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Effects of the *ABCC8* R1420H loss-of-function variant on beta-cell function, diabetes incidence, and retinopathy

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ABSTRACT

Introduction The *ABCC8* gene regulates insulin secretion and plays a critical role in glucose homeostasis. The effects of an *ABCC8* R1420H loss-of-function variant on beta-cell function, incidence of type 2 diabetes, and age-at-onset, prevalence, and progression of diabetes complications were assessed in a longitudinal study in American Indians.

Research design and methods We analyzed beta-cell function through the relationship between insulin secretion and insulin sensitivity in members of this population without diabetes aged ≥5 years using standard major axis regression. We used hierarchical logistic regression models to study cross-sectional associations with diabetes complications including increased albuminuria (albumin-to-creatinine ratio (ACR) ≥30 mg/g), severe albuminuria (ACR ≥300 mg/g), reduced estimated glomerular filtration rate (eGFR <60 mL/min/1.73 m^2), and retinopathy. This study included 7675 individuals (254 variant carriers) previously genotyped for the R1420H with available phenotypic data and with a median follow-up time of 13.5 years (IQR 4.5–26.8).

Results Variant carriers had worse beta-cell function than non-carriers (p=0.0004; on average estimated secretion was 22% lower, in carriers), in children and adults, with no difference in insulin sensitivity (p=0.50). At any body mass index and age before 35 years, carriers had higher type 2 diabetes incidence. This variant did not associate with prevalence of increased albuminuria (OR 0.87, 95% CI 0.66 to 1.16), severe albuminuria (OR 0.96, 95% CI 0.55 to 1.68), or reduced eGFR (OR 0.44, 95% CI 0.18 to 1.06). By contrast, the variant significantly associated with higher retinopathy prevalence (OR 1.74, 95% CI 1.19 to 2.53) and this association was only partially mediated (<11%) by glycemia, duration of diabetes, risk factors of retinopathy, or insulin use. Retinopathy prevalence in carriers was higher regardless of diabetes presence.

Conclusions The *ABCC8* R1420H variant is associated with increased risks of diabetes and of retinopathy, which may be partially explained by higher glycemia levels and worse beta-cell function.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In a Southwestern American Indian community that has a high prevalence of type 2 diabetes and a high frequency of an *ABCC8* R1420H loss-of-function variant (~3% of population are carriers), variant carriers had a doubling risk of diabetes at a younger age and lower body mass index.

WHAT THIS STUDY ADDS

⇒ This variant contributes to worsening of beta-cell function and accelerates appearance of retinopathy.

⇒ The variant did not associate with prevalence of increased albuminuria, severe albuminuria, or reduced estimated glomerular filtration rate.

⇒ The increased risk of retinopathy in variant carriers is only partially explained by diabetes duration and hyperglycemia.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Identifying people with this variant and closely monitoring their glycemia levels might help reduce the incidence of diabetes and retinopathy.

INTRODUCTION

ABCC8 and the adjacent gene *KCNJ11* encode the subunits SUR1 and KIR6.2, respectively, of the heteroctomer K_{ATP} channel.[1 2] These K_{ATP} channels regulate membrane K^+ flux for various cell types including pancreatic β-cells, where increased glucose metabolism resulting in high ATP levels causes closure of these K_{ATP} channels and hyperpolarization of the cell membrane leading to calcium influx, and subsequent insulin secretion.[1 3] In contrast, low glucose levels and the concomitant low ATP levels opens the K_{ATP} channel, resulting in an efflux of K^+ causing depolarization of the cell membrane and closure of the voltage-gated Ca^+ channel such that insulin is not secreted.[2] Gain-of-function variants in the *ABCC8* gene cause K_{ATP} channels to remain open even in presence of excess glucose, preventing insulin secretion and leading to type 2 diabetes.[1 4] Loss-of-function variants cause an impaired response to opening these channels, thus, insulin secretion continues even with low glucose concentrations.[1 4] Generally, loss-of-function variants in the *ABCC8* gene result in hyperinsulinemia and hypoglycemia and some of these variants cause hyperinsulinism in the neonatal period followed by type 2 diabetes later in life.[1 3–6]

An R1420H loss-of-function variant in *ABCC8* (rs1272388614) was previously detected in ~3% of people in a Southwestern

American Indian Community with a high prevalence of type 2 diabetes (a frequency of the allele coding for the histidine substitution of ~1.5%).[6] This variant is not completely private to this community, but the gnomAD V.3.1.2 database reports 5 carriers (observed in 4/15 280 alleles among Latino/Admixed Americans and 1 allele/68 032 among Europeans). The American Indian Community carriers have an increased type 2 diabetes risk with a younger age-at-onset, despite having lower body mass index (BMI).[6] They also had higher birth weights which suggests possible exposure to insulin (due to oversecretion) in utero.[6] In this population, beta-cell function studied through the relationship between insulin secretion and sensitivity declines as diabetes progresses.[7–9] Measures of insulin secretion compensation and insulin secretion demand estimated through the relationship between insulin secretion and sensitivity have also been studied.[9]

