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Genetic Variations in Magnesium-Related Ion Channels May Affect Diabetes Risk among African American and Hispanic American Women^{1–3}

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Abstract

Background: Prospective studies consistently link low magnesium intake to higher type 2 diabetes (T2D) risk.

Objective: We examined the association of common genetic variants [single nucleotide polymorphisms (SNPs)] in genes related to magnesium homeostasis with T2D risk and potential interactions with magnesium intake.

Methods: Using the Women's Health Initiative-SNP Health Association Resource (WHI-SHARe) study, we identified 17 magnesium-related ion channel genes (583 SNPs) and examined their associations with T2D risk in 7287 African-American (AA; $n = 1949$ T2D cases) and 3285 Hispanic-American (HA; $n = 611$ T2D cases) postmenopausal women. We performed both single- and multiple-locus haplotype analyses.

Results: Among AA women, carriers of each additional copy of SNP rs6584273 in cyclin mediator 1 (*CNNM1*) had 16% lower T2D risk [OR: 0.84; false discovery rate (FDR)-adjusted $P = 0.02$]. Among HA women, several variants were significantly associated with T2D risk, including rs10861279 in solute carrier family 41 (anion exchanger), member 2 (*SLC41A2*) (OR: 0.54; FDR-adjusted $P = 0.04$), rs7174119 in nonimprinted in Prader-Willi/Angelman syndrome 1 (*NIPA1*) (OR: 1.27; FDR-adjusted $P = 0.04$), and 2 SNPs in mitochondrial RNA splicing 2 (*MRS2*) (rs7738943: OR = 1.55, FDR-adjusted $P = 0.01$; rs1056285: OR = 1.48, FDR-adjusted $P = 0.02$). Even with the most conservative Bonferroni adjustment, two 2-SNP-haplotypes in *SLC41A2* and *MRS2* region were significantly associated with T2D risk (rs12582312-rs10861279: $P = 0.0006$; rs1056285-rs7738943: $P = 0.002$). Among women with magnesium intake in the lowest 30% (AA: ≤ 0.164 g/d; HA: ≤ 0.185 g/d), 4 SNP signals were strengthened [rs11590362 in claudin 19 (*CLDN19*), rs823154 in *SLC41A1*, rs5929706 and rs5930817 in *membra*; HA: ≥ 0.313 g/d), rs6584273 in *CNNM1* (OR: 0.71; FDR-adjusted $P = 0.04$) and rs1800467 in potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) (OR: 2.50; FDR-adjusted $P = 0.01$) were significantly associated with T2D risk.

Conclusions: Our findings suggest important associations between genetic variations in magnesium-related ion channel genes and T2D risk in AA and HA women that vary by amount of magnesium intake. *J Nutr* 2015;145:418–24.

Keywords: genes, ion channel, magnesium intake, diabetes, women

Introduction

Magnesium is an essential mineral found in whole grains, leafy green vegetables, legumes, and nuts. Large prospective studies of men and women have associated greater magnesium intake with

lower risk of type 2 diabetes (T2D)¹⁵ and metabolic abnormalities (1, 2). Absorption of magnesium, a positively charged cation (Mg^{2+}), relies on ion channels, including the transient receptor potential (TRP) family of cation channels. In patients with hypomagnesemia and secondary hypocalcemia, several mutations in TRP cation channel, subfamily M, member 6 (*TRPM6*) were identified, indicating the importance of genes in

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³ Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

the TRP family (*TRPM6* and *TRPM7*) in the regulation of magnesium homeostasis. In particular, 2 variants of the *TRPM6* coding region were recently identified as T2D susceptibility loci in Caucasian women with low magnesium intake (<250 mg), further highlighting a potential nutrient-gene interaction in affecting T2D risk. However, this magnesium-gene interaction was not consistently observed nor examined (2, 3) in other racial/ethnic groups.

Moreover, pancreatic β -cell ATP-sensitive K^+ (K_{ATP}) channels play a central role in glucose-induced insulin secretion (4). Common variants in potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) and ATP-binding cassette, sub-family C, member 8 (*ABCC8*) genes that encode the major K_{ATP} channel subunits SUR1 and Kir6.2, respectively, were identified as T2D susceptibility loci (5). K_{ATP} channel activity is regulated by the intracellular balance of magnesium-ATP and magnesium-ADP and depends on the presence of Mg^{2+} ions (6). Low magnesium intake and imbalance in intracellular Mg^{2+} ion concentrations may interact with K_{ATP} ion channel variants in affecting T2D risk; however, no studies to date have examined these interactions.

According to their activity at the protein level (e.g., magnesium and other divalent cation transport, reabsorption, mitochondrial magnesium homeostasis, regulating magnesium-dependent enzymes, and handling renal sodium and magnesium), at least 13 additional genes (in the families of claudin, solute carrier family, cyclin, nonimprinted in Prader-Willi/Angelman syndrome, zinc finger DHHC-type, mitochondrial RNA splicing, membrane magnesium transporter, and FXYP domain-containing ion transport regulator) may play a role in magnesium homeostasis (Supplemental Table 1).

