The C Allele of *ATM* rs11212617 Does Not Associate With Metformin Response in the Diabetes Prevention Program

Jose C. Florez, md, phd[1,2,3]
Kathleen A. Jablonski, phd[4]
Andrew Taylor, ba[1,2]
Kieren Mather, md[5]
Edward Horton, md[3,6]
Neil H. White, md[7]
Elizabeth Barrett-Connor, md[8]
William C. Knowler, md, drph[9]
Alan R. Shuldiner, md[10]
Toni I. Pollin, phd[10]
for the Diabetes Prevention Program Research Group*

OBJECTIVE—The C allele at the rs11212617 polymorphism in the ataxia-telangiectasia–mutated (*ATM*) gene has been associated with greater clinical response to metformin in people with type 2 diabetes. We tested whether this variant modified the effect of metformin in the Diabetes Prevention Program (DPP), in which metformin reduced diabetes incidence by 31% in volunteers with impaired glucose tolerance.

RESEARCH DESIGN AND METHODS—We genotyped rs11212617 in 2,994 DPP participants and analyzed its effects on diabetes incidence and related traits.

RESULTS—Contrary to expectations, C carriers enjoyed no preventive advantage on metformin; their hazard ratio, compared with A carriers, was 1.17 ([95% CI 0.96–1.42], $P = 0.13$) under metformin. There were no significant differences by genotype in metformin's effects on insulin sensitivity, fasting glucose, glycated hemoglobin, or disposition index.

CONCLUSIONS—The reported association of rs11212617 with metformin response was not confirmed for diabetes prevention or for effects on relevant physiologic parameters in the DPP.

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Metformin is an effective, cheap, and safe drug used as the first-line agent for treating type 2 diabetes (T2D) (1–3). Nevertheless, hyperglycemia eventually progresses in many patients, causing escalation of therapy (4). The reasons for such failure, which takes some time to become apparent, are unknown. Genetic factors may contribute to this process.

Recently, a genome-wide association study (GWAS) for metformin response has been published (5). The Genetics of Diabetes Audit and Research Tayside (GoDARTS) investigators analyzed 705,125 single nucleotide polymorphisms (SNPs) in 1,024 individuals who had a definable metformin response in a retrospective clinical database. Treatment success (analyzed as a categorical trait) was declared if glycated hemoglobin (A1C) became ≤7% within 18 months of starting therapy; change in A1C was also analyzed as a quantitative trait. Fourteen SNPs, concentrated around the ataxia-telangiectasia–mutated (*ATM*) gene, were associated with categorical metformin response at a suggestive level of $P < 10^{-6}$. Consistent results were obtained for metformin response as a quantitative trait. The top SNP (rs11212617) was genotyped in 1,783 additional GoDARTS participants and 1,113 participants in the UK Prospective Diabetes Study (UKPDS) clinical trial (6). Again, in both cohorts, its minor C allele (frequency 44%) was nominally associated with greater metformin response, either as a categorical or quantitative trait. Joint analysis exceeded conventional genome-wide statistical significance ($P = 2.9 \times 10^{-9}$), i.e., this *P* value withstands correction for multiple comparisons. We therefore tested this SNP for metformin response in the Diabetes Prevention Program (DPP), a clinical trial with participants from five U.S. ethnic groups who were at high risk of T2D and were treated with an intensive lifestyle intervention or metformin for diabetes prevention.

RESEARCH DESIGN AND METHODS

Participants

The DPP enrolled 3,234 overweight or obese, nondiabetic people with impaired glucose tolerance. They were randomized to placebo, metformin (850 mg twice daily), or a lifestyle intervention. The participants' mean age was 51 years and mean BMI was 34.0 kg/m^2, 68% were women, and 45% belonged to U.S. ethnic minority groups; their demographic characteristics are shown in Table 1. The lifestyle and metformin interventions reduced the incidence of diabetes by 58 and 31%, respectively, versus placebo (7). In total, 2,994 participants (988 on metformin) consented to genetic investigation. All procedures were approved by institutional review boards at the 27 study sites.

