*Journal of the Endocrine Society*, 2022, **6**, 1–9
https://doi.org/10.1210/jendso/bvac092
Advance access publication 29 June 2022
**Research Article**

# Variants of *SLC2A10* may be Linked to Poor Response to Metformin

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## Abstract

**Purpose:** A study among Filipinos revealed that only 15% of patients with diabetes achieved glycemic control, and poor response to metformin could be one of the possible reasons. Recent studies demonstrate how genetic variations influence response to metformin. Hence, the present study aimed to determine genetic variants associated with poor response to metformin.

**Methods:** Using a candidate variant approach, 195 adult Filipino participants with newly diagnosed type 2 diabetes mellitus (T2DM) were enrolled in a case-control study. Genomic DNA from blood samples were collected. Allelic and genotypic associations of variants with poor response to metformin were determined using exact statistical methods.

**Results:** Several polymorphisms were nominally associated with poor response to metformin ($P_{uncorr} < 0.05$). The most notable is the association of multiple variants in the *SLC2A10* gene—rs2425911, rs3092412, and rs2425904—with common additive genetic mode of inheritance. Other variants that have possible associations with poor drug response include rs340874 (*PROX-AS1*), rs815815 (*CALM2*), rs1333049 (*CDKN2B-AS1*), rs2010963 (*VEGFA*), rs1535435 and rs9494266 (*AHI1*), rs11128347 (*PDZRN3*), rs1805081 (*NPC1*), and rs13266634 (*SLC30A8*).

**Conclusion:** In Filipinos, a trend for the association for several variants was noted, with further observation that several mechanisms may be involved. The results may serve as pilot data for further validation of candidate variants for T2DM pharmacotherapy.

**Key Words:** metformin response, *SLC2A10*, pharmacogenetics

Metformin is one of the most prescribed drugs for type 2 diabetes mellitus (T2DM) in the Philippines, as inexpensive generic equivalents of metformin are readily accessible and affordable to patients. In a 2008 survey on glycemic control, approximately 73.8% of patients with diabetes were on metformin medication [1]. Although there are no local studies on glycemic control on metformin alone, variations in response have been observed in different populations, with about 35% of the patients failing to achieve glycemic control and other patients becoming less responsive over time [2]. While the mechanisms behind poor response to metformin are not fully understood, it is hypothesized that genetic factors contribute to this. Studies show that some variants affect genes coding for receptors, transporters, and enzymes important in drug absorption, metabolism, distribution, and excretion of oral hypoglycemic agents [3]. Thus, it is important to identify the variants that may affect an individual's poor response to metformin.

Studies on poor response to metformin suggest that genetic variants are mainly involved with transport proteins, specifically the *SLC* (solute-carrier) gene family. Polymorphisms in different SLC genes (*SLC22A2*, *SLC22A3*, and *SLC2A2*)

**Received:** 8 December 2021. **Editorial Decision:** 9 June 2022. **Corrected and Typeset:** 13 July 2022

were found to be associated with poor metformin response among African-American and Caucasian ancestries [4]. OCT1 is one of the most studied transporter proteins related to metformin action, and some variants of the gene encoding this transporter, *SLC22A1* (solute carrier family 22 member 1), are associated with metformin response [5]. In addition, 2 variants of *SLC22A1* are associated with metformin efficacy in the Japanese populations: -43T > G (rs4646272) is a negative predictor, while 1222A > G (rs628031) is a positive predictor for metformin efficacy. The study also indicated a trend toward reduced expression of *SLC22A1* among those who have the A allele of rs620831 [6]. A genome-wide association study (GWAS) study conducted among Europeans, GoDARTS (Genetics of DARTS—Diabetes Audit and Search in Tayside, Scotland), showed that the ataxia telangiectasia mutated (*ATM*) gene, which is responsible for encoding serine/threonine kinase, may also be involved in the regulation of the enzymes responsible for metformin response [2].

Despite availability of numerous studies on the possible mechanisms of metformin poor response, the results from those studies do not reflect the genetic variations in Filipino population since the sample populations were mostly of Caucasians, African-American, and European ancestries. In the Philippines, such pharmacogenetics studies on oral hypoglycemic agents are scarce; thus, the current study looked at the association of some known genetic variants with poor response to metformin. This knowledge is important for the creation of gene-based technologies for personalized medicine, which aims to achieve therapeutic targets and to reduce occurrence of adverse reactions. Lastly, this effort may also justify the integration of genetics in clinical guidelines for prescribing metformin to patients with T2DM.

