



Leslie J. Baier,¹ Yunhua Li Muller,¹ Maria Sara Remedi,² Michael Traurig,¹ Paolo Piaggi,¹ Gregory Wiessner,¹ Ke Huang,¹ Alyssa Stacy,¹ Sayuko Kobes,¹ Jonathan Krakoff,¹ Peter H. Bennett,¹ Robert G. Nelson,¹ William C. Knowler,¹ Robert L. Hanson,¹ Colin G. Nichols,² and Clifton Bogardus¹

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



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Missense variants in *KCNJ11* and *ABCC8*, which encode the KIR6.2 and SUR1 subunits of the β -cell K_{ATP} 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_{ATP} 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 heterooctomer K_{ATP} channel (8). K_{ATP} channels regulate membrane K^+ flux for various cell types including pancreatic β -cells, where increased glucose metabolism results in the closure of the K_{ATP} 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 *HNF4 α* mutations that cause maturity-onset diabetes of the young (MODY), which may be due to previously unrecognized fetal hyperinsulinemia (18–20).

¹Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, AZ

²Department of Cell Biology and Physiology, Washington University in St. Louis, St. Louis, MO

Corresponding author: Leslie J. Baier, lbaier@phx.niddk.nih.gov.

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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-I; 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 ($P > 1.0 \times 10^{-3}$). 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 ($P > 1.0 \times 10^{-3}$), 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. COSm6 cells maintained in DMEM plus 10 mmol/L glucose supplemented with 10% FCS were transfected with *ABCC8* (0.5 μ g), *KCNJ11* (0.3 μ g), and green fluorescent protein (*gfp*, marker for transfection efficiency, 0.3 μ g) plasmids using FuGENE 6 (Roche Diagnostics, Indianapolis, IN). Forty-eight hours posttransfection, the cells were incubated overnight in medium containing $^{86}\text{RbCl}$ (1 $\mu\text{Ci}/\text{mL}$) and then washed twice with Ringer's solution (118 mmol/L NaCl, 2.5 mmol/L CaCl_2 , 1.2 mmol/L KH_2PO_4 , 4.7 mmol/L KCl, 25 mmol/L NaHCO_3 , 1.2 mmol/L MgSO_4 , 10 mmol/L HEPES; pH 7.4) with or without metabolic inhibition (1 mmol/L 2-deoxy-D-glucose and 2.5 $\mu\text{g}/\text{mL}$ oligomycin) or with 100 $\mu\text{mol}/\text{L}$ diazoxide. At selected time points, the Ringer's solution was removed for subsequent $^{86}\text{Rb}^+$ measurements and replaced with fresh Ringer's solution. At the end of the assay, the cells were lysed with 1% SDS, and $^{86}\text{Rb}^+$ levels in the saved samples and cell lysates were measured in a scintillation solution. The relative $^{86}\text{Rb}^+$ efflux ratio at each time point was calculated as the cumulative $^{86}\text{Rb}^+$ counts in the saved samples divided by the total $^{86}\text{Rb}^+$ counts from the solutions and cell lysates.

RESULTS

Previously analyzed whole-genome sequence data ($\sim 40\times$ 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 noncoding 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 ($r^2 \geq 0.98$) (Supplementary Fig. 2). The other 6 missense variants (all in *ABCC8*: K1565E, 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 ($r^2 > 0.85$) 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 $P \leq 0.0004$), 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, birth weight, or adult BMI (Table 2), and these variants were not associated with insulin secretion among the 298 subjects with normal glucose tolerance (Supplementary Table 3).

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], $P = 3.6 \times 10^{-5}$) (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, $P = 1.1 \times 10^{-7}$) (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.