In this study, we determine whether the relationship between insulin secretion and sensitivity differs between *ABCC8* R1420H loss-of-function variant carriers and non-carriers. We examine the association of this variant with type 2 diabetes incidence stratified by age and BMI. We investigate how the variant associates with incidence of type 2 diabetes complications by age, including increased and severe albuminuria, reduced estimated glomerular filtration rate (eGFR), and retinopathy. We analyze the association of the variant with prevalence and progression of these complications.

RESEARCH DESIGN AND METHODS

Data collection

Members of an American Indian Community in the Southwest USA participated in a longitudinal epidemiological study of type 2 diabetes conducted from 1965 to 2007.[10] All community members aged ≥5 years were invited to participate in biennial physical examinations that included medical history and laboratory tests. DNA collection was started as part of the study in 1982. Participants with physical examinations since then who were at least one-eighth American Indian (by self report) were genotyped unless there was insufficient quantity or quality of DNA. The current study only includes participants who were genotyped and that had data available for the corresponding analyses described below: inclusion criteria varied within each analysis and are described in online supplemental table 1.

Surrogate measures of insulin secretion and insulin sensitivity were obtained from plasma glucose (mg/dL) and serum insulin (µU/mL) concentrations measured at fasting (G_0 and I_0) and 120 min (G_{120} and I_{120}) during 75 g oral glucose tolerance tests. Insulin sensitivity and insulin secretion were estimated by the insulin sensitivity index ($ISI_0 = \left[10^4 / \left(I_0 \times G_0\right)\right]$) and the 120 min corrected insulin response ($CIR_{120} = \left[I_{120} / \left(G_{120} \times \left[G_{120} - 70 mg/dL\right]\right)\right]$), respectively. Urine albumin-to-creatinine ratio (ACR in mg/g) concentrations were used to define increased albuminuria (ACR ≥30 mg/g) and severe albuminuria (ACR ≥300 mg/g). The eGFR was calculated using the 2021 chronic kidney disease epidemiology collaboration equation from age, sex, and creatinine concentrations[11] eGFR(AS), with an eGFR of <60 mL/min/1.73 m^2 of estimated body surface area defined as reduced. Diabetes was defined as a 2-hour plasma glucose (2-hr PG) ≥11.1 mmol/L or a fasting plasma glucose (FPG) ≥7.0 mmol/L based on the 2020 American Diabetes Association guidelines.[12]

Physical examinations included direct fundoscopic examinations performed on all participants, and with pupillary dilation on those aged ≥15 years. We defined diabetic retinopathy by the presence of at least one microaneurysm or hemorrhage or proliferative retinopathy in at least one eye; hard exudates alone were not classified as retinopathy, and the number of microaneurysms was not counted.[13] Although retinopathy was also assessed by retinal photography, we used data from fundoscopic examinations in the current study because retinal photography was only performed in a subset of the population.

Relationship between insulin secretion and sensitivity

The relationship between insulin secretion and sensitivity was studied in all examinations in which CIR_{120} and ISI_0 were assessed and participants did not have diabetes; linear models with $\ln(CIR_{120})$ as a function of $\ln(ISI_0)$ were fit using standardized major axis regression.[9 14] These models do not assume a hyperbolic relationship between insulin secretion and sensitivity. We investigated whether this relationship differed between carriers and non-carriers by testing whether their respective lines shared common slopes and/or intercepts.[14] Significantly different slopes and/or intercepts indicated that the relationships differed. These comparisons were made using the whole sample and also with adjustment for age and separately in six age groups.

Insulin secretion compensation and insulin secretion demand were estimated in participants who had at least one measurement of ISI_0 and CIR_{120}, using standardized major axis regression[9] (see online supplemental material). Estimates of insulin secretion compensation (distances from the regression line) quantify the ability of insulin secretion to compensate for insulin sensitivity and represent a measure of homeostatic response to glucose.[8 9] Estimates of insulin secretion demand (distances along the regression line) quantify the demand on insulin secretion imposed by low insulin sensitivity and represent a measure of allostatic load/physiological stress on the beta-cells when compensating for diminished insulin sensitivity.[8 9] Participants included in this analysis did not have diabetes on their first measurement of ISI_0 and CIR_{120} (baseline measurement). Cox proportional hazards models were used to determine whether the variant modified the association of insulin secretion compensation and insulin secretion demand with diabetes incidence.