## Materials and Methods

### Study Design and Enrollment of Participants

Volunteer participants were enrolled from March 2014 to January 2019 from the Philippine General Hospital in Manila, Corazon Locsin Montelibano Memorial Regional Hospital in Bacolod City, Southern Philippines Medical Center in Davao City, and other government hospitals, health centers, and private clinics in metro Manila and nearby provinces. All procedures were implemented in compliance with the University of Philippines Manila's Research Ethics Board (study protocol code: UPMREB-2012-0187-NIH).

Enrollment of cases and controls was completed following the inclusion and exclusion criteria. Participants who satisfied the following conditions were included in the study: aged ≥18, diagnosed with T2DM within the past 3 years with fasting blood sugar (FBS) of 126 to 255mg/dL or hemoglobin A1c (HbA1c) of 6.5% to 10.5%, and drug-naïve or started on the lowest dose of metformin or combination but with a drug-free period of at least 4 weeks. On the contrary, participants were excluded due to the following conditions: previously diagnosed as type 1 diabetes mellitus; currently pregnant or lactating; with active cancer or had cancer but disease-free for <5 years; diagnosed with congestive heart failure NYHA Functional Class III or IV; diagnosed with chronic kidney disease stage ≥3 or with an estimated glomerular filtration rate < 30 mL/min/1.73 $m^2$; and with active liver disease, which is defined as serum levels of either alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase >3× the upper limit of normal values. In addition, this study also excluded participants who had used systemic steroids within the past 3 months, with active drug or alcohol abuse within the past 3 months, or with previous use of maintenance insulin.

Prior to interview and sample collection, informed consent was obtained from the participants. A standard case report form was used to collect information on demographic data, medical history, and clinical characteristics of the participants. FBS, HbA1c, fasting serum insulin, C-peptide, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and creatine results were obtained and recorded.

Metformin response was measured based on changes in their HbA1c after 3 months of metformin therapy. Participants were started in metformin following the study's treatment algorithm, under the discretion of the attending physician based on standard of care. Pills were counted on the day of follow-up for that month to check for medication adherence, which is computed as follows: number of packets consumed/number of packets prescribed multiplied by 100%. Responders were those whose HbA1c levels changed by ≥0.5% (absolute value difference) from baseline after 3 months of treatment, while poor responders were those whose HbA1c changed <0.5% from baseline after 3 months of treatment [6-8].

## DNA Extraction and Quantification

DNA extraction was performed using QIAamp DNA Blood Mini Kit (QIAGEN, Victoria, Australia) following the spin protocol for blood buffy coat specified in the manufacturer's instruction manual. The eluted DNA was quantified using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) at 260 nm. DNA samples that have an $A_{260/280}$ ratio of at least 1.7 to 2.0 and minimum concentration of 50 ng/uL were stored at –20°C until microarray genotyping.

## Genotyping

Customized genotyping of candidate variants was performed using DNA microarray technology following the manufacturer's manual of the Illumina Infinium iSelect Assay. The variants included in both bead chip designs were selected after an extensive search was done in different databases such as PharmGKB (Pharmacogenetics Knowledgebase), NHGRI GWAS Catalog (National Human Genome Research Institute Genome-wide Association Study), PubMed, and patent databases (eg, Patentscope and Escapenet). The variants associated with T2DM, response and adverse effects due to metformin, and other cardiometabolic traits were included in the final analysis. The HiScan system was utilized to produce the images for GenomeStudio analysis.

GenomeStudio v2.0 and GPLINK v2.05.10 were used to evaluate the quality of sample data and identify or remove participants and single nucleotide polymorphisms (SNPs) with incomplete data. Genotyping data with a call frequency of ≥95% were only included in the study [9]. Participants with a missingness rate > 5%, identified using individual missingness test, were excluded from further analysis. Furthermore, the following tests were performed to determine SNPs that have incomplete genotype data and needed to be excluded from further analyses: frequency tests (minor allele frequency < 1%),

genotypic missingness test (genotype missingness rate > 5%), and Hardy-Weinberg equilibrium test (significant Hardy-Weinberg disequilibrium among controls > 0.001).