Table 1—Subjects from the Gila River Indian Community genotyped and analyzed for all missense variants

	N	Males/females	Age (years)	Adult BMI (kg/m ²) or birth weight (g)
Type 2 diabetes				
With type 2 diabetes	2,549	982/1,567	46.5 ± 14.6	38.7 ± 8.6
Full-heritage Pima Indian	1,684	623/1,061	49.1 ± 14.0	38.7 ± 8.6
Non-full-heritage Pima Indian	865	359/506	41.2 ± 14.1	38.7 ± 8.7
Without type 2 diabetes	5,161	2,444/2,717	27.6 ± 13.5	34.6 ± 8.6
Full-heritage Pima Indian	1,941	930/1,011	32.1 ± 14.6	36.0 ± 8.5
Non-full-heritage Pima Indian	3,220	1,514/1,706	24.9 ± 11.9	33.5 ± 8.4
BMI				
Full-heritage Pima Indian	2,862	1,239/1,623	32.2 ± 11.8	36.3 ± 8.3
Non-full-heritage Pima Indian	3,056	1,367/1,689	27.3 ± 10.5	34.0 ± 8.4
Birth weight				
Full-heritage Pima Indian	988	439/549	—	3,468 ± 523
Non-full-heritage Pima Indian	1,389	619/770	—	3,483 ± 521
Metabolic studies (full-heritage Pima Indian)	298	207/119	26.3 ± 6.2	33.4 ± 7.4

Data are shown as *n* or mean ± SD.

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 nondiabetic 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 ($P = 7 \times 10^{-4}$) (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 ABCC8 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 *ABCC8* (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 $^{86}\text{Rb}^+$ efflux across the plasma membrane of COSm6 cells cotransfected with *KCNJ11* and either *ABCC8* R1420R or *ABCC8* 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 KCNJ11 and ABCC8 variants with type 2 diabetes, birth weight, and adult maximum BMI in American Indians from the Gila River Indian Community

Gene	Variant	Diabetes (N = 7,710)			Birth Weight (N = 2,377)			Adult BMI* (N = 5,918)		
		Freq†	OR (95% CI) _{minor allele}	P‡	B _{minor allele} (g)	P§	B _{minor allele} (log _e)	P		
KCNJ11	rs5215	0.39	1.03 (0.94–1.12)	0.54	–21.2	0.16	–0.0001	0.98		
KCNJ11	rs5219	0.39	1.04 (0.95–1.13)	0.43	–18.4	0.22	0.002	0.72		
ABCC8	chr11:17414594T>C	0.01	0.88 (0.56–1.36)	0.56	44.9	0.52	–0.051	0.01		
ABCC8	chr11:17417205C>T	0.017	2.02 (1.45–2.82)	3.6 × 10 ^{–5}	169.1	1.5 × 10 ^{–3}	–0.049	2.5 × 10 ^{–3}		
ABCC8	rs757110	0.38	1.03 (0.94–1.12)	0.56	–15.5	0.35	0.002	0.60		
ABCC8	chr11:17418781C>T	0.0001	1.04 (0.32–3.38)	0.94	–	–	0.037	0.40		
ABCC8	chr11:17435016G>T	0.03	1.29 (0.98–1.71)	0.07	–72.9	0.08	0.009	0.53		
ABCC8	chr11:17449457T>A	0.001	0.42 (0.07–2.70)	0.36	–	–	–0.162	4.1 × 10 ^{–3}		
ABCC8	chr11:17485070G>A	0.01	1.59 (1.07–2.35)	0.02	71.0	0.32	0.078	1.8 × 10 ^{–4}		

OR (95% CI) per copy of the minor allele. B_{minor allele} is the regression coefficient of the difference in the mean value of the trait per copy of the minor allele. *Maximum BMI measured at a nondiabetic exam. †Frequency (Freq) of the variant allele based on the genotypes for 7,710 American Indians. B and P values were adjusted as follows: ‡adjusted for age, sex, birth year, family membership, and ancestry (American Indian/European admixture based on genetic markers and self-reported fraction Pima Indian heritage); §adjusted for 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 N = 2 and 8 for G1316Q and D691E, respectively; therefore, these substitutions were not analyzed for this trait.

analyzed for comparison. Basal ⁸⁶Rb⁺ 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, ⁸⁶Rb⁺ effluxes were lower for both R1420H and R1420C K_{ATP} channels (Fig. 6). Maximal ⁸⁶Rb⁺ 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