From the [1]Center for Human Genetic Research and Diabetes Research Center, Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts; the [2]Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts; the [3]Department of Medicine, Harvard Medical School, Boston, Massachusetts; the [4]Department of Epidemiology and Biostatistics, The Biostatistics Center, George Washington University, Rockville, Maryland; the [5]Department of Medicine, Division of Endocrinology and Metabolism, Indiana University School of Medicine, Indianapolis, Indiana; the [6]Section on Clinical, Behavioral & Outcomes Research, Joslin Diabetes Center, Boston, Massachusetts; the [7]Departments of Pediatrics and Medicine, Washington University School of Medicine in St. Louis, St. Louis, Missouri; the [8]Department of Family Medicine, University of California, San Diego, California; the [9]Diabetes Epidemiology and Clinical Research Section, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, Arizona; and the [10]Department of Medicine, Division of Endocrinology, Diabetes, and Nutrition, and Program in Genetics and Genomic Medicine, University of Maryland School of Medicine, Baltimore, Maryland.
Corresponding author: Jose C. Florez, jcflorez@partners.org.
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*A complete list of the Diabetes Prevention Program Research Group investigators is provided in the Supplementary Data online.

Table 1—*Demographic characteristics of the DPP cohort by treatment arm and genotype at rs11212617*

	Placebo	Metformin	Lifestyle	*P* value
n	997	988	999	
rs11212617 genotype, *n* (%)				
AA	281 (28)	289 (29)	274 (27)	0.16
AC	489 (49)	442 (45)	455 (46)	
CC	227 (23)	257 (26)	270 (27)	
Male sex, *n* (%)	308 (31)	343 (35)	325 (33)	0.19
Age, years (mean ± SD)	50 ± 10	51 ± 10	51 ± 11	0.58
Waist circumference, cm (mean ± SD)	105 ± 14	105 ± 15	105 ± 15	0.84
Weight, kg (mean ± SD)	94.8 ± 20.2	94.6 ± 19.9	94.5 ± 20.8	0.95
Self-reported ethnicity, *n* (%)				
White	555 (56)	568 (57)	546 (55)	0.31
African American	210 (21)	206 (21)	193 (19)	
Hispanic	163 (16)	158 (16)	176 (18)	
Asian	39 (4)	33 (3)	55 (6)	
American Indian	30 (3)	23 (2)	29 (3)	

P values are based on F tests for continuous variables and χ^2 tests for categorical variables.

Genotyping

We genotyped rs11212617 on a Sequenom iPLEX platform as previously described (8,9). Genotyping success was 99.9%.

Measurements

Diabetes incidence was determined by semiannual measurements of fasting glucose and an annual oral glucose tolerance test. The principal study outcome was the development of diabetes by American Diabetes Association criteria, including confirmation. Besides diabetes incidence as a categorical outcome, we selected the insulin sensitivity index (ISI), fasting glucose, A1C, weight, and oral disposition index (DIo) as indices of metformin response. We calculated the ISI as 22.5/([fasting insulin × fasting glucose]/18.01) (or 1/homeostasis model assessment of insulin resistance) (10), and the DIo as 1/fasting insulin × insulinogenic index (Δinsulin/Δglucose over the first 30 min of the oral glucose tolerance test) (11). Quantitative traits were natural log–transformed.

Statistical analysis

We tested the additive effect of genotype at rs11212617 on diabetes incidence by Cox proportional hazards regression models with genotype and intervention and their interactions as the independent variables predicting time to diabetes over mean 3.2 years follow-up, adjusted for sex, ethnicity, treatment arm, baseline age, and waist circumference. We included all three treatment arms and an interaction test to simultaneously rule out a main effect of this variant on diabetes incidence independent of metformin, or under the action of a lifestyle intervention. We used generalized mixed models to test additive effect of genotype on baseline log-transformed traits and, to model change under metformin action, on the same traits after 1 year of intervention adjusted for the baseline value of the respective trait, age, sex, ethnicity, treatment arm, and waist circumference. Post hoc power calculations (which should be interpreted with caution) show that the sample size in the DPP metformin arm has >99% power to detect the change in A1C of 0.61% that was reported in the UKPDS (5). To control for the potential effect of ethnicity, we performed sensitivity analyses in the largest race/ethnic group (white participants), which is most closely related to the populations examined in the original report and whom we have previously shown to be essentially free of non-European admixture (12).