## Data Analyses

### Allelic association testing and determination of risk allele

Allelic association tests using Fisher-Irwin exact tests were performed through gPLINK 2.05.10 to assess initially the significant differences in the frequencies of the alleles. Correcting for multiple testing was done via Holm-Bonferroni adjustments, if possible [10]. *P*-values or false discovery rate were reported when available [11, 12].

The crude odds ratios (OR) were used to infer the impact of an allele on phenotypic outcome. An OR > 1.0 denoted susceptibility, and an OR < 1.0 denoted protection.

### Genotypic association testing and determination of genetic effect

Complex multifactorial diseases follow an additive trend where the presence of more copies of the risk allele confers higher risk of the associated disease. Other models include the recessive model, where the presence of 2 risk alleles is required for the phenotypic effect to develop, and the dominant model, which requires the presence of only 1 risk allele [13]. These models were inferred based on the frequency and distribution of the genotypes among participants.

Fisher-Irwin exact test of association was performed to determine the best possible mode of genetic effect. Correcting for multiple testing was done either in the same procedure as in allelic association testing, via Holm-Bonferroni adjustments.

## Results

A total of 205 participants were enrolled under the metformin treatment group. After quality control, 10 participants were removed due to low genotyping rate (MIND > 0.05). This gave the study a total of 195 participants (165 responders and 30 poor responders) for further analyses. Those identified responders were compared with poor responders in terms of clinical characteristics and laboratory values (Table 1). As observed, there is a comparable age and sex distribution between both groups. The differences in medication adherence between the 2 groups are not statistically significant. The baseline HbA1c of the poor response group is significantly lower from that of the normal response group. Interestingly, higher baseline HbA1c and FBS levels were observed in the responder group, which subsequently had lower levels after 3 months. HbA1c is significantly decreased from the baseline to third month among responders by 19%.

Quality control tests were also done for the variants. Out of 2842 SNPs, 322 failed the missingness test (ie, GENO > 0.05), 883 failed the frequency test (ie, minor allele frequency < 0.05), and 120 failed the Hardy-Weinberg equilibrium test in controls ($P \leq 0.001$). Of the remaining 1587 variants, 12 with a previous link to diabetes and related conditions were found to have nominal association with poor response to metformin ($P < 0.05$) (Table 2). Interestingly, 3 variants of *SLC2A10* (solute carrier family 2 member 10

**Table 1.** Clinical profile of participants classified by response to metformin

| Characteristics | Poor response to metformin (n = 30) | Normal response to metformin (n = 165) | *P*-value[a] |
|---|---|---|---|
| Age, years, mean (SD) | 51.20 (7.20) | 52.27 (11.38) | ns |
| Sex, % males | 16.67 | 29.09 | ns |
| Hypertension, % | 43.33 | 53.33 | ns |
| Ever smoked, % | 16.67 | 22.42 | ns |
| Alcohol use, % | 23.33 | 30.91 | ns |
| BMI, kg/m$^2$, mean (SD) | 28.47 (2.91) | 28.64 (4.19) | ns |
| Waist circumference, cm, mean (SD) | 97.67 (8.27) | 96.30 (9.92) | ns |
| Average adherence to medication, %[a] | 89.29 (19.35) | 94.80 (8.43) | ns |
| First month | 89.97 (21.97) | 94.00 (12.19) | ns |
| Second month | 91.21 (20.34) | 96.39 (6.59) | ns |
| Third month | 86.70 (25.73) | 94.00 (16.31) | ns |
| Adverse drug effect, % (n) | 0 | 0.61 (1) | ns |
| Baseline | | | |
| FBS, mg/dL, mean (SD) | 146.92 (27.85) | 160.24 (30.24) | 0.026 |
| HbA1c, %, mean (SD) | 7.55 (1.09) | 8.57 (1.09) | <0.001 |
| Creatinine, mg/dL, mean (SD) | 0.71 (0.16) | 0.74 (0.19) | ns |
| Third month | | | |
| FBS, mg/dL, mean (SD) | 142.14 (47.44) | 121.33 (22.96)[b] | 0.0002 |
| HbA1c, %, mean (SD) | 7.72 (1.48) | 6.92 (0.79)[b] | <0.0001 |

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ns, not significant.
[a]Significant at $P < 0.05$ using Student's *t*-test (age, BMI, waist circumference, FBS, HbA1c, creatinine), Wilcoxon rank-sum test/Mann-Whitney test (medication adherence) or Fisher's exact test.
[b]Third month values are significantly different compared with baseline values at $P < 0.05$ using paired *t*-test.