We therefore hypothesized that genetic variations in ion channels related to magnesium homeostasis and glucose metabolism are associated with an increased risk of T2D. Because sufficient magnesium intake may partially compensate for magnesium deficiency caused by genetic mutations (2), we further hypothesized that any potential effect on T2D risk because of magnesium-related genetic variants may be more evident among individuals with low magnesium intake. To test these hypotheses, we conducted a genetic association study of T2D risk in 7287 African American (AA) and 3285 Hispanic American (HA) postmenopausal women aged 50–79 y from the National Women's Health Initiative SNP Health Association Resource (WHI-SHARe). With the use of a whole-genome scan, we identified 583 common single nucleotide polymorphisms (SNPs) in 17 candidate genes of ion channels, including *TRPM6*, *TRPM7*, *ABCC8*, *KCNJ11*, claudin 16 (*CLDN16*), *CLDN19*, solute carrier family 41 (anion exchanger), member 1 (*SLC41A1*), *SLC41A2*, solute carrier family 12 (anion exchanger), member 3

(*SLC12A3*), cyclin mediator 1 (*CNNM1*), *CNNM2*, nonimprinted in Prader-Willi/Angelman syndrome 1 (*NIPAI1*), *NIPA2*, zinc finger, DHHC-type containing 17 (*ZDHHC17*), mitochondrial RNA splicing 2 (*MRS2*), membrane magnesium transporter 1 (*MMGT1*), and FXYP domain-containing ion transport regulator 2 (*FXYP2*), and investigated their associations with T2D risk.

Methods

Study population. The WHI is a long-term national health study that focuses on strategies to prevent cardiovascular disease, cancer, diabetes, and fractures in postmenopausal women. Details on the design and recruitment of the WHI are published elsewhere (7). The WHI-SHARe includes 7287 AA and 3285 HA participants aged 50–79 y who participated in the WHI clinical trials or observational study and for whom genome-wide association study genotyping was conducted and who provided supplemental consent that allowed for data sharing. Prevalent T2D cases were identified from self-report of diabetes treatment. Incident T2D was identified as first-time use of hypoglycemic medication (insulin or oral hypoglycemic agents) or hospitalization for previously unreported T2D (7). The procedures of this study were in accordance with the ethical standards of Brown University on human experimentation, and approval was obtained from the institutional review board on human subjects.

Data collection. Standardized questionnaires, including information on age, ethnicity, education, income, occupation, medical and family history, smoking status, alcohol use, recreational physical activity, and medication and supplement use, were administered at the baseline visit. Dietary magnesium intake was assessed with a semiquantitative FFQ that estimated average dietary intake for the past 3 months. Total magnesium represented the sum of magnesium intake from both dietary and supplemental sources.

Fasting blood specimens were collected from all participants at baseline according to a standardized protocol. Participants were instructed to fast for 12 h before collection, take all regular medications except for diabetes medication, take no aspirin or nonsteroidal anti-inflammatory drugs for 48 h before the visit except for those medications taken regularly, refrain from smoking for 1 h before the visit, and perform no vigorous physical activity for 12 h before the visit.

Genotyping. DNA extraction and genotyping were conducted at the Fred Hutchinson Cancer Research Center in Seattle. The Affymetrix Genome-wide Human SNP Array 6.0 was used to genotype >1.8 million markers of genetic variation. To test the hypotheses in this study, we identified a total of 583 SNPs that include 68 SNPs on the *TRPM6* gene, 36 on *TRPM7*, 65 on *ABCC8*, 12 on *KCNJ11*, 40 on *CLDN16*, 5 on *CLDN19*, 19 on *SLC41A1*, 62 on *SLC41A2*, 29 on *SLC12A3*, 48 on *CNNM1*, 36 on *CNNM2*, 23 on *NIPAI1*, 18 on *NIPA2*, 71 on *ZDHHC17*, 23 on *MRS2*, 14 on *MMGT1*, and 14 on *FXYP2*.

Statistical analyses. Baseline demographic and lifestyle characteristics were examined by T2D case-control status with the use of χ^2 or *t* tests. Deviations from Hardy-Weinberg equilibrium were assessed with a χ^2 goodness-of-fit test in PLINK (8). Relatedness was determined with the method-of-moments approach with an identity-by-descent model (8). Confirmatory analysis (9) was also performed with a pairwise kinship coefficient estimator. On the basis of these coefficients, pairs of parent-offspring (22 pairs and 2 trios), monozygotic twins (5 pairs), and siblings (192 pairs and 5 trios) were identified. The ones with the largest call rate of each pair of relatives were included in the subsequent analysis; 234 individuals with lower call rates of each pair of relatives (parent-offspring pairs, monozygotic twins, and siblings) were excluded on the basis of the relatedness analysis. To correct for population stratification because of admixture within AA and HA populations, we conducted principal component analyses (10) of global ancestry and included 3 principal components in all multivariable-adjusted models.

We used logistic regression to calculate ORs and 95% CIs for single-locus (SNP) associations with T2D risk under an additive genetic model.