RESULTS—The frequency of the C (metformin-responsive) allele was 42.4, 72.4, 40.1, 51.6, and 41.5% in 1,669 white, 609 African American, 497 Hispanic, 127 Asian/Pacific Islander, and 82 American Indian participants, respectively (Table 1). We found no association of genotype with diabetes incidence in all arms combined, either in unadjusted analyses (hazard ratio [HR] per copy of the C allele 0.98 [95% CI 0.88–1.10], $P = 0.76$) or after adjusting for age, sex, ethnicity, and treatment arm (HR per

Table 2—*Diabetes incidence in the DPP by genotype at rs11212617, treatment arm, and self-reported ethnicity*

		Genotype at rs11212617: No. of cases of diabetes (cases/100 person-years)			HR for C vs. A	
	Total participants (*n*)	AA	AC	CC	(95% CI)	*P* value
Overall cohort	2,984	186 (8.1)	293 (7.8)	157 (7.7)	0.94 (0.84–1.05)	0.25
By treatment arm						
Placebo	997	87 (12.2)	131 (10.2)	64 (10.7)	0.84 (0.70–1.01)	0.71
Metformin	988	56 (6.9)	90 (7.6)	62 (9.1)	1.17 (0.96–1.42)	0.13
Lifestyle	999	43 (5.5)	72 (5.6)	31 (4.1)	0.84 (0.66–1.07)	0.16
By self-reported ethnicity						
White	1,669	116 (7.6)	182 (8.2)	51 (6.2)	0.91 (0.78–1.07)	0.24
African American	609	<15 (9.1)	56 (8.8)	74 (8.7)	1.03 (0.78–1.35)	0.85
Hispanic	497	45 (9.3)	38 (6.0)	18 (7.8)	0.83 (0.62–1.12)	0.22
Asian	127	<15 (9.0)	<15 (7.1)	<15 (10.0)	1.59 (0.88–2.89)	0.13
American Indian	82	<15 (6.6)	<15 (4.9)	<15 (12.0)	1.68 (0.80–3.69)	0.20

Cox proportional hazards results are reported in additive models adjusted by sex, age at randomization, treatment group, and waist circumference. In accordance with DPP privacy policies, cells with <15 individuals do not report the exact number of participants.

Table 3—*Association of rs11212617 with quantitative glycemic traits at 1 year*

	SNP effect per C allele by treatment group						SNP × treatment interaction *P* value	
	Placebo		Metformin		Lifestyle			
Trait	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	MET	ILS
ISI	+0.028 ± 0.024	0.24	−0.019 ± 0.022	0.39	+0.001 ± 0.025	0.97	0.25	0.19
Fasting glucose	−0.006 ± 0.005	0.19	+0.003 ± 0.004	0.43	+0.002 ± 0.004	0.65	0.62	0.31
A1C	−0.003 ± 0.003	0.22	+0.002 ± 0.002	0.32	−0.001 ± 0.002	0.75	0.37	0.77
Weight	−0.004 ± 0.003	0.16	+0.005 ± 0.002	0.04	0.000 ± 0.004	0.92	0.07	0.05
DIo	+0.065 ± 0.032	0.04	−0.025 ± 0.031	0.43	+0.004 ± 0.033	0.90	0.27	0.28

Genotype at rs11212617 has no detectable effect on change in quantitative traits relative to metformin response after 1 year of metformin treatment. The β-coefficients and SEs are shown for the C allele under additive genetic models for the natural log of the given trait adjusted for the natural log of the baseline value of the trait, age, sex, ethnicity, treatment arm, and waist circumference. *P* values are shown for main SNP effects in each of the treatment arms, as well as for the SNP × intervention interaction terms. MET, metformin; ILS, intensive lifestyle.

copy of the C allele 0.95 [0.85–1.07], *P* = 0.42); further adjustment for waist circumference produced indistinguishable results (Table 2). There was no significant genotype × metformin interaction in the unadjusted model. Though there was a nominal SNP × metformin interaction in the fully adjusted model (*P* = 0.04), the observed trend was in the opposite direction from the expected prevention effect; the C allele conferred no detectable advantage on metformin recipients in diabetes prevention but was associated with a nonsignificant trend toward increased risk of diabetes (HR per copy of the C allele 1.17 [0.96–1.42], *P* = 0.13). We found no significant associations of genotype with relevant quantitative glycemic traits at baseline; similarly, there were no significant differences across genotype groups in change in ISI, fasting glucose, A1C, or DIo after 1 year of metformin (Table 3). The C allele was associated with greater weight gain in the metformin arm. In this arm, there were no statistically significant interactions between the C allele and BMI or waist circumference on diabetes incidence. Analyses stratified by ethnic group failed to show any ethnic-specific beneficial effects of the C allele with regard to diabetes incidence on metformin-treated participants (Table 4).