**Table 2.** Variants nominally associated with poor metformin response in the study

| Variant | Nearest Gene | Location | Genotype | Genotype frequencies, % (n) | | Genotypic models | Crude OR (95% CI) | $P^*$ | Adjusted OR (95% CI) | $P^a$ | Adjusted OR (95% CI) | $P^b$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cases | Controls | | | | | | | |
| rs2425911 | *SLC2A10* | Intron | GG<br>CG<br>CC | 16.67 (5)<br>46.67 (14)<br>36.67 (11) | 32.73 (54)<br>47.27 (78)<br>20.00 (33) | CG vs GG (additive) | 1.93 (0.61, 7.26) | 0.334 | 2.02 (0.67, 6.088) | 0.211 | 1.98 (0.62, 6.30) | 0.248 |
| | | | | | | CC vs GG (additive) | 3.55 (1.03, 14.26) | 0.044 | 4.20 (1.31, 13.48) | 0.016 | 4.44 (1.26, 15.62) | 0.020 |
| rs3092412 | *SLC2A10* | Intron | TT<br>AT<br>AA | 16.67 (5)<br>46.67 (14)<br>36.67 (11) | 32.73 (54)<br>47.27 (78)<br>20.00 (33) | AT vs TT (additive) | 1.93 (0.61, 7.26) | 0.334 | 2.02 (0.67, 6.08) | 0.211 | 1.98 (0.62, 6.29) | 0.248 |
| | | | | | | AA vs TT (additive) | 3.55 (1.03, 14.26) | 0.044 | 4.20 (1.31, 13.47) | 0.016 | 4.44 (1.26, 15.61) | 0.020 |
| rs2425904 | *SLC2A10* | Intron/downstream | TT<br>CT<br>CC | 16.67 (5)<br>46.67 (14)<br>36.67 (11) | 32.12 (53)<br>47.88 (79)<br>20.00 (33) | CT vs TT (additive) | 1.87 (0.59, 7.04) | 0.366 | 1.97 (0.65, 5.94) | 0.228 | 1.96 (0.61, 6.23) | 0.256 |
| | | | | | | CC vs TT (additive) | 3.49 (1.01, 14.00) | 0.048 | 4.14 (1.29, 13.31) | 0.017 | 4.41 (1.25, 15.55) | 0.021 |
| rs340874 | *PROX1-AS1* | intron/ upstream | TC and TT<br>CC | 66.67 (20)<br>33.33 (10) | 91.46 (150)<br>8.54 (14) | CC vs TC and TT (recessive) | 5.29 (1.84, 14.91) | 0.002 | 6.36 (2.43, 16.66) | <0.001 | 10.71 (3.34, 34.38) | <0.001 |
| rs815815 | *CALM2* | Intron/regulatory region | AG and AA<br>GG | 93.33 (28)<br>6.67 (2) | 100 (163)<br>0 | GG vs AG and AA (recessive) | 13.49 (1.03, +inf) | 0.047 | — | | — | |
| rs1333049 | *CDKN2B-AS1* | Intron/downstream | GG<br>CC and GC | 0 100 (30) | 16.97 (28)<br>83.03 (137) | CC and GC vs GG (dominant) | 8.56 (1.44, +inf) | 0.013 | — | | — | |
| rs2010963 | *VEGFA* | 5'-UTR/regulatory region/ upstream | CG and GG<br>CC | 90.00 (27)<br>10.00 (3) | 99.39 (164)<br>0.61 (1) | CC vs CG and GG (recessive) | 17.77 (1.37, 962.07) | 0.024 | 18.88 (1.85, 192.73) | 0.013 | 11.64 (0.93, 145.19) | 0.057 |
| rs1535435 | *AHI1* | Intron/regulatory region | GG<br>AG<br>AA | 46.67 (14)<br>53.33 (16)<br>0 | 67.27 (111)<br>28.48 (47)<br>4.24 (7) | AG vs GG (dominant deviant) | 2.68 (1.13, 6.47) | 0.024 | 2.49 (1.11, 5.59) | 0.027 | 2.41 (1.01, 5.78) | 0.048 |
| | | | | | | AA vs GG (dominant deviant) | 0.85 (0, 6.15) | 0.894 | -- | | -- | |
| rs9494266 | *LINC00271* | intron/ downstream | GG<br>AG<br>AA | 46.67 (14)<br>53.33 (16)<br>0 | 66.06 (109)<br>29.70 (49)<br>4.24 (7) | AG vs GG (dominant deviant) | 2.53 (1.06, 6.09) | 0.035 | 2.35 (1.05, 5.28) | 0.038 | 2.28 (0.95, 5.46) | 0.064 |
| | | | | | | AA vs GG (dominant deviant) | 0.84 (0, 6.04) | 0.883 | — | | — | |
| rs11128347 | *PDZRN3* | intron/ upstream/ regulatory region | GG<br>CC and GC | 53.33 (16)<br>46.67 (14) | 75.15 (124)<br>24.85 (41) | CC and GC vs GG (dominant) | 2.63 (1.09, 6.33) | 0.031 | 2.46 (1.09, 5.57) | 0.030 | 2.94 (1.19, 7.26) | 0.020 |
| rs1805081 | *NPC1* | missense/ upstream | AA<br>GG and AG | 56.67 (17)<br>43.33 (17) | 76.97 (127)<br>23.03 (38) | GG and AG vs AA (dominant) | 2.54 (1.03, 6.14) | 0.041 | 2.57 (1.13, 5.85) | 0.024 | 2.38 (0.98, 5.79) | 0.057 |
| rs13266634 | *SLC30A8* | missense/ regulatory region | TC and CC<br>TT | 63.33 (19)<br>36.67 (11) | 81.82 (135)<br>18.18 (30) | TT vs TC and CC (recessive) | 2.59 (1.00, 6.46) | 0.049 | 2.85 (1.21, 6.73) | 0.017 | 3.17 (1.22, 8.23) | 0.018 |