¹⁵ Abbreviations used: AA, African American; *ABCC8*, ATP-binding cassette, subfamily C, member 8 gene; *CLDN16*, claudin 16 gene; *CLDN19*, claudin 19 gene; *CNNM1*, cyclin mediator 1 gene; *CNNM2*, cyclin mediator 2 gene; FDR, false discovery rate; *FXYP2*, FXYP domain-containing ion transport regulator 2 gene; HA, Hispanic American; K_{ATP} , ATP-sensitive K^+ ; *KCNJ11*, potassium inwardly rectifying channel, subfamily J, member 11 gene; *MMGT1*, membrane magnesium transporter 1 gene; *MRS2*, mitochondrial RNA splicing 2 gene; *NIPAI1*, nonimprinted in Prader-Willi/Angelman syndrome 1 gene; *NIPA2*, nonimprinted in Prader-Willi/Angelman syndrome 2 gene; *SLC12A3*, solute carrier family 12 (anion exchanger), member 3 gene; *SLC41A2*, solute carrier family 41 (anion exchanger), member 2 gene; SHARe, SNP Health Association Resource; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; TRP, transient receptor potential; *TRPM6*, transient receptor potential cation channel, subfamily M, member 6 gene; *TRPM7*, transient receptor potential cation channel, subfamily M, member 7 gene; WHI, Women's Health Initiative; *ZDHHC17*, zinc finger, DHHC-type containing 17 gene.

We also used dominant model for assessing single-SNP association with T2D risk. All multivariable models were adjusted for age, geographic region, and 3 principal components of global ancestry. To account for potential false positives because of multiple comparisons in this study, we calculated the false discovery rate (FDR) by incorporating all *P* values from multiple tests performed for the association of SNPs in each gene and T2D risk. FDR is defined as the proportion of false positives among all significant results and is estimated by setting some rejection region so that on average $FDR < \alpha$ (the level of significance ($\alpha = 0.05$)); another commonly used and more conservative multiple comparison adjustment method, Bonferroni's correction, sets the significance cutoff at α/n , where *n* is the number of hypotheses to test. The FDR statistics were obtained for each *P* value, and the FDR statistics with adjusted $P \leq 0.05$ were considered significant (11). All models were run separately in AA and HA women. Stratified analyses were performed to examine whether the genetic associations with T2D were modified by magnesium intake by using the magnesium intake in the lowest 30% (≤ 0.164 g/d for AA women and ≤ 0.185 g/d for HA women) and highest 70% (≥ 0.291 g/d for AA women and ≥ 0.313 g/d for HA women) categories as cutoffs. Sliding window haplotype-based analyses (12) (window width = 2) were used to examine the joint contribution of 2-SNP windows on T2D risk. For each window, a Wald test based on *t* distribution was used. The test performed N-1 haplotype-specific tests for N haplotypes (of each vs. all others), depending on the number of SNPs in each gene. All statistical analyses were conducted with PLINK (8) and R open access software version 2.13.0 (13).

Results

Baseline characteristics of the study population are shown in **Table 1**. Compared with controls, cases had a higher BMI, lower current alcohol intake, a lower level of physical activity, and higher percentage of family history of T2D ($P < 0.05$). Total magnesium intake was lower among cases than among controls (mean intake = 0.248 g/d for AA women and 0.267 g/d for HA women), and AA women had lower magnesium intake than HA women.

After adjustment for age, region, principal components of global ancestry, and multiple comparisons with the FDR, carriers of each additional copy of a variant allele of the SNP rs6584273 (in the *CNNM1* gene) had 16% lower T2D risk in AA women. Among HA women, 4 variants were significantly associated with T2D risk; these include rs10861279 in *SLC41A2*, rs7174119 in *NIPAI1*, and 2 SNPs in *MRS2* gene (rs7738943 and rs1056285) (**Tables 2, 3**). Results were similar with the use of a dominant genetic model (**Supplemental Tables 2, 3**). Sliding window analyses revealed two 2-SNP sub-haplotypes (*SLC41A2* rs12582312-rs10861279: $P = 0.0006$; *MRS2* rs1056285-rs7738943: $P = 0.002$) significantly associated with T2D risk after the most conservative Bonferroni adjustment [threshold for *SLC41A2* = $-\log_{10}(0.05/61) = 3.09$; threshold for *MRS2* = $-\log_{10}(0.05/22) = 2.64$] (**Figure 1A, B**).