Genotypes

Genotypes were generated for a genome-wide association study (GWAS) including 7701 individuals from the population, as previously described.[15] An Axiom genotyping array (ThermoFisher Scientific, Waltham, Massachusetts, USA), custom-designed to capture common variation in this population, was used which resulted in 515 729 markers, including the *ABCC8* R1420H variant. Missing genotypes (0.05% of participants for rs1272388614) were imputed using IMPUTE2.[16]

Complications

Diabetes incidence rates stratified by age and BMI were estimated separately for carriers and non-carriers using multiplicative Poisson regression models. Participants included in this analysis did not have diabetes at baseline and had at least one follow-up observation for diabetes diagnosis. Since participants were examined at intervals of ~2 years, several measurements of age and BMI were made during participants' period at risk. Incidence rates were computed from new cases and person-years at risk of diabetes over all interexamination intervals with age and BMI as time-dependent covariates. We stratified by the BMI determined at the beginning of each interexamination interval, and for age attained at the middle point of the intervals.

We investigated the association of the variant with cumulative incidence of complications by age accounting for type 2 diabetes development as an intervening event that could alter risk of complications. We studied the risk of each complication based on time-dependent cohorts of people with and without diabetes with and without the variant using time-varying Cox models with age-at-onset of the complication as the time-to-event variable. We used the extended Kaplan-Meier estimator for time-dependent covariates to generate cumulative incidence curves.[17] Participants included in these analyses were assessed for the complication of interest at least once during the study.

The cross-sectional associations of the variant with prevalence of complications were investigated using hierarchical logistic regression models that accounted for familial and individual correlation by including random effects for sibships and for repeated examinations of participants nested within sibships. Two models were fit for each complication, one that adjusted for glycemia through FPG and another that did not. FPG was used as the measure of glycemia because it had fewer missing data than 2-hr PG and hemoglobin A1c (HbA1c). Missing values of FPG, 2-hr PG, and HbA1c were imputed using the fully conditional specification multiple imputation method (20 imputed datasets), no other variables were imputed. These analyses included all observations from carriers and non-carriers where the complication of interest was assessed.

The variant consistently associated with retinopathy prevalence. Thus, we studied its associations with risk factors of retinopathy (systolic and diastolic blood pressure, triglycerides, and low-density lipoprotein (LDL) cholesterol) and with diabetes' severity through glycemia levels (FPG) and current insulin use (taking insulin within 48 hours prior to research examination) as possible mediators of the association. We identified mediators following the causal steps approach.[18 19] We calculated the proportion of the association mediated as explained in the online supplemental material.

The association of the variant with disease progression for all complications was studied using Markov multistate regression models with frailty terms for sibships that accounted for familial correlation. When modeling progression of complications, there were four possible states: no diabetes and no complication, diabetes and no complication, no diabetes and complication, and diabetes and complication. We modeled the eight possible transitions depicted in online supplemental figure 1. We considered diabetes an absorbing state, that is, once a participant was diagnosed with diabetes, they always had it. Participants included in these analyses had to have ≥2 examinations with data on the same complication; they could be at any state at baseline, and some were at risk of the same transition more than once.

Covariates

Most models investigating complications adjusted for sex, exposure to diabetes in utero, and population structure (first five genetic principal components derived from the GWAS array). Exposure to diabetes in utero was quantified through two indicator variables: an indicator that was '1' if the individual's mother was diagnosed with diabetes before or during the pregnancy resulting in the individual's birth; another indicator that was '1' if the mother had an exam without diabetes >1 year after the individual's birth. Models also adjusted for age (baseline or at examination) and presence and duration of diabetes as appropriate. Table footnotes and figure legends indicate which covariates were included in each model.

RESULTS

There were 7675 community members previously genotyped for the R1420H with available phenotypic data and with a median follow-up time of 13.5 years (IQR 4.5–26.8). Among them, 1 homozygote and 253 heterozygotes were observed; in the current report, they are combined as 'carriers'. Most participants were female (61% of carriers and 56% of non-carriers). Median baseline age for carriers was 10.9 years (IQR 7.9–19.9) and was lower than that of non-carriers (12.6 years; IQR 8.3–24.0). Median baseline BMI for carriers (23.4 kg/m^2; IQR 18.1–29.4) was slightly lower than that of non-carriers (24.1 kg/m^2; IQR 18.1–30.8). Online supplemental table 2 includes a more detailed description of all 7675 participants at baseline. Online supplemental table 3 shows descriptive statistics for participants included in each analysis by genotype.