Exact logistic regression was done to determine whether the crude odds ratio gathered from the 2xn analysis is statistically significant ($P^*$). Simple logistic regression with adjustment based on medication adherence alone ($P^a$) or based on medication adherence and baseline HbA1c ($P^b$) was done to determine whether statistical significance is retained.
Abbreviations: *AHI1*, Abelson helper integration site 1; *CALM2*, calmodulin 2; *CDKN2B*-AS1, cyclin dependent kinase inhibitor 2B antisense RNA 1; *LINC00271*, long intergenic nonprotein coding RNA 271; *NPC1*, NPC intracellular cholesterol transporter 1; *PDZRN3*, PDZ domain containing ring finger 3; *PROX1-AS1*, prospero-related homeobox antisense RNA 1; *SLC2A10*, solute carrier family 2 member 10; *SLC30A8*, solute carrier family 30 member 8; VEGFA, vascular endothelial growth factor.

gene)— rs2425911, rs3092412, and rs2425904—were observed to be nominally associated with a poor response to metformin in an additive manner ($P < 0.05$). However, statistical significance of these variants was not retained after correcting for multiple testing ($P < 3.15 \times 10^{-5}$).

Adjusting for multiple testing may prove to be too constricting, however, and may prevent true positives from being recognized. The enrichment from the multiple variant associations of *SLC2A10* may be considered to circumvent this limitation.

## Discussion

One of the most prescribed drugs is metformin, which is a commonly used oral hypoglycemic agent due to its accessibility to different societal classes in the Philippines. However, drug response varies in different individuals, and several studies suggest that genetic polymorphisms contribute to these interindividual differences. Despite the worldwide availability of studies regarding interindividual differences of response to metformin, the specific cause for the poor response is still unknown. As for the Filipino population, such study is limited; thus, the present study investigated the association of genetic variants with metformin response.