Among women with total magnesium intake in the lowest 30% (≤ 0.164 g/d for AA women; ≤ 0.185 g/d for HA women) and after FDR adjustment, 4 SNPs signals were significantly strengthened among HA women (*CLDN19* rs11590362, *SLC41A1* rs823154, *MMGT1* rs929706 and *MMGT1* rs5930817) (**Table 4**). *TRPM6* coding region variants, *Ile1393Val* and *Lys1584Glu*, previously identified as susceptibility loci for T2D among women with low magnesium intake (2), were not statistically significant among women with low magnesium intake in single-SNP or haplotype sliding window analyses. Among women with magnesium intake in the highest 70% of magnesium intake (≥ 0.291 g/d for AA women; ≥ 0.313 g/d for HA women) and after FDR adjustment, *CNNM1* rs6584273

was significantly associated with T2D risk among AA women with magnesium intake ≥ 291 mg/d. A missense mutation in *KCNJ11* (rs1800467) was also significantly associated with T2D risk among HA women with magnesium intake ≥ 0.313 g/d. Results were similar with the use of a dominant genetic model (**Supplemental Table 4**).

Discussion

In this large prospective study of postmenopausal women, we observed genetic associations between magnesium-related ion channel genes and risk of T2D that were modified by total magnesium intake and ethnicity.

The significant signal in the *KCNJ11* gene (rs1800467) identified in our study is consistent with a previous report that identified *KCNJ11* as an important susceptibility locus for T2D (14, 15). Mutations in *KCNJ11* cause familial persistent hyperinsulinemic hypoglycemia in infancy and permanent neonatal diabetes (16–18). In addition, the E23K single nucleotide variant (K23, rs5219) in the *KCNJ11* gene is also associated with diabetes (19, 20) and impaired insulin action and secretion (21–24). The rs1800467 polymorphism is a missense mutation (C-G substitution) located on chromosome 11 (codon 3), which appears to result in an L/V substitution that is not predicted to be deleterious (25). In particular, this SNP was significantly associated with T2D risk with high magnesium intake (≥ 313 mg/d) among HA women. Although there are as yet no functional links about this particular SNP, rs1800467 is in tight linkage disequilibrium ($D' = 0.913$) with rs5125, a known type 1 diabetes risk variant. Previously, rs5215 was linked to T2D risk among 924 diabetic cases and 2938 population controls in the Wellcome Trust Case Control Consortium study (28). Previous work has also associated genetic variants and micro-RNA involved in β cells with T2D development. A haplotype consisting of SNPs within *KCNJ11* and *ABCC8* genes (rs5210, rs5115, and rs5219), involved in pancreatic β -cell development and function, was reported to be significantly associated with T2D among 2025 North Indian subjects of Indo-European ancestry (27). A β -cell commotion of the micro-RNA network, which allowed for normal β -cell development, led to impairment of insulin secretion, impaired glucose homeostasis, and development of diabetes in mice (28).

Several rare mutations in *TRPM6* lead to autosomal recessive hypomagnesemia with secondary hypocalcemia, a severe magnesium-wasting disorder caused by impaired intestinal magnesium reabsorption through *TRPM6* channels. Oral magnesium intake can compensate for severe magnesium deficiency caused by this disorder through the non-*TRPM6*-mediated paracellular uptake pathway. Experimental evidence suggests this compensation may be because of dual pathways of intestinal magnesium absorption; a passive paracellular route active when intestinal magnesium concentrations are high and an active transport system when magnesium intake is low. In our sample of AA and HA women, however, we did not observe *TRPM6* SNPs to pose significant increased T2D risk among those with low magnesium intake as previously reported in white women (2). In contrast, SNPs in *CNNM1*, *SLC41A2*, and *MRS2*, genes that code for proteins that function in divalent cation and magnesium homeostasis, were significantly associated with T2D risk among AA and HA women. Genetic variants in *SLC41A2* and *MRS2* lead to changes in corresponding proteins that control Mg^{2+} influx. Previous studies have shown that increased Mg^{2+} efflux through dysregulation of the vascular exchange of

TABLE 1 Baseline characteristics of AA and HA women in the WHI-SHARe cohort by diabetes status¹

| | AA women | | | HA women | | <i>P</i> ² |
|--|----------------------------|-----------------------------|-----------------------|-------------------------|-----------------------------|-----------------------|
| | Cases (<i>n</i> = 1949) | Controls (<i>n</i> = 6161) | <i>P</i> ² | Cases (<i>n</i> = 611) | Controls (<i>n</i> = 2855) | |
| Total magnesium intake, ³ g/d | 0.243 ± 0.151 ⁴ | 0.254 ± 0.162 | 0.005 | 0.255 ± 0.154 | 0.276 ± 0.169 | 0.003 |
| Total energy intake, kcal/d | 1490 ± 962 | 1490 ± 881 | 0.96 | 1550 ± 964 | 1550 ± 932 | 0.99 |
| Age, y | 62.4 ± 6.80 | 61.4 ± 7.10 | <0.001 | 60.4 ± 6.70 | 60.2 ± 6.70 | 0.52 |
| BMI, kg/m ² | 33.1 ± 6.50 | 30.4 ± 6.20 | <0.001 | 31.5 ± 5.80 | 28.3 ± 5.40 | <0.001 |
| Smoking, % | | | 0.23 | | | 0.49 |
| Never | 46.7 | 48.8 | | 60.8 | 63.1 | |
| Past | 41.6 | 39.5 | | 31.6 | 30.2 | |
| Current | 11.7 | 11.7 | | 7.68 | 6.68 | |
| Alcohol consumption, % | | | <0.001 | | | <0.001 |
| Never | 18.0 | 15.5 | | 25.1 | 17.2 | |
| Past | 43.0 | 30.2 | | 32.6 | 19.1 | |
| Current | 39.1 | 54.3 | | 42.3 | 63.7 | |
| Physical activity, METs/wk | 7.97 ± 11.2 | 10.2 ± 13.1 | <0.001 | 8.52 ± 12.4 | 11.2 ± 14.0 | <0.001 |
| Family history of diabetes, % | 63.0 | 41.2 | <0.001 | 62.4 | 38.5 | <0.001 |