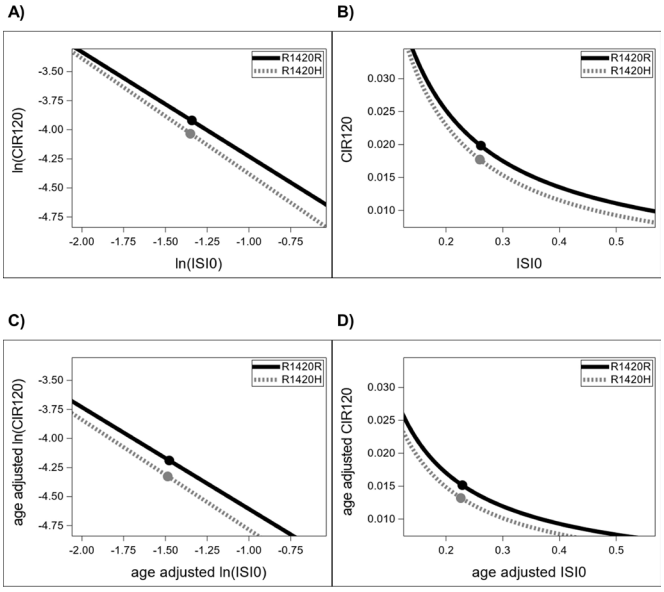

Figure 1 Relationship between insulin secretion and sensitivity. (A) Relationship between insulin secretion and insulin sensitivity in logarithmic scale; the equations of the lines in ln scale were: R1420R: $\ln(CIR_{120})=-5.12-0.90\times\ln(ISI_0)$ and R1420H: $\ln(CIR)=-5.37-0.99\times\ln(ISI_0)$. (B) Relationship between insulin secretion and sensitivity in original scale. (C) Relationship between insulin secretion and sensitivity in logarithmic scale adjusted for age; the equations were: R1420R: $\ln(CIR_{120})=-5.48-0.88\times\ln(ISI_0)$ and R1420H: $\ln(CIR_{120})=-5.73-0.94\times\ln(ISI_0)$. (D) Relationship between insulin secretion and sensitivity in original scale adjusted for age at time of examination (exponentiated lines from C). In panels (A) and (C), the gray and black points represent average $\ln(ISI_0)$, average $\ln(CIR_{120})$ in R1420H carriers and non-carriers, respectively. In panels (B) and (D), the gray and black points represent geometric mean of ISI_0, geometric mean of CIR_{120} in R1420H carriers and non-carriers, respectively. ISI_0 estimates insulin sensitivity and CIR_{120} estimates insulin secretion. CIR, corrected insulin response; ISI, insulin sensitivity index.

Relationship between insulin secretion and sensitivity

There were 5527 non-carriers with data on insulin secretion and sensitivity with a total of 14 585 observations, and 174 carriers with 423 observations. The regression lines for carriers and non-carriers describing the relationship between $\ln(CIR_{120})$ and $\ln(ISI_0)$ in all observations had significantly different slopes and intercepts (p=0.034 and p=0.006); adjusted for age, they had significantly different intercepts (p=0.0004) (figure 1). Insulin sensitivity was not significantly different (p=0.500) (figure 1). This indicates lower insulin secretion for a given degree of insulin sensitivity in carriers compared with non-carriers; estimated secretion was 22% lower, on average, in carriers. In age-group analysis, this relationship differed significantly in group 15 to <25 years (p=0.0004) (online supplemental table 4 and figure 2). Generally, however, the curves describing this relationship for non-carriers were above and to the right of the curves for carriers, even in the age groups in which these differences were not statistically significant (figure 1 and online supplemental figure 2). In observations with normal glucose regulation (FPG <5.6 mmol/L and 2-hr PG <7.8 mmol/L) in groups 5 to <10 years and 15 to <25 years, curves for carriers were also below and to the left of curves for non-carriers (online supplemental figure 3). In observations with impaired glucose regulation (FPG ≥5.6 mmol/L or 2-hr PG ≥7.8 mmol/L), curves for carriers and non-carriers overlapped across age groups (online supplemental figure 4).

Insulin secretion demand and insulin secretion compensation were estimated in 4154 participants (133=3.20% carriers). In the whole sample, higher insulin secretion compensation significantly reduced diabetes risk while higher insulin secretion demand significantly increased it (table 1). HRs for both insulin secretion compensation and insulin secretion demand were the same in models fit to the whole sample with and without the variant. In both carriers and non-carriers, insulin secretion compensation significantly associated with diabetes risk; insulin secretion demand only associated significantly with diabetes risk in non-carriers (table 1).