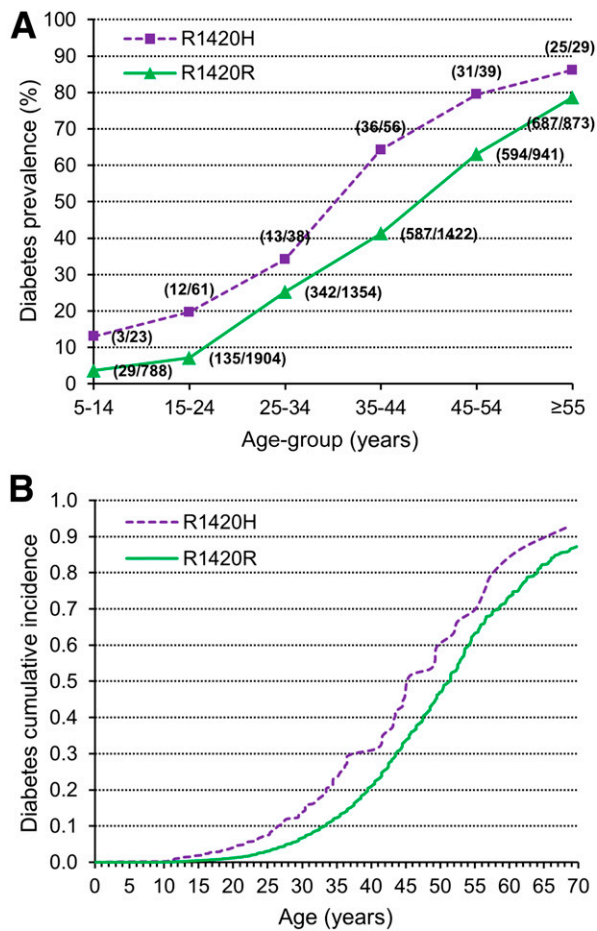


Figure 1—Type 2 diabetes status for R1420H carriers and noncarriers. *A*: Prevalence of type 2 diabetes shown in individuals grouped by their age at last exam. Numbers in parentheses indicate the number of subjects with diabetes (numerator) and total number of subjects (denominator) for each age-group. *B*: Type 2 diabetes cumulative incidence adjusted for sex, birth year, and ancestry (American Indian/European admixture based on genetic markers and self-reported fraction Pima Indian heritage). Mean onset age was calculated from the parameters of the Weibull model for an individual born in 1940, and the mean age for all other covariates was 45.0 years for R1420H and 52.1 years for R1420R.

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

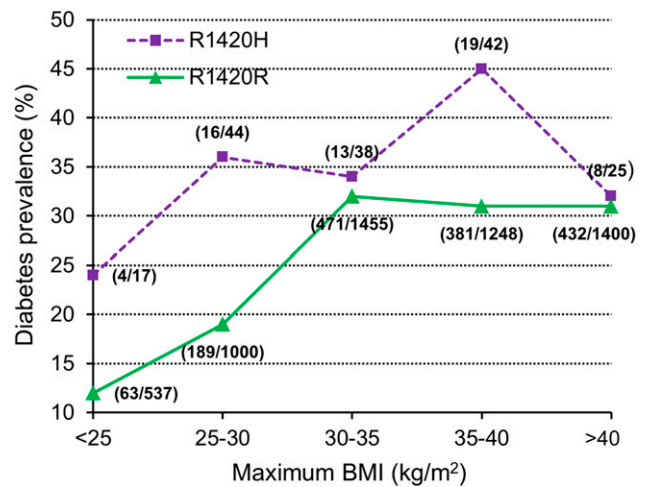


Figure 2—The prevalence of type 2 diabetes for R1420H carriers and noncarriers in relation to BMI. Diabetes prevalence is shown in individuals stratified by their maximum BMI measured at age ≥ 15 years at a nondiabetic exam. Numbers in parentheses indicate the number of subjects with diabetes (numerator) and total number of subjects (denominator) for the BMI group. As a comparison of prevalence between R1420H and R1420R at each level of BMI, ORs (95% CI) are as follows: BMI < 25 kg/m², 2.32 (0.73–7.32); BMI 25–30 kg/m², 2.45 (1.30–4.62); BMI > 30 –35 kg/m², 1.09 (0.55–2.14); BMI > 35 –40 kg/m², 1.88 (1.01–3.49); and BMI > 40 kg/m², 1.04 (0.45–2.46).

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 *HNF4 α* that cause fetal hyperinsulinemia have higher birth weights (18–20).