¹ *n* = 11,576. AA, African American; HA, Hispanic American; MET, metabolic equivalent; SHARe, SNP (single nucleotide polymorphism) Health Association Resource; WHI, Women's Health Initiative.

² Determined by χ^2 or *t* tests.

³ Includes dietary and supplemental magnesium.

⁴ Means ± SDs (all such values).

Na⁺ and Mg²⁺ and decreased Mg²⁺ influx because of defective vascular *TRPM6* and *TRPM7* expression may lead to blood pressure regulation. This alteration may also contribute to hypomagnesemia and intracellular Mg²⁺ deficiency in hypertension, diabetes, and cardiovascular diseases (29).

We also observed that polymorphisms in the genes related to magnesium transport, including *CLDN19*, *SLC41A1*, and *MMGT1*, lead to significantly increased T2D risk among AA and HA women with lower magnesium intake, which seems to support the notion that alteration in Mg²⁺ transport may lead to impaired insulin response and thus increase T2D risk. In low-magnesium media in the kidney cortex of mice maintained on low-magnesium diets, *MMGT1* mRNA levels do increase, leading to regulated pathways for Mg²⁺ transport in the Golgi and post-Golgi organelles of epithelium-derived cells (30). Among women with higher magnesium intake, some carriers

of variants in *NIPA1* and *NIPA2* appear to have a lower T2D risk, which is consistent with a previous observation that links high magnesium to decreased cell surface of *NIPA1* (31). Future work to confirm our observations in large multiethnic cohorts or consortia is warranted.

Measurement of magnesium intake estimated by the FFQ is known to work best only to provide a relative ranking of long-term average intake among participants in well-characterized populations. Moreover, magnesium intake assessed by our FFQ has a correlation of 0.7 when validated against dietary records (32, 33). Thus, the magnitude of relation may be underestimated in our study, given that this error is not likely to differ across specific genotypes or the T2D outcome of interest. Some may speculate that magnesium intake may not necessarily reflect the amount of magnesium available to the body; therefore, magnesium concentration may provide further information to investigate

TABLE 2 Top 10 associations (ranked by nominal *P* values among AA women) between ion channel-related genes and diabetes risk in the WHI-SHARe cohort¹

| Locus | SNP | Major/minor allele | Minor allele frequency (AA/HA) | | AA women (<i>n</i> = 7287) | | HA women (<i>n</i> = 3285) | |
|---------------|-----------|--------------------|--------------------------------|-----------|-----------------------------|-----------------------------------|-----------------------------|----------|
| | | | Cases | Controls | OR (95% CI) | <i>P</i> (FDR-adjusted <i>P</i>) | OR (95% CI) | <i>P</i> |
| <i>CNNM1</i> | rs6584273 | G/A | 0.20/0.04 | 0.22/0.05 | 0.84 (0.76, 0.92) | 0.0005 (0.02)* | 1.05 (0.76, 1.44) | 0.76 |
| <i>TRPM6</i> | rs577787 | T/C | 0.30/0.09 | 0.27/0.08 | 1.15 (1.10, 1.25) | 0.002 (0.09) | 1.14 (0.91, 1.44) | 0.26 |
| <i>ABCC8</i> | rs7947462 | G/A | 0.07/0.23 | 0.09/0.24 | 0.79 (0.68, 0.92) | 0.002 (0.14) | 0.92 (0.79, 1.08) | 0.29 |
| <i>TRPM6</i> | rs546862 | T/C | 0.30/0.09 | 0.27/0.08 | 1.14 (1.00, 1.25) | 0.003 (0.09) | 1.17 (0.93, 1.47) | 0.19 |
| <i>CLDN16</i> | rs4438632 | G/A | 0.04/0.25 | 0.03/0.22 | 1.38 (1.10, 1.70) | 0.003 (0.11) | 1.06 (0.90, 1.24) | 0.48 |
| <i>TRPM6</i> | rs7046143 | T/C | 0.30/0.24 | 0.27/0.22 | 1.13 (1.00, 1.24) | 0.004 (0.10) | 1.05 (0.90, 1.23) | 0.53 |
| <i>TRPM7</i> | rs2011064 | C/G | 0.19/0.46 | 0.17/0.45 | 1.16 (1.00, 1.28) | 0.005 (0.09) | 1.02 (0.89, 1.16) | 0.80 |
| <i>CLDN16</i> | rs1593313 | T/C | 0.06/0.32 | 0.05/0.29 | 1.28 (1.10, 1.53) | 0.006 (0.12) | 1.01 (0.87, 1.18) | 0.87 |
| <i>TRPM7</i> | rs3109894 | C/T | 0.30/0.47 | 0.28/0.46 | 1.13 (1.00, 1.23) | 0.007 (0.09) | 1.00 (0.88, 1.15) | 0.95 |
| <i>TRPM7</i> | rs1395297 | T/C | 0.30/0.47 | 0.28/0.46 | 1.12 (1.00, 1.22) | 0.008 (0.09) | 0.99 (0.87, 1.14) | 0.95 |