Online supplemental table 5 shows crude incidence rates of diabetes by age in carriers and non-carriers

Table 1 *ABCC8* R1420H variant, insulin secretion compensation, and insulin secretion demand associations with risk of diabetes

Sample	Model	Variant HR (95% CI)	Insulin secretion compensation HR (95% CI)	Insulin secretion demand HR (95% CI)
Whole	Model 1	1.33 (0.95 to 1.87)	--	--
	Model 2	–	0.58 (0.54 to 0.62)	1.29 (1.20 to 1.39)
	Model 3	1.26 (0.89 to 1.78)	0.58 (0.54 to 0.62)	1.29 (1.20 to 1.39)
R1420H*	Model 2	–	0.49 (0.32 to 0.75)	1.13 (0.66 to 1.94)
R1420R	Model 2	–	0.58 (0.54 to 0.62)	1.30 (1.21 to 1.40)

All models adjusted for sex, age at baseline observation with insulin secretion and sensitivity, two indicator variables quantifying exposure to diabetes in utero, and first five principal components for population structure. HRs for insulin secretion compensation and insulin secretion demand were obtained per 1 SD difference of these measures. For samples with variant carriers and non-carriers, SMA lines were calculated within samples and insulin secretion compensation and insulin secretion demand were standardized within samples.
*The one homozygous individual had diabetes at baseline and not included in these analyses.

based on data from all 7675 participants included in this study. Of the 254 carriers included, 124 (49%) developed diabetes during the study. Carriers had the highest diabetes incidence at ages 10 to <15 years, 15 to <25 years, and 25 to <35 years (34.8, 16.7, and 20.6 cases/1000 person years, respectively).

There were 6108 participants (204 carriers) included in the analysis describing diabetes incidence rates stratified by age and BMI. In both carriers and non-carriers, diabetes incidence was significantly associated with BMI (p<0.001 and p<0.0001, respectively) and age (p=0.016 and p<0.0001, respectively) and there was an age and BMI interaction (p=0.028 and p<0.0001, respectively) (online supplemental figure 5). Diabetes incidence rates were consistently higher with increasing BMI up to age 35 years for carriers, and at all ages for non-carriers (online supplemental figure 5); carriers had lower BMIs than non-carriers at any age ≥5 years regardless of whether they developed diabetes (online supplemental table 6).

Cumulative incidence of complications by age

People with diabetes developed complications earlier in life (figure 2 and online supplemental figures 6–8). Time-varying Cox models suggested that compared with the group of people without the variant, those with the variant had higher retinopathy risk, regardless of the presence of diabetes (figure 2). In those with diabetes, carriers had higher incidence of albuminuria and severe albuminuria compared with non-carriers (online supplemental figures 6 and 7).

Prevalence of complications

At the baseline assessment for prevalence of complications, 19% of carriers and 17% of non-carriers had albuminuria, 5% of carriers and 3% of non-carriers had severe albuminuria, 0% of carriers and 0.13% of non-carriers had reduced eGFR, 2% of carriers and 1% of non-carriers had retinopathy. With adjustment for diabetes and its duration, the variant did not associate significantly with the prevalence of albuminuria, severe albuminuria, or reduced eGFR (table 2). By contrast, the variant associated significantly with prevalence of retinopathy. In the whole sample, carriers had significantly higher odds of retinopathy when not adjusting for FPG (OR 1.74, 95% CI 1.19 to 2.53) and when adjusting for it (OR 1.68, 95% CI 1.16 to 2.45). Separate analysis in observations with and without diabetes showed that in both subsamples the variant increased odds of retinopathy, even when adjusting for FPG (table 2). Retinopathy in observations without diabetes was mostly characterized by microaneurysms alone, while in observations with diabetes it was mostly characterized by the presence of both microaneurysms and exudates. This was the case for both carriers and non-carriers (exudates alone were not classified as retinopathy).

In all participants assessed for retinopathy, carriers had significantly higher FPG (b=0.34 mmol/L, 95% CI 0.16 to 0.51) (online supplemental table 7). Inclusion