Despite being hyperinsulinemic as infants, some individuals with HHI (including the *ABCC8* 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

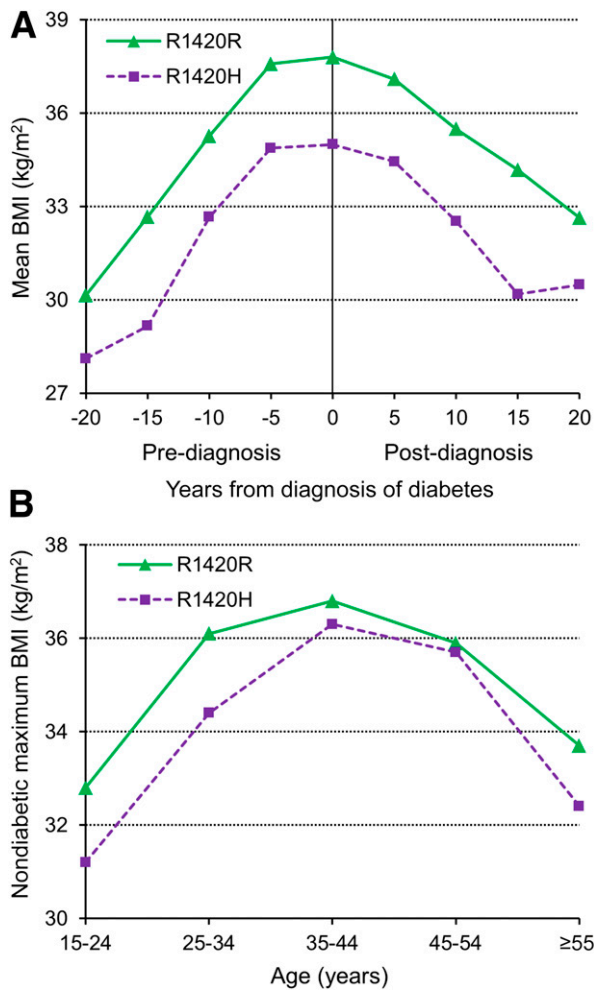


Figure 3—Comparison of mean BMI for R1420H carriers and non-carriers in relation to diabetes status and age. *A*: Analysis of BMI restricted to subjects who developed diabetes. Mean BMI at all exams before and after diagnosis of diabetes in 2,458 individuals who eventually developed diabetes (117 R1420H 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 ($P = 7 \times 10^{-4}$ 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).

diet (52). Inactivating mutations in *ABCC8* lead to HHI predominately through reduced K_{ATP} channel activity in pancreatic β -cells, which leads to abnormal membrane polarization, activation of voltage-gated calcium channels, and increased Ca^{2+} concentrations causing insulin

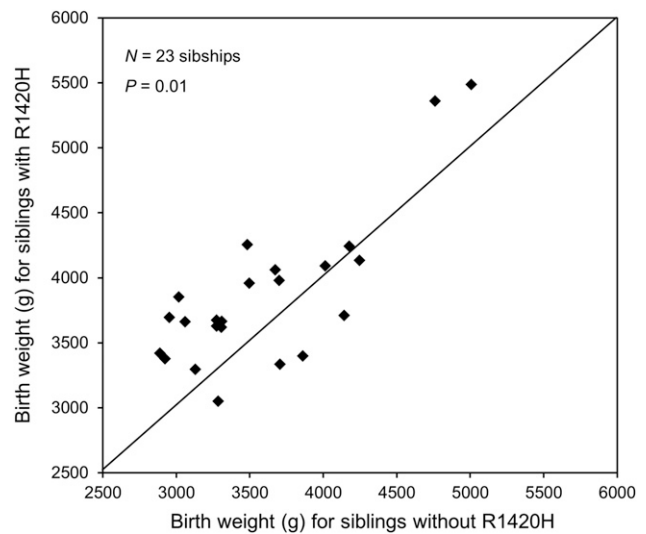


Figure 4—Mean birth weight for siblings discordant for the R1420H variant. Birth weight is adjusted for gestational age, birth year, sex, genetically derived estimate of American Indian/European ancestry, and maternal diabetes status during pregnancy (see RESEARCH DESIGN AND METHODS). Diagonal line is 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.