¹ *n* = 10,572. ORs and 95% CIs were computed by using additive model with multivariate logistic regression models and were adjusted for age, region, and 3 principal components of global ancestry. *FDR-adjusted *P* ≤ 0.05. AA, African American; FDA, false discovery rate; HA, Hispanic American; SHARe, SNP Health Association Resource; SNP, single nucleotide polymorphism; WHI, Women's Health Initiative.

TABLE 3 Top 10 associations (ranked by nominal *P* values among HA women) between ion channel-related genes and diabetes risk in the WHI-SHARe cohort¹

| Locus | SNP | Major/minor allele | Minor allele frequency (AA/HA) | | AA women (<i>n</i> = 7287) | | HA women (<i>n</i> = 3285) | |
|----------------|------------|--------------------|--------------------------------|------------|-----------------------------|----------|-----------------------------|-----------------------------------|
| | | | Cases | Controls | OR (95% CI) | <i>P</i> | OR (95% CI) | <i>P</i> (FDR-adjusted <i>P</i>) |
| <i>SLC41A2</i> | rs10861279 | T/G | 0.08/0.03 | 0.07/0.07 | 1.11 (0.96, 1.28) | 0.17 | 0.54 (0.38, 0.77) | 0.0006 (0.04)* |
| <i>MRS2</i> | rs7738943 | G/C | 0.13/0.08 | 0.12/0.06 | 1.02 (0.90, 1.14) | 0.79 | 1.55 (1.20, 2.00) | 0.0006 (0.01)* |
| <i>NIPA1</i> | rs7174119 | G/A | 0.22/0.30 | 0.22/0.27 | 1.00 (0.91, 1.10) | 1.00 | 1.27 (1.10, 1.47) | 0.002 (0.04)* |
| <i>MRS2</i> | rs1056285 | G/A | 0.14/0.08 | 0.14/0.06 | 1.02 (0.91, 1.14) | 0.74 | 1.48 (1.20, 1.90) | 0.002 (0.02)* |
| <i>FXSD2</i> | rs479991 | T/C | 0.27/0.19 | 0.28/0.23 | 0.99 (0.91, 1.08) | 0.84 | 0.79(0.67, 0.93) | 0.005 (0.07) |
| <i>NIPA1</i> | rs6606830 | C/T | 0.21/0.29 | 0.22/0.27 | 1.00 (0.91, 1.10) | 0.94 | 1.23 (1.10, 1.43) | 0.01 (0.07) |
| <i>SLC41A2</i> | rs11112191 | T/C | 0.27/0.17 | 0.27/0.20 | 1.00 (0.91, 1.09) | 0.96 | 0.78 (0.66, 0.93) | 0.01 (0.15) |
| <i>KCNJ11</i> | rs1800467 | G/C | 0.01/0.06 | 0.009/0.04 | 1.01 (0.65, 1.55) | 0.98 | 1.49 (1.10, 2.01) | 0.01 (0.11) |
| <i>SLC12A3</i> | rs8061631 | C/T | 0.38/0.22 | 0.37/0.25 | 1.02 (0.94, 1.11) | 0.62 | 0.81 (0.69, 0.95) | 0.01 (0.32) |
| <i>SLC41A2</i> | rs11112211 | G/C | 0.09/0.05 | 0.08/0.07 | 1.05 (0.92, 1.21) | 0.47 | 0.67 (0.49, 0.92) | 0.01 (0.15) |

¹ *n* = 10,572. ORs and 95% CIs were computed by using additive model with multivariate logistic regression models and were adjusted for age, region, and 3 principal components of global ancestry. *FDR-adjusted *P* ≤ 0.05. AA, African American; FDA, false discovery rate; HA, Hispanic American; SHARe, SNP Health Association Resource; SNP, single nucleotide polymorphism WHI, Women's Health Initiative.

whether magnesium differs according to genetic variants for a given magnesium intake. In the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, genomic regions that included *TRPM6* and *CNNM2*, *CNNM3*, and *CNNM4* were associated with magnesium concentrations (34).