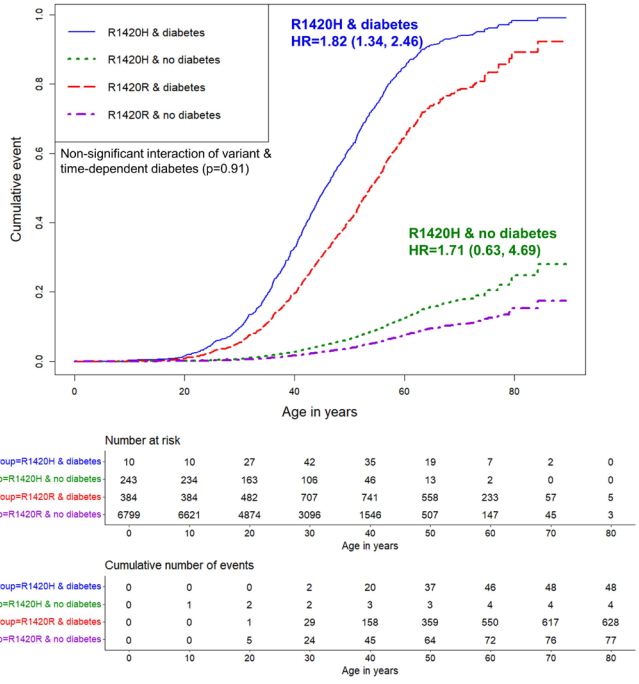

Figure 2 Retinopathy cumulative incidence by age. Kaplan-Meier (KM) curves were built based on Cox models fit to age-at-onset of retinopathy that adjusted for sex, two variables quantifying exposure to diabetes in utero, and the first five principal components. Diabetes was analyzed as a time-dependent variable, that is, it changed from 0 to 1 at the diagnosis of diabetes. The survival probabilities used to create these KM curves were estimated at the average values of the covariates. HRs for R1420H and diabetes are comparing this group with the group R1420R and diabetes. HRs for R1420H and no diabetes are comparing this group with the group of R1420R and no diabetes. Cohorts at risk for people with the variant (both with and without diabetes) at age 50 years or later were small leading to large variances in the KM curves. Because of these large variances, findings of the KM curves and the time-varying Cox models should be observed with caution after that age.

of FPG in the model slightly attenuated the association of the variant with retinopathy prevalence in the whole sample and in observations with diabetes only; the association was not attenuated in observations without diabetes (table 2). However, the proportion of the association of the variant with retinopathy prevalence mediated by FPG was ≤0.067 (online supplemental table 9). The proportion of the association mediated by 2-hr PG or HbA1c was ≤0.106 (online supplemental table 9).

The variant did not associate with systolic or diastolic blood pressure or with triglycerides (online supplemental table 7). Carriers had significantly higher LDL cholesterol levels in the whole sample (b=0.16 mmol/L, 95% CI 0.06 to 0.25) and in the sample of observations with diabetes only (b=0.19 mmol/L, 95% CI 0.03 to 0.34); higher LDL cholesterol levels increased retinopathy prevalence in both samples. However, the proportion of the association of the variant with retinopathy prevalence mediated by LDL cholesterol was ≤0.022 (online supplemental tables 8 and 9).

Table 2 Prevalence of diabetes complications

Outcome	Whole sample		Only observations with diabetes		Only observations without diabetes	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Increased albuminuria (ACR ≥30)	0.87 (0.66 to 1.16)	0.83 (0.62 to 1.10)	0.85 (0.57 to 1.28)	0.82 (0.54 to 1.23)	0.78 (0.52 to 1.16)	0.78 (0.52 to 1.17)
Severe albuminuria* (ACR ≥300)	0.96 (0.55 to 1.68)	0.91 (0.56 to 1.50)	1.02 (0.64 to 1.64)	0.92 (0.54 to 1.56)	—	—
Reduced eGFR* (eGFR <60)	0.44 (0.18 to 1.06)	0.46 (0.19 to 1.11)	0.43 (0.18 to 1.03)	0.45 (0.18 to 1.08)	—	—
Retinopathy	1.74 (1.19 to 2.53)	1.68 (1.16 to 2.45)	1.66 (1.14 to 2.44)	1.61 (1.08 to 2.41)	1.73 (0.61 to 4.91)	1.74 (0.62 to 4.92)

OR describes the association of the variant with the prevalence of each complication. In whole sample: model 1 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, population structure (first five principal components), and presence and duration of diabetes duration at examination; model 2 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, population structure, presence and duration of diabetes at examination, and fasting plasma glucose at time of examination. In observations with diabetes only: model 1 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, population structure and diabetes duration; model 2 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, population structure, diabetes duration, and fasting plasma glucose at time of examination. In observations without diabetes: model 1 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, and population structure; model 2 adjusted for sex, age at examination, two variables quantifying exposure to diabetes in utero, population structure, and fasting plasma glucose at time of examination.
*In observations without diabetes of *ABCC8* R1420H carriers, there were only two cases of severe albuminuria and there were no cases of reduced eGFR (— not enough data to calculate ORs).
ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

In participants assessed for retinopathy, 1124 were treated with insulin (66 carriers), 1015 were treated with sulfonylureas (55 carriers), 715 took metformin (35 carriers), and 303 were treated with a thiazolidinedione (17 carriers), at some point at or after diabetes diagnosis. In observations with diabetes only, the variant did not associate significantly with current use of insulin (OR 1.55, 95% CI 0.95 to 2.51), sulfonylureas (OR 0.93, 95% CI 0.64 to 1.34), metformin (OR 0.68, 95% CI 0.43 to 1.09), or thiazolidinedione (OR 1.19, 95% CI 0.64 to 2.18), when looking at their use separately (online supplemental table 7). Based on the causal steps approach, the association of the variant with retinopathy prevalence was not mediated by the use of any of these medications.