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 $SUR1 K_{ATP}$ channel

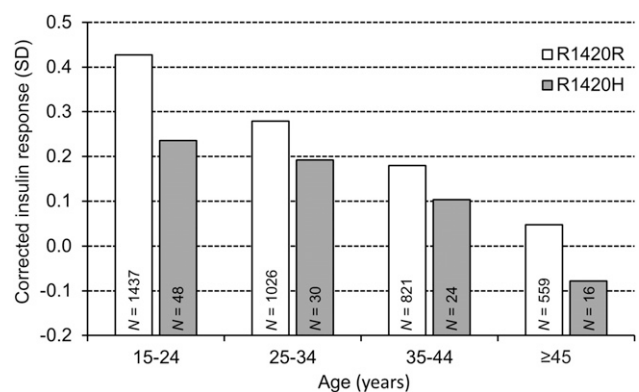


Figure 5—CIR for R1420H carriers and noncarriers grouped by their age at last nondiabetic OGTT. CIR is standardized for insulin assay (three different assays were used in this longitudinal study) after logarithmic transformation. Means are adjusted for sex, BMI, HOMA-IR, and ancestry (American Indian/European 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_{ATP} channel activity, we cannot rule out the possibility that altered K_{ATP} channel function in other tissues may also contribute to diabetes risk. For example, K_{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_{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_{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_{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_{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_{ATP} channel activity by affecting adenine nucleotide binding in NBF2.

Although the *in vitro* study shows that the R1420H variant affects K_{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

