also impacts other American Indian tribes. However, our recent screening of 2,576 individuals from the greater Phoenix area who have an American Indian heritage ≥50% but are not Pima Indian identified only two siblings heterozygous for the R1420H variant, suggesting that the variant may be rare in other Southwestern American Indian tribes. The R1420H variant also does not appear to occur at a detectable frequency in
other ethnic groups. The variant is not listed in the 1000 Genomes (41) or National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project (42) public databases, nor was it detected among 12,940 exomes or 583 genomes analyzed as part of the Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multi-Ethnic Samples (T2D-GENES) and Genetics of T2D (GoT2D) consortia from individuals representing European, African American, Hispanic, East Asian, and South Asian ancestry groups (M. Boehnke on behalf of the T2D-GENES and GoT2D investigators, personal communication). However, there is a report of a Japanese pedigree with three family members who carry the R1420H variant (43). The proband had HHI but was a compound heterozygote who also carried a maternally inherited frameshift mutation. The father, who was a R1420H heterozygote, was apparently healthy, but his cousin, also a heterozygote, had a history of hypoglycemia as an infant (43).

It is not known whether individuals heterozygous for the R1420H variant in our study were hyperinsulinemic as infants; however, their increased birth weights are suggestive of insulin oversecretion in utero. Consistent with our observation of higher birth weights among R1420H carriers, increased birth weight has also been reported for newborns with other loss-of-function ABCC8 mutations (44–46). The "fetal insulin hypothesis" proposes that variation in birth weight is, in part, a result of fetal hypo- or hyperinsulinemia (17). Insulin is a major growth factor in utero, and newborns with genetic variants in GCK, CDKAL1, HHEX-IDE, and TCF7L2 that reduce insulin secretion and increase risk for type 2 diabetes have lower birth weights (47–49). Conversely, newborns with mutations in HNF4α that cause fetal hyperinsulinemia have higher birth weights (18–20).

Despite being hyperinsulinemic as infants, some individuals with HHI (including the ABC8 R1420H homozygous individual in this study) eventually develop diabetes. It has also been shown that Abcc8 (SUR1) knockout mice and mice carrying a knock-in of the hyperinsulinemia-associated SUR1 loss-of-function E1506K mutation all progress to insulin undersecretion or diabetes with age (50,51). Transgenic mouse models of congenital hyperinsulinemia with a dominant-negative K_{ATP} channel mutation also progress to hypoinsulinemic hyperglycemia later in life either spontaneously or in response to a high-fat

![Figure 2](image-url)
diet (52). Inactivating mutations in ABCC8 lead to HHI predominately through reduced K\textsubscript{ATP} channel activity in pancreatic β-cells, which leads to abnormal membrane polarization, activation of voltage-gated calcium channels, and increased Ca\textsuperscript{2+} concentrations causing insulin hypersecretion (9). However, the physiological mechanism by which these mutations subsequently cause the remission of HHI and future hyperglycemia leading to diabetes remains unclear. Possible mechanisms for the reversal could be either β-cell apoptosis or ensuing negative regulation of insulin secretion (53). In an autopsy study of fetuses and infants with and without HHI, an increased frequency of apoptosis in the affected β-cell samples was noted, pointing to β-cell death as a possible cause of progressive insulin secretion dysfunction and eventual diabetes (54). Closure of the SUR1 K\textsubscript{ATP} channel

**Figure 3**—Comparison of mean BMI for R1420H carriers and noncarriers in relation to diabetes status and age. A: Analysis of BMI restricted to subjects who developed diabetes. Mean BMI at all exams before and after diagnosis of diabetes in 2,458 individuals who eventually developed diabetes (117 R1420H carriers [the one H1420H was included with the heterozygote carriers] and 2,341 noncarriers). Mean BMI was calculated using data from all longitudinal exams (total exams = 9,086). Diabetes diagnosis is at 0 years; negative years represent time before diagnosis and positive years represent time following diagnosis. BMI is significantly lower in all exams from R1420H carriers ($P = 7 \times 10^{-7}$ adjusted for age, sex, birth year, time category, and ancestry in a mixed model that accounts for sibship and repeated examinations within individuals). B: Analysis of BMI restricted to subjects not known to have diabetes. The maximum BMI by age among 4,210 subjects who did not have diabetes as of their last exam (106 R1420H carriers and 4,104 noncarriers). The number of R1420H carriers/noncarriers at each age category are 15–24 years (50/1,922), 25–34 years (26/1,042), 35–44 years (19/747), 45–54 years (8/259), and $>55$ years (3/134). R1420H carriers had a lower BMI compared with noncarriers ($P = 0.01$ adjusted for age, sex, birth year, and ancestry in a model that accounts for sibship).

**Figure 4**—Mean birth weight for siblings discordant for the R1420H variant. Birth weight is adjusted for gestational age, birth year, sex, genetically derived estimate of American Indian/European ancestry, and maternal diabetes status during pregnancy (see Research Design and Methods). Diagonal line is line of identity. The mean birth weight of siblings with R1420H genotype was higher than that of siblings with the R1420R genotype in 18 of 23 sibships. $P = 0.01$ for within-sibship difference in birth weight.