Progression of complications

Online supplemental table 10 shows states at baseline for participants included in the multistate models; for all complications, most participants at baseline were in the state of no diabetes and no complication. For participants without the complications at baseline (states 1 or 2), online supplemental table 11 presents their state at first occurrence of the complication, if they experienced it during the study, or their state at last observation if they did not experience it. For participants with the complication at baseline (states 3 or 4), online supplemental table 11 presents their state at last observation. Table 3 shows percentage of transitions in carriers and non-carriers and results of the multistate model describing progression of retinopathy; online supplemental table 12 presents this information for increased albuminuria, severe albuminuria, and reduced eGFR.

When modeling the progression of increased albuminuria, carriers had significantly higher risk of transitioning from no diabetes and normal albuminuria to diabetes and normal albuminuria (T1 HR=1.65, 95% CI 1.16 to 2.35) and from no diabetes and normal albuminuria to diabetes and increased albuminuria (T3 HR=2.84, 95% CI 1.59 to 5.06) (online supplemental table 12). Similar findings were observed for development of severe albuminuria and reduced eGFR (online supplemental table 12). In the progression of retinopathy, the variant associated significantly with an increased risk of transitioning from no diabetes and no retinopathy to diabetes and no retinopathy (T1 HR=1.53, 95% CI 1.17 to 2.00) and from no diabetes and no retinopathy to diabetes and retinopathy (T3 HR=4.17, 95% CI 1.74 to 10.00) (table 3).

DISCUSSION

This study describes a detailed investigation of the phenotypic characteristics of the *ABCC8* R1420H loss-of-function variant in a Southwestern American Indian community with high prevalence of type 2 diabetes. We expand on previous results by studying the associations of this variant with beta-cell function and prevalence and progression of diabetes complications, which were not were considered before. In this population, R1420H

Table 3 Progression of retinopathy

Transition	From	To	# of transitions in R1420R	# of transitions in R1420H	HR (95% CI)
T1	No diabetes, no retinopathy	Diabetes, no retinopathy	1641/21 594 (7.6%)	76/683 (11.1%)	1.53 (1.17 to 2.00)
T2	No diabetes, no retinopathy	No diabetes, retinopathy	67/21 594 (0.3%)	4/683 (0.6%)	1.98 (0.71 to 5.56)
T3	No diabetes, no retinopathy	Diabetes, retinopathy	61/21 594 (0.3%)	6/683 (0.9%)	4.17 (1.74 to 10.00)
T4	Diabetes, no retinopathy	Diabetes, retinopathy	631/4698 (13.4%)	43/273 (15.8%)	1.22 (0.84 to 1.77)
T5*	No diabetes, retinopathy	No diabetes, no retinopathy	37/45 (82.2%)	2/2 (100%)	1.55 (–)
T6*	No diabetes, retinopathy	Diabetes, no retinopathy	5/45 (11.1%)	0/2 (0%)	0 (–)
T7*	No diabetes, retinopathy	Diabetes, retinopathy	2/45 (4.4%)	0/2 (0%)	0 (–)
T8	Diabetes, retinopathy	Diabetes, no retinopathy	285/757 (37.6%)	25/73 (34.2%)	0.97 (0.59 to 1.60)

For all transitions, the multistate model adjusted for age at first assessment of complication, sex, two variables quantifying exposure to hyperglycemia in utero, and population structure. For transitions 4 and 8, from diabetes and no retinopathy to diabetes and retinopathy and vice versa, the multistate model also adjusted for diabetes duration.
* CIs were too imprecise because of small sample sizes.

carriers had higher prevalence of retinopathy and worse beta-cell function than non-carriers. They had higher incidence of type 2 diabetes at younger ages and especially at higher BMI. Also, carriers with diabetes had the highest risk of retinopathy, increased albuminuria, and severe albuminuria.