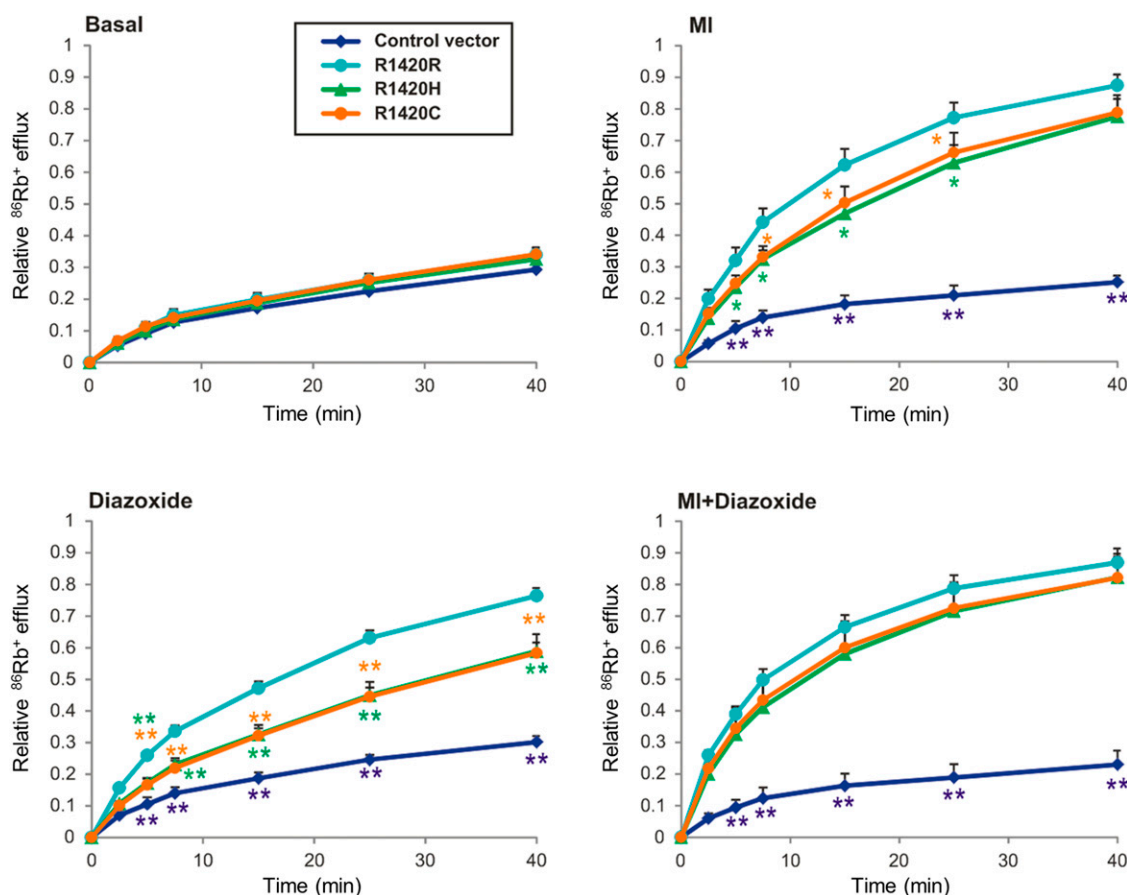


Figure 6—Decreased K_{ATP} activity for SUR1 (*ABCC8*) channels harboring R1420H or R1420C mutations. Relative $^{86}\text{Rb}^+$ efflux is shown as a function of time under basal conditions, in the presence of metabolic inhibition (MI), in the presence of K_{ATP} channel opener diazoxide, and for both control cells expressing green fluorescent protein and cells expressing R1420R, R1420H, or R1420C channels. Data are shown as mean \pm 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 β -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_{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_{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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References

- Knowler WC, Pettitt DJ, Saad MF, Bennett PH. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 1990;6:1–27
- Hanson RL, Muller YL, Kobes S, et al. A genome-wide association study in American Indians implicates DNER as a susceptibility locus for type 2 diabetes. *Diabetes* 2014;63:369–376
- Muller YL, Piaggi P, Hoffman D, et al. Common genetic variation in the glucokinase gene (GCK) is associated with type 2 diabetes and rates of carbohydrate oxidation and energy expenditure. *Diabetologia* 2014;57:1382–1390
- Hanson RL, Guo T, Muller YL, et al. Strong parent-of-origin effects in the association of *KCNQ1* variants with type 2 diabetes in American Indians. *Diabetes* 2013;62:2984–2991
- Bian L, Traurig M, Hanson RL, et al. MAP2K3 is reproducibly associated with body mass index in American Indians and Caucasians and may mediate hypothalamic inflammation. *Hum Mol Genet* 2013;22:4438–4449
- Thearle MS, Muller YL, Hanson RL, et al. Greater impact of melanocortin-4 receptor deficiency on rates of growth and risk of type 2 diabetes during childhood compared with adulthood in Pima Indians. *Diabetes* 2012;61:250–257
- Traurig M, Mack J, Hanson RL, et al. Common variation in *SIM1* is reproducibly associated with BMI in Pima Indians. *Diabetes* 2009;58:1682–1689
- Shyng S, Nichols CG. Octameric stoichiometry of the K_{ATP} channel complex. *J Gen Physiol* 1997;110:655–664
- Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest* 2005;115:2047–2058
- Gloyn AL, Weedon MN, Owen KR, et al. Large-scale association studies of variants in genes encoding the pancreatic β -cell K_{ATP} channel subunits *Kir6.2* (*KCNJ11*) and *SUR1* (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* 2003;52:568–572
- Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007;316:1341–1345
- Hanson RL, Rong R, Kobes S, et al. Role of established type 2 diabetes-susceptibility genetic variants in a high prevalence American Indian population. *Diabetes* 2015;64:2646–2657
- Kapoor RR, Flanagan SE, James C, Shield J, Ellard S, Hussain K. Hyperinsulinaemic hypoglycaemia. *Arch Dis Child* 2009;94:450–457
- Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. *Eur J Endocrinol* 2013;168:557–564
- Snider KE, Becker S, Boyajian L, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab* 2013;98:E355–E363
- Gussinyer M, Clemente M, Cebrián R, Yeste D, Albus M, Carrascosa A. Glucose intolerance and diabetes are observed in the long-term follow-up of nonpancreatectomized patients with persistent hyperinsulinemic hypoglycemia of infancy due to mutations in the *ABCC8* gene. *Diabetes Care* 2008;31:1257–1259
- Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 1999;353:1789–1792