The top polymorphisms appeared to be involved in magnesium transport and homeostasis; it is speculated that they may also modify individual risk for diabetes despite a difference in magnesium intake (35). However, the literature lacks a report of difference in magnesium concentrations according to susceptible

FIGURE 1 (A) Sliding window analysis for *SLC41A2* haplotype-T2D associations among 3285 Hispanic American WHI women. Window size is 2 SNPs. Sliding window haplotype-based analyses (12) (window width = 2) were used to examine the joint contribution of 2 SNP windows on T2D risk. For each window, a Wald test on the basis of *t* distribution was used. The test performed N-1 haplotype-specific tests for N haplotypes (of each vs. all others), depending on the number of SNPs in each gene. The dashed line represents a $-\log_{10}P$ of 3.08 (corresponding to *P* = 0.001), which was used as the global significance threshold by Bonferroni's correction for 61 window frames. (B) Sliding window analysis for *MRS2* haplotype-T2D associations among 3285 Hispanic American WHI women. Window size is 2 SNPs. Sliding window haplotype-based analyses (12) (window width = 2) were used to examine the joint contribution of 2 SNP windows on T2D risk. For each window, a Wald test on the basis of *t* distribution was used. The test performed N-1 haplotype-specific tests for N haplotypes (of each vs. all others), depending on the number of SNPs in each gene. The dashed line represents a $-\log_{10}P$ of 2.64 (corresponding to *P* = 0.002), which was used as the global significance threshold by Bonferroni's correction for 22 window frames. *MRS2*, mitochondrial RNA splicing 2 gene; *SLC41A2*, solute carrier family 41 (anion exchanger), member 2 gene; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; WHI, Women's Health Initiative.

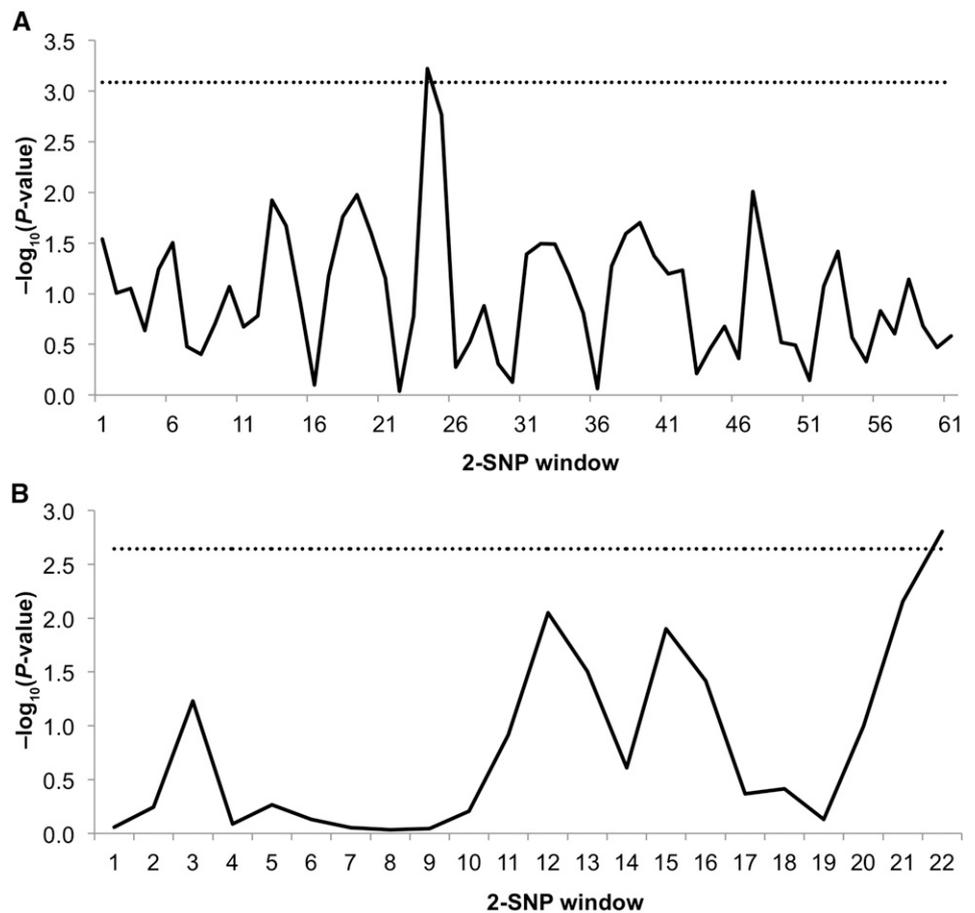


TABLE 4 Significant associations (FDR $q \leq 0.05$) between ion channel-related genes and diabetes risk stratified by total magnesium intake in the WHI-SHARe cohort¹