The relationship between insulin secretion and sensitivity differed significantly between carriers and non-carriers and suggested that, at any age, carriers had worse beta-cell function, even though insulin sensitivity was not significantly different between the two groups. Carriers' worse beta-cell function is consistent with their higher diabetes risk[6] and with beta-cell fatigue with aging. Consistent with previous findings in this population, higher insulin secretion compensation decreased diabetes risk while higher insulin secretion demand increased it.[9] The variant did not modify associations of insulin secretion compensation and insulin secretion demand with diabetes risk. However, insulin secretion compensation associated significantly with diabetes risk in both groups, while in carriers, insulin secretion demand did not. This is consistent with previous results that indicated that in people with increased diabetes risk (in this case carriers), the most important factor for delaying or preventing diabetes is the ability to reach glucose homeostasis by adequately compensating for decreased insulin sensitivity (insulin secretion compensation).[20]

The variant associated significantly with the prevalence of retinopathy, but not with the other complications. R1420H carriers had a higher prevalence (but not significantly) of retinopathy even in observations without diabetes; signs of diabetic retinopathy have been previously found in people without diabetes in this population.[21] Carriers had more severe diabetes as they had greater hyperglycemia and were more likely to be treated with insulin. They also had higher LDL cholesterol levels. However, the association of the variant with prevalence of retinopathy was not fully mediated by glycemia or LDL cholesterol. The use of insulin, sulfonylureas, metformin, or thiazolidinedione were not identified as mediators of this association. Similar findings were observed in the subsample of observations where retinopathy was assessed by retinal photography (results not shown). The possibility of ABCC8 mutations contributing to the rapid progression to proliferative retinopathy when an individual is started on insulin was previously suggested.[22] Consistent with that finding, our results showed that among participants with diabetes who had ever been treated with insulin, R1420H carriers had higher retinopathy prevalence (OR 1.62, 95% CI 1.01 to 2.59).

Previous studies found a high prevalence of retinopathy in people with mutations on the *ABCC8* and other genes that cause maturity onset diabetes of the young.[22–24] However, the sample sizes in those studies were small. Our work adds to the previous literature by using data from a large longitudinal population study to confirm in a more statistically robust way the associations of this variant with retinopathy.

Retinopathy is closely related to the duration and degree of hyperglycemia. In this population, higher values of FPG and 2-hr PG increased the prevalence of retinopathy.[25] In a randomized clinical trial of control of hyperglycemia in type 1 diabetes, there was a continuously increasing risk of sustained progression of retinopathy with increasing mean glycosylated hemoglobin values over time, and progression of retinopathy was decreased by tighter control of hyperglycemia—evidence that hyperglycemia is a cause of retinopathy.[26] A possible explanation for higher retinopathy prevalence in R1420H carriers might be that they had worse beta-cell function since a young age. Thus, through most of their lives they were less able to maintain glucose homeostasis than non-carriers. Also, carriers had the highest diabetes incidence at earlier ages. This might have led glucose levels to be higher in carriers through most of their lifespan, as shown by their higher FPG levels in this study. Another possible explanation may be that the SUR1 subunit of the K_{ATP} channels is expressed in the retinal vessels and in all retinal layers.[27] Glibenclamide, an antidiabetes drug of the sulfonylurea class, exerted neuroprotective effects on the retina of rodent models of type 2 diabetes, by avoiding cell death and preserving visual function through direct binding to its receptor SUR1.[27 28] If confirmed, the beneficial effect of a drug stimulating the SUR1 receptor, coded by the *ABCC8* gene, would support the hypothesis that a loss-of-function variant, such as R1420H, could be associated with retinopathy.

In the progression of complications, the variant increased the risk of transitioning from the state of no complication and no diabetes to the state of no complication and diabetes, for all complications. In the progression of increased albuminuria and retinopathy, the variant also increased the risk of transitioning from the state of no complication and no diabetes to complication and diabetes. People with the variant had a higher risk of developing retinopathy before and after developing diabetes. However, the small number of variant carriers that experienced certain transitions in the multistate models used to study progression of retinopathy made it difficult to estimate precisely the HRs associated with those transitions.

A limitation is that the variant investigated is rarely found in other populations which does not allow for replication or comparison of our findings. We cannot determine whether these results generalize to other American Indians, because most American Indian communities do not have DNA available on a population basis for such research.

In conclusion, in this population-based study, *ABCC8* R1420H variant carriers had worse beta-cell function than non-carriers. Further work is needed to better understand the transition from hyperinsulinemia in infancy to hyperglycemia at later ages since our results suggest that in this population, such transition occurred prior to age 5 years; in the present study, R1420H carriers had lower insulin secretion relative to insulin sensitivity than non-carriers in all age groups. The variant generally accelerated the age-of-onset of most complications. Also, the impact of this variant, beyond its effect on diabetes risk, was marked in retinopathy prevalence and was minimal on prevalence of albuminuria, severe albuminuria and reduced eGFR. The association of the variant with retinopathy prevalence is only partially explained by worse beta-cell function and higher levels of glycemia.

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