The presence of 3 SNPs from the *SLC2A10* gene were shown to be associated nominally with poor metformin response—rs2425911, rs3092412, and rs2425904. These intron variants all exhibited additive mode of inheritance; hence, as the number of risk allele increases for each variant, the odds of being poorly responsive to metformin also increases (Table 2). The frequency of the risk alleles of the 3 variants is 0.44 in the current study.

The observed multiple nominal associations of *SLC2A10* variants enrich and appear to strengthen confidence of the association with the trait of interest, even if statistical significance is not retained after adjustment. This enrichment may offset the known limitation of conservative nature of multiple testing adjustment that often disregards true positives by lowering the *P*-value cutoff.

The *SLC2A10* gene encodes a member of Class III glucose facilitative transporters—namely, glucose transporter 10, a transporter protein essential in the regulation of glucose homeostasis in the body [14]. The expression of *SLC2A10* in pancreas and liver may contribute to the pharmacokinetics of metformin since the drug is widely distributed into body tissues, including the liver and kidney via organic cation transporters [15, 16]. The lack of response to metformin may be linked to variants related to glucose regulation. However, further studies related to its contribution to metformin pharmacodynamics are still needed.

The intron variant of rs3430874 in the upstream region of prospero homeobox 1 antisense RNA 1 gene (*PROX1-AS1*) is also associated with poor response to metformin ($P < 0.002$). Its CC genotype conferred 5.29× higher likelihood of poor response to metformin as compared with either TC or TT genotypes, and the frequency of the risk allele C is lower than the allele frequencies among the different populations reported in the 1000 Genomes Project (Table 3). *PROX1-AS1* is an antisense RNA of the *PROX1* gene, which is an important transcription factor for the development of various organs, such as liver and pancreas [17]. The variant rs340874 is believed to modulate the expression of *PROX1-AS1* in different body tissues, such as thyroid, testis, pancreas, pituitary, and liver, among others [18]. The *PROX1-AS1* CC variant was not previously associated with metformin response but rather with increased fasting blood glucose and decreased islet beta-cell function among individuals of European ancestry [19]. The CC genotype was associated with altered postprandial glucose and lipid metabolism, as well as accumulation of visceral fat [20]. While the mechanisms behind the involvement of *PROX1-AS1* to the development of T2DM and to metformin response are still unknown, it can be hypothesized that *PROX1-AS1* might be a silencer of the *PROX1* gene. Increased expression of *PROX1-AS1* among those with the rs340875-C variant may lead to reduced *PROX1* expression as shown in quantitative trait locus studies [18]. This may result in decreased islet beta-cell function and decreased function of metformin in certain individuals, which may manifest

**Table 3.** Risk allele frequencies of variants associated with poor response to metformin among study participants and other populations

| Variants | Risk allele | Risk allele frequency[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | This study | Global | AFR | AMR | EAS | EUR | SAS |
| rs2425911 | C | 0.44 | 0.69 | 0.95 | 0.60 | 0.42 | 0.67 | 0.71 |
| rs3092412 | A | 0.44 | 0.69 | 0.94 | 0.60 | 0.42 | 0.67 | 0.71 |
| rs2425904 | C | 0.44 | 0.69 | 0.95 | 0.60 | 0.42 | 0.67 | 0.71 |
| rs340874 | C | 0.32 | 0.38 | 0.09 | 0.41 | 0.42 | 0.53 | 0.53 |
| rs815815 | G | 0.10 | | 0.19 | 0.13 | 0.11 | 0.14 | 0.07 |
| rs1333049 | C | 0.61 | 0.42 | 0.21 | 0.46 | 0.54 | 0.47 | 0.49 |
| rs2010963 | C | 0.15 | 0.33 | 0.32 | 0.35 | 0.41 | 0.31 | 0.25 |
| rs1535435 | A | 0.18 | 0.25 | 0.70 | 0.13 | 0.10 | 0.07 | 0.10 |
| rs9494266 | A | 0.25 | 0.28 | 0.80 | 0.15 | 0.10 | 0.07 | 0.09 |
| rs11128347 | C | 0.13 | 0.13 | 0.12 | 0.23 | 0.14 | 0.12 | 0.09 |
| rs1805081 | G | 0.14 | 0.22 | 0.02 | 0.26 | 0.26 | 0.38 | 0.25 |
| rs13266634 | T | 0.47 | 0.26 | 0.07 | 0.27 | 0.46 | 0.28 | 0.25 |

Abbreviations: AFR, African 1000 Genomes Project participants; AMR, admixed American 1000 Genomes Project participants; EAS, East Asian 1000 Genomes Project participants; EUR, European 1000 Genomes Project participants; SAS, South Asian 1000 Genomes Project participants.
[a]Presented are the risk allele frequencies among the control group of this study, compared with the findings from the 1000 Genomes Project.

as poor response. Expression studies may be done to verify the results.