CONCLUSIONS—In the DPP, the effect of metformin to prevent diabetes or improve relevant glycemic traits was not magnified among carriers of the C allele at rs11212617 in the *ATM* gene. Our findings do not support the previously reported association of this allele with improved metformin action on glycemic control. The original association was consistent in three different datasets (the discovery sample and two follow-up cohorts) and has been reported recently in other clinical cohorts similarly ascertained (13). Inconsistent results in the DPP could be due to multiple reasons. First, metformin response is defined differently in a prediabetic cohort (impact on diabetes incidence or quantitative glycemic traits) than it is in a disease cohort (ability to reach A1C ≤7% under treatment). Second, metformin may be more effective in individuals with a higher A1C at baseline, and thereby the effects of genotype on response might be easier to detect in the disease setting. Third, the reported effect might be confined to populations of European descent, e.g., if rs11212617 tags a low-frequency variant unique to white populations, further diminishing statistical power in the DPP multiethnic cohort. And fourth, the previously reported GWAS was based on a retrospective evaluation of clinical records, where potential confounders (e.g., if genotype were to influence comorbidities that affect patient adherence, continuity of care, or frequency of A1C measurements) are harder to control than in a clinical trial.

To address potential ethnic differences in the genomic architecture of this region that might explain our negative results, we examined the haplotype structure around this locus in the HapMap European (CEU) and West African (YRI) datasets. The full *ATM* gene and the rs11212617 variant share a segment of tight linkage disequilibrium in both the CEU and YRI populations; there is a recombination hot spot downstream from rs11212617, beyond which SNPs display equally low correlations with rs11212617 in CEU and YRI, indicating that major differences in linkage disequilibrium patterns would be unlikely to account for potentially discrepant findings in Europeans and Africans. Furthermore, the region distal to this hot spot was well captured by

Table 4—*Ethnic-specific effects of rs11212617 on diabetes incidence in the DPP*

	Placebo		Metformin		Lifestyle	
Ethnic group	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
White	0.97 (0.76–1.25)	0.83	0.94 (0.72–1.22)	0.61	0.76 (0.56–1.04)	0.09
African American	0.69 (0.47–1.01)	0.05	1.43 (0.85–2.39)	0.17	1.74 (0.85–3.57)	0.13
Hispanic	0.50 (0.31–0.81)	0.005	1.79 (1.10–2.89)	0.02	0.63 (0.32–1.23)	0.18
Asian	3.58 (1.11–11.56)	0.03	1.72 (0.54–5.51)	0.36	1.05 (0.36–3.09)	0.93
American Indian	2.45 (0.74–8.06)	0.14	2.77 (0.42–18.37)	0.29	0.79 (0.14–4.52)	0.79

Per-allele HR and 95% CI of the metformin-responsive C allele vs. the A allele at rs11212617 in the DPP, analyzed under an additive genetic model, adjusted for age, sex, and waist circumference, and stratified by treatment arm and self-reported ethnic group. In metformin-treated participants, the C allele confers no diabetes-protective advantage in any ethnic group; indeed, there is a suggestion of increased risk in Hispanics, although the wide and overlapping 95% CI precludes us from making any meaningful ethnic comparisons.

the original GWAS array, suggesting that a true signal emerging from this region (and which might have explained a stronger association in Europeans than in Africans) would have also been detected by the original GWAS.

Nevertheless, this previously reported association merits additional follow-up in independent cohorts. More generally, a better-powered genome-wide assessment of pharmacogenetic responses in T2D is needed; whether genetic information will prove useful in diabetes prevention or therapeutics must be tested in prospective clinical trials.

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