**Figure 5**—CIR for R1420H carriers and noncarriers grouped by their age at last nondiabetic OGTT. CIR is standardized for insulin assay (three different assays were used in this longitudinal study) after logarithmic transformation. Means are adjusted for sex, BMI, HOMA-IR, and ancestry (American Indian/European admixture based on genetic markers and self-reported fraction Pima Indian heritage).
by sulfonylureas also has been reported to induce β-cell apoptosis in cultured human islets (55), and this has led to concern that long-term sulfonylurea treatment may exacerbate β-cell failure (56). However, in extensive rodent studies that include the examination of knockout mice lacking SUR1 or Kir6.2, mice expressing a dominant-negative Kir6.2 subunit in β-cells, and mice implanted with slow-release sulfonylurea pellets, there was little evidence for enhanced β-cell death or loss of insulin content; rather, there was an unexplained downregulation of insulin secretion (52,57–59). Although β-cells are the cell type most sensitive to changes in $K_{\text{ATP}}$ channel activity, we cannot rule out the possibility that altered $K_{\text{ATP}}$ channel function in other tissues may also contribute to diabetes risk. For example, $K_{\text{ATP}}$ channels are expressed in the hypothalamus (60), and it has been reported that channel activation in the mediobasal hypothalamus inhibits hepatic glucose production through a glucagon-$K_{\text{ATP}}$ channel signaling pathway that, in turn, lowers blood glucose levels (61,62).

Our in vitro functional study showed that the R1420H substitution caused decreased $K_{\text{ATP}}$ channel activity in response to metabolic inhibition or diazoxide administration. R1420H is positioned in the second nucleotide binding fold (NBF2) of the SUR1 protein where other amino acid substitutions have been identified including R1420C, which was previously shown to be associated with a sporadic case of persistent HHI (15,63) (note: R1420C is referred to as R1421C in [63]). In our study, a similar decrease in $K_{\text{ATP}}$ channel activity was also seen for R1420C, which is consistent with earlier reports showing diminished activity for this variant (32,33). R1420C may reduce $K_{\text{ATP}}$ channel activity by lowering NBF2 binding affinities for ATP and ADP and impairing the cooperative ATP binding between NBF2 and NBF1 (32,33). While further studies are required, it seems likely that R1420H also may impair $K_{\text{ATP}}$ channel activity by affecting adenine nucleotide binding in NBF2.

Although the in vitro study shows that the R1420H variant affects $K_{\text{ATP}}$ channel function, we did not detect statistical evidence of decreased insulin secretory function for the eight R1420H carriers whose insulin response was measured by an IVGTT. In addition to the small sample size, these data may be difficult to interpret because this

**Figure 6**—Decreased $K_{\text{ATP}}$ activity for SUR1 (ABCC8) channels harboring R1420H or R1420C mutations. Relative $^{86}$Rb$^+$ efflux is shown as a function of time under basal conditions, in the presence of metabolic inhibition (MI), in the presence of $K_{\text{ATP}}$ channel opener diazoxide, and for both control cells expressing green fluorescent protein and cells expressing R1420R, R1420H, or R1420C channels. Data are shown as mean ± SEM from four independent transfections. $^*P < 0.05$, $^**P < 0.01$ vs. R1420R for each case.
cross-sectional measure of insulin response is only assessed in adults who have normal glucose tolerance. The rate at which insulin secretion declines from hyper-to hypoinsulinemia due to loss-of-function mutations in SUR1 is unknown and may be highly variable, and it is possible that these eight R1420H carriers were able to maintain their normal glucose tolerance into adulthood because they were leaner and more insulin sensitive ($P = 1 \times 10^{-4}$ and $P = 0.03$, for percent body fat and insulin sensitivity, respectively) (Supplementary Table 3) and therefore were protected from the added $\beta$-cell stress needed to respond to greater degrees of obesity and insulin resistance. Further analysis using a surrogate measure of insulin secretion (CIR) in 3,961 individuals (including 118 R1420H carriers) provided modest evidence that the R1420H carriers, on average, may indeed have reduced insulin secretory function as compared with noncarriers at all ages between 15 and 45 years and older.

Although variants in ABCC8 and KCNJ11 have predominately been studied in relation to diseases of impaired insulin secretion, the current study identified an association between several variants in ABCC8 and BMI (Table 2). For the R1420H variant, carriers had lower BMIs than noncarriers, even after stratifying subjects by diabetes status (Fig. 3). $K_{\text{ATP}}$ channels have been detected in various areas of the hypothalamus important for regulating body weight, and both leptin and insulin have been shown to activate $K_{\text{ATP}}$ channels in glucose-responsive neurons (60). There has been one report of an association with the KCNJ11 E23K variant and increased BMI in humans, and it was suggested that this association may result from the variant affecting glucose-sensing neurons in the hypothalamus, which, in turn, influences BMI (64). In mouse models, Kcnj11 knockouts have significantly reduced adipose tissue mass and are resistant to obesity induced by a high-fat diet (65,66).

In summary, we report a loss-of-function ABCC8 R1420H variant with a high carrier rate (3.3%) in a Southwestern American Indian Community. R1420H carriers have increased birth weight presumably due to fetal hyperinsulinemia and a twofold increased risk of diabetes with a younger age of onset. As there is a high prevalence of R1420H heterozygotes in this community and R1420H homozygotes are potentially at risk for HHI, medical care providers should be aware of the increased risk of HHI in infants born to parents from this ethnic group.

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