| Locus | SNP | AA women | | | | HA women | | | |
|----------------|------------|--|----------|---|-----------------------------------|--|--------------------------|--|-----------------------------------|
| | | Low intake (≤ 0.164 g/d; $n = 2154$) | | High intake (≥ 0.291 g/d; $n = 2120$) | | Low intake (≤ 0.185 g/d; $n = 1015$) | | High intake (≥ 0.313 g/d; $n = 954$) | |
| | | OR (95% CI) | <i>P</i> | OR (95% CI) | <i>P</i> (FDR-adjusted <i>P</i>) | OR (95% CI) | <i>P</i> (FDR <i>q</i>) | OR (95% CI) | <i>P</i> (FDR-adjusted <i>P</i>) |
| <i>KCNJ11</i> | rs1800467 | 0.97 (0.46, 2.04) | 0.93 | 1.13 (0.55, 2.32) | 0.74 | 1.11 (0.66, 1.86) | 0.70 | 2.50 (1.50, 4.28) | <0.001 (0.01)* |
| <i>CLDN19</i> | rs11590362 | 1.24 (0.79, 1.93) | 0.34 | 1.23 (0.80, 1.88) | 0.34 | 2.01 (1.20, 3.37) | 0.01 (0.04)** | 1.38 (0.79, 2.41) | 0.26 |
| <i>SLC41A1</i> | rs823154 | 1.06 (0.9, 1.26) | 0.47 | 1.02 (0.85, 1.23) | 0.84 | 1.46 (1.10, 1.86) | 0.002 (0.04)** | 1.01 (0.75, 1.37) | 0.93 |
| <i>CNNM1</i> | rs6584273 | 0.88 (0.74, 1.06) | 0.18 | 0.71 (0.59, 0.87) | <0.001 (0.04)** | 0.93 (0.52, 1.66) | 0.80 | 0.88 (0.46, 1.71) | 0.71 |
| <i>NIPA2</i> | rs7170784 | 1.05 (0.88, 1.26) | 0.58 | 0.94 (0.77, 1.13) | 0.50 | 1.07 (0.86, 1.34) | 0.55 | 0.62 (0.47, 0.80) | <0.001 (0.01)** |
| <i>NIPA2</i> | rs8028189 | 1.05 (0.88, 1.26) | 0.60 | 0.93 (0.77, 1.13) | 0.46 | 1.08 (0.87, 1.35) | 0.48 | 0.65 (0.50, 0.84) | 0.001 (0.01)* |
| <i>MMGT1</i> | rs5929706 | 0.89 (0.60, 1.32) | 0.57 | 0.73 (0.47, 1.14) | 0.17 | 4.66 (1.50, 14.3) | 0.01 (0.05)** | 1.42 (0.29, 6.90) | 0.67 |
| <i>MMGT1</i> | rs5930817 | 1.09 (0.83, 1.43) | 0.54 | 0.90 (0.66, 1.23) | 0.52 | 3.10 (1.40, 6.85) | 0.01 (0.05)** | 0.88 (0.19, 4.03) | 0.87 |

¹ ORs and 95% CIs were computed by using additive model with multivariate logistic regression models and were adjusted for age, region, and 3 principal components of global ancestry. *FDR-adjusted $P \leq 0.01$; **FDR-adjusted $P \leq 0.05$. AA, African American; FDA, false discovery rate; HA, Hispanic American; SHARe, SNP Health Association Resource; SNP, single nucleotide polymorphism WHI, Women's Health Initiative.

genetic variants for a given magnesium intake. Until now, because of feasibility difficulties (assay characteristics, specimen requirement, and costs), no data on magnesium concentrations are available in the WHI cohort. Although blood concentrations of total magnesium are commonly used for defining magnesium deficiency, they are usually within a narrow range under tight homeostatic regulation in the human body and cannot reliably reflect both inter- and intra-individual magnesium status. Thus, there is an urgent need to develop accurate, reliable, and affordable biomarker measures to assess individual magnesium status in population studies, which would provide more informative answers about magnesium status in future studies. Further, previous studies showed that magnesium plays an important role in the synthesis and metabolism of vitamin D, which receptors exist on pancreatic β cells and insulin-sensitive tissues, and which repletion improves glucose and insulin homeostasis in animal models of vitamin D deficiency (36, 37). Given the biological interconnection between magnesium and vitamin D, disentangling the independent and potentially interactive effects of these factors is of critical importance, and further research may provide insights into the mechanism of the development of diabetes through ion channel pathways. Strengths of this study include a well-characterized population of >10,000 minority women with complete genome scans. Although false positives remain a possibility, the candidate gene approach with careful adjustment for multiple comparisons and strong a priori biological information will continue to shed light on the genetic susceptibility of complex diseases. Further, as a complement to single-marker analysis (12), we performed sliding window haplotype analyses which suggested that HA women who were carriers of the haplotypes *SLC41A2* rs12582312-rs10861279 and *MRS2* rs1056285-rs7738943 were associated with T2D risk.

In conclusion, findings from this national cohort of AA and HA postmenopausal women indicate that genetic variations in several magnesium-related ion channels may affect T2D risk with possible effect modifications by magnesium intake and ethnicity. In particular, among HA women with low magnesium intake, *CLDN19*, *SLC41A1*, and *MMGT1* were associated with increased risk of T2D. Among HA women with high magnesium intake, the *KCNJ11* gene was associated with an increased T2D risk and *NIPA2* was associated with a decreased T2D risk. Among AA women with high magnesium intake, the *CNNM1*

gene was found to have a protective association with T2D. Further confirmation of our observations in WHI-SHARe is clearly warranted in future mechanistic studies.

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