18. Pearson ER, Boj SF, Steele AM, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med* 2007;4:e118
19. Arya VB, Rahman S, Senniappan S, Flanagan SE, Ellard S, Hussain K. HNF4A mutation: switch from hyperinsulinaemic hypoglycaemia to maturity-onset diabetes of the young, and incretin response. *Diabet Med* 2014;31:e11–e15
20. McGlacken-Byrne SM, Hawkes CP, Flanagan SE, Ellard S, McDonnell CM, Murphy NP. The evolving course of HNF4A hyperinsulinaemic hypoglycaemia—a case series. *Diabet Med* 2014;31:e1–e5
21. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2003;26(Suppl. 1):S5–S20
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419
23. Hanson RL, Pratley RE, Bogardus C, et al. Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 2000;151:190–198
24. Pettitt DJ, Knowler WC, Baird HR, Bennett PH. Gestational diabetes: infant and maternal complications of pregnancy in relation to third-trimester glucose tolerance in the Pima Indians. *Diabetes Care* 1980;3:458–464
25. Lindsay RS, Hanson RL, Bennett PH, Knowler WC. Secular trends in birth weight, BMI, and diabetes in the offspring of diabetic mothers. *Diabetes Care* 2000;23:1249–1254
26. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993;329:1988–1992
27. Hanis CL, Chakraborty R, Ferrell RE, Schull WJ. Individual admixture estimates: disease associations and individual risk of diabetes and gallbladder disease among Mexican-Americans in Starr County, Texas. *Am J Phys Anthropol* 1986;70:433–441
28. Tian C, Hinds DA, Shigeta R, et al. A genomewide single-nucleotide-polymorphism panel for Mexican American admixture mapping. *Am J Hum Genet* 2007;80:1014–1023
29. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073–1081
30. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248–249
31. Looker HC, Knowler WC, Hanson RL. Changes in BMI and weight before and after the development of type 2 diabetes. *Diabetes Care* 2001;24:1917–1922
32. Matsuo M, Trapp S, Tanizawa Y, et al. Functional analysis of a mutant sulfonylurea receptor, SUR1-R1420C, that is responsible for persistent hyperinsulinemic hypoglycemia of infancy. *J Biol Chem* 2000;275:41184–41191
33. Tanizawa Y, Matsuda K, Matsuo M, et al. Genetic analysis of Japanese patients with persistent hyperinsulinemic hypoglycemia of infancy: nucleotide-binding fold-2 mutation impairs cooperative binding of adenine nucleotides to sulfonylurea receptor 1. *Diabetes* 2000;49:114–120
34. Shyng SL, Ferrigni T, Shepard JB, et al. Functional analyses of novel mutations in the sulfonylurea receptor 1 associated with persistent hyperinsulinemic hypoglycemia of infancy. *Diabetes* 1998;47:1145–1151
35. Masia R, Nichols CG. Functional clustering of mutations in the dimer interface of the nucleotide binding folds of the sulfonylurea receptor. *J Biol Chem* 2008;283:30322–30329
36. Bowman P, Flanagan SE, Edghill EL, et al. Heterozygous ABCC8 mutations are a cause of MODY. *Diabetologia* 2012;55:123–127
37. Johansson S, Irgens H, Chudasama KK, et al. Exome sequencing and genetic testing for MODY. *PLoS One* 2012;7:e38050
38. Abdulhadi-Atwan M, Bushman J, Tornovsky-Babaey S, et al. Novel de novo mutation in sulfonylurea receptor 1 presenting as hyperinsulinism in infancy followed by overt diabetes in early adolescence [published correction in *Diabetes* 2008;57:2552]. *Diabetes* 2008;57:1935–1940
39. Vieira TC, Bergamin CS, Gurgel LC, Moisés RS. Hyperinsulinemic hypoglycemia evolving to gestational diabetes and diabetes mellitus in a family carrying the inactivating ABCC8 E1506K mutation. *Pediatr Diabetes* 2010;11:505–508
40. Glaser B, Blech I, Krakinovsky Y, et al. ABCC8 mutation allele frequency in the Ashkenazi Jewish population and risk of focal hyperinsulinemic hypoglycemia. *Genet Med* 2011;13:891–894
41. Abecasis GR, Auton A, Brooks LD, et al.; 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012;491:56–65
42. National Heart, Lung, and Blood Institute. NHLBI GO Exome Sequencing Project (ESP) Exome Variant Server. Available from <http://evs.gs.washington.edu/EVS/>. Accessed December 2014