Another interesting variant shown to be associated with poor response to metformin is rs815815, an intronic enhancer of calmodulin 2 (*CALM2*). Participants with the GG genotype were 13.49× more likely to be poorly responsive to metformin, and the frequency of the risk allele is 0.10, which is lower as compared to the allele frequencies among different populations in the 1000 Genomes Project, with the exception of the South Asian population (Table 3). *CALM2* is a gene responsible for encoding a calcium binding protein, which plays important roles in signaling pathways, a cell cycle progression and proliferation. Previous studies demonstrated the association of rs815815 with disease-related mortality among T2DM patients [21] and further considered the variant as a novel SNP associated with glucose metabolism [22]. Although there are no previous association studies between this variant and metformin response, the influence of *CALM2* expression on adenosine monophosphate-activated protein kinase (AMPK) signaling pathway may suggest a probable molecular mechanism by which the variant can affect metformin response [23]. The AMPK signaling pathway, together with phosphoinositide 3 kinase (PI3K)/v-akt murine thymoma viral oncogene homologue and mitogen-activated protein kinase, was proven to be essential in glucose homeostasis thru promoting translocation of GLUT4 transporter protein; thus, the unchecked expression of this pathway often results to diabetes and obesity [23]. Interestingly, activation of the *AMPK* signaling pathway has been primarily the function of metformin to inhibit glucose and lipid synthesis [14]. Quantitative trait loci analysis showed that rs815815-A significantly increased *CALM2* expression by 2-fold. Furthermore, *CALM2* expression and *AMPK* are negatively correlated [22], which means that the increase in *CALM2* expression inhibits *AMPK* signaling and causes a surge in glucose. The GG phenotype may exert its effect by lowering the expression of *CALM2* and increasing the activity of *AMPK*, inhibiting the action of metformin. Functional characterization of the variant or expression studies are needed to verify this speculation.

Other than the possible mechanisms presented, numerous variants have also shown significant association with poor metformin response. These variants include rs1333049 found at the intron of cyclin dependent kinase inhibitor 2B antisense RNA 1 (*CDKN2B-AS1*), rs2010963 located at the 5'-untranslated region of vascular endothelial growth factor A (*VEGFA*), ra1535435 and rs9494266 at the intron regions of Abelson helper integration site 1 (*AHI1*), rs11128347 at the intron region of PDZ domain containing ring finger 3 (PDZRN3), rs1805081 located at the Niemann-Pick disease type C1 (*NPC1*), and rs13266634 of the zinc protein member solute carrier family 30 member 8 (*SLC30A8*). These variants have not previously demonstrated association with poor metformin response; however, some of them have shown significant association with diabetes; for example, rs1333049 is found to be significantly associated with HbA1c level in Mexican nonobese T2DM patients [24]. On the other hand, the rs2010963-G is associated with protection from nonproliferative retinopathy, but the same findings have not been consistently found in proliferative retinopathy or the composite of the 2 types [25-30]. Both variants of the *AHI1* gene have been studied for their possible association with T2DM among Caucasians. The *AHI1-LOC441171* gene region has been linked to T2DM susceptibility among Chinese and Native Americans [31, 32]. An initial GWAS done among Finns, Ashkenazi Jews, English, German, and French individuals [33] has shown that the variants rs1535435 and rs9494266 found in that locus are significantly associated with T2DM (rs1535435, $P = 1.86 \times 10^{-5}$; rs9494266, $P = 2.67 \times 10^{-5}$); yet another GWAS done among the Danish population showed opposite results [34]. In addition, neither the *PDZRN3* gene nor its variant rs11128347 has been linked to poor metformin response; previously, the variant was associated with survival among African-Americans with T2DM on dialysis [21]. The variant rs1805081 (His215Arg) is a nonsynonymous variant linked to increased risk for obesity found in a GWAS done among European populations; this association has been consistently replicated [35, 36]. The variant has also been linked to T2DM and glycemic parameters such as fasting insulin, insulin sensitivity index, and homeostatic model assessment of insulin resistance [37-39]. Lastly, the T allele of rs13266634 (c.973C > T or p.Trp325Arg) has been associated with T2DM among the Japanese [40, 41] and Chinese populations [42].