43. Saito-Hakoda A, Yorifuji T, Kanno J, Kure S, Fujiwara I. Nateglinide is effective for diabetes mellitus with reactive hypoglycemia in a child with a compound heterozygous ABCC8 mutation. *Clin Pediatr Endocrinol* 2012;21:45–52
44. Kapoor RR, Flanagan SE, James CT, et al. Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant ABCC8/KCNJ11 mutations. *Diabetologia* 2011;54:2575–2583
45. Khoriaty D, Arya VB, Flanagan SE, Ellard S, Hussain K. Prematurity, macrosomia, hyperinsulinaemic hypoglycaemia and a dominant ABCC8 gene mutation. *BMJ Case Rep* 2013;2013:bcr2013008767
46. Ince DA, Sahin NM, Ecevit A, et al. Congenital hyperinsulinism in a newborn with a novel homozygous mutation (p.Q392H) in the ABCC8 gene. *J Pediatr Endocrinol Metab* 2014;27:1253–1255
47. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S. Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat Genet* 1998;19:268–270
48. Andersson EA, Pilgaard K, Pisinger C, et al. Type 2 diabetes risk alleles near ADCY5, CDKAL1 and HHEX-IDE are associated with reduced birthweight. *Diabetologia* 2010;53:1908–1916
49. Freathy RM, Weedon MN, Bennett A, et al. Type 2 diabetes TCF7L2 risk genotypes alter birth weight: a study of 24,053 individuals. *Am J Hum Genet* 2007;80:1150–1161
50. Seghers V, Nakazaki M, DeMayo F, Aguilar-Bryan L, Bryan J. Sur1 knockout mice. A model for K(ATP) channel-independent regulation of insulin secretion. *J Biol Chem* 2000;275:9270–9277
51. Shimomura K, Tusa M, Iberl M, et al. A mouse model of human hyperinsulinism produced by the E1506K mutation in the sulphonylurea receptor SUR1. *Diabetes* 2013;62:3797–3806
52. Remedi MS, Koster JC, Markova K, et al. Diet-induced glucose intolerance in mice with decreased beta-cell ATP-sensitive K⁺ channels. *Diabetes* 2004;53:3159–3167
53. Yorifuji T. Congenital hyperinsulinism: current status and future perspectives. *Ann Pediatr Endocrinol Metab* 2014;19:57–68
54. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B. β -cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 2000;49:1325–1333
55. Maedler K, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY. Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J Clin Endocrinol Metab* 2005;90:501–506
56. Qian L, Zhang S, Xu L, Peng Y. Endoplasmic reticulum stress in beta cells: latent mechanism of secondary sulfonylurea failure in type 2 diabetes? *Med Hypotheses* 2008;71:889–891
57. Nichols CG, Remedi MS. The diabetic β -cell: hyperstimulated vs. hyperexcited. *Diabetes Obes Metab* 2012;14(Suppl. 3):129–135
58. Remedi MS, Nichols CG. Chronic antidiabetic sulfonylureas in vivo: reversible effects on mouse pancreatic beta-cells. *PLoS Med* 2008;5:e206
59. Nichols CG, Koster JC, Remedi MS. beta-Cell hyperexcitability: from hyperinsulinism to diabetes. *Diabetes Obes Metab* 2007;9(Suppl. 2):81–88

60. Acosta-Martínez M, Levine JE. Regulation of KATP channel subunit gene expression by hyperglycemia in the mediobasal hypothalamus of female rats. *Am J Physiol Endocrinol Metab* 2007;292:E1801–E1807
61. Pocai A, Lam TK, Gutierrez-Juarez R, et al. Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 2005;434:1026–1031
62. Abraham MA, Yue JT, LaPierre MP, et al. Hypothalamic glucagon signals through the KATP channels to regulate glucose production. *Mol Metab* 2014;3:202–208
63. Verkarre V, Fournet JC, de Lonlay P, et al. Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. *J Clin Invest* 1998;102:1286–1291
64. Nielsen EM, Hansen L, Carstensen B, et al. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 2003;52:573–577
65. Park YB, Choi YJ, Park SY, et al. ATP-sensitive potassium channel-deficient mice show hyperphagia but are resistant to obesity. *Diabetes Metab J* 2011;35:219–225
66. Alekseev AE, Reyes S, Yamada S, et al. Sarcolemmal ATP-sensitive K(+) channels control energy expenditure determining body weight. *Cell Metab* 2010;11:58–69