Other variants were also included in the analysis. For example, the *KCNJ11* E23K (rs5219) and *ABCC8* A1369S (rs757110) variants are both shown to be associated with T2D, with KCNJ11 E23K lysine carriers (i.e. T allele carriers) being shown to be less protected by metformin [43, 44]. However, they were not found to have associations with poor response to metformin in our population. Table 4 summarizes the results of the Fisher exact tests done using Plink.

Several factors should also be considered that may affect end glucose control. It is interesting to note that medication adherence is lower among poor responders than normal responders, although the difference is not statistically significant. The presence of adverse drug reactions (ADRs) was also almost negligible in both groups. These factors may affect a person's response to metformin. Thus, additional analysis was done to determine whether the statistical significance of the individual SNPs are retained after adjustment to medication adherence (Table 2). Except for *CALM2*, which could not be assessed due to very small sample sizes per genotype, all variants retained their nominal association after adjusting for medication adherence.

It is also noted that poor responders had significantly lower baseline HbA1c levels compared to normal responders. Despite the difference in baseline HbA1c values between responders and poor responders, compliance with definitions were carried out: baseline values of both groups still fall within the hyperglycemia category, with poor responders showing a positive change from the baseline instead of the expected decrease after initiation of metformin therapy. This difference may be explained by the nonprobabilistic sampling done to search for participants who will fulfill the criteria of responder or poor responder. Nevertheless, this is a limitation of the study that may be considered in follow-up studies.

To adjust for this, additional analysis was done to determine whether the associations of the individual SNPs are retained after adjustment to both medication adherence and baseline HbA1c (Table 2). The variants rs2010963 (VEGFA), rs9494266 (LINC00271), and rs1805081 (NPC1) did not retain individual nominal association when adjusted for both

**Table 4.** Results of Fisher exact tests done for *KCNJ11* E23K (rs5219) and *ABCC8* A1369S (rs757110)

| Chr | SNP | Minor allele | Major allele | Test | Cases | Controls | *P* |
|---|---|---|---|---|---|---|---|
| 11 | rs5219 | T | C | Geno | 4/10/16 | 15/69/81 | 0.5885 |
| 11 | rs5219 | T | C | Trend | 18/42 | 99/231 | 1.0000 |
| 11 | rs5219 | T | C | Allelic | 18/42 | 99/231 | 1.0000 |
| 11 | rs5219 | T | C | Dom | 14/16 | 84/81 | 0.6959 |
| 11 | rs5219 | T | C | Rec | 4/26 | 15/150 | 0.5023 |
| 11 | rs757110 | G | T | Geno | 5/10/15 | 17/75/73 | 0.3686 |
| 11 | rs757110 | G | T | Trend | 20/40 | 109/221 | 0.9637 |
| 11 | rs757110 | G | T | Allelic | 20/40 | 109/221 | 1.0000 |
| 11 | rs757110 | G | T | Dom | 15/15 | 92/73 | 0.6905 |
| 11 | rs757110 | G | T | Rec | 5/25 | 17/148 | 0.3451 |

Abbreviations: Allelic, Fisher exact test comparing the 2 alleles; Chr, chromosome; Dom, Fisher exact test using the dominant model; Geno, Fisher exact test using the genotypic model; Rec, Fisher exact test using the recessive model; SNP, single nucleotide polymorphism; Trend, Cochrane-Armitage trend test.

medication adherence and baseline HbA1c. The nonretention of nominal association may be due to loss of statistical power either because of the relative small sample size of the cohort or because of the additional variables added into the model.

Despite discoveries and advances in the understanding the effect of these drugs on T2DM, more questions remain to be answered. The lack of variants with statistically significant associations after multiple testing is heavily influenced by the small sample size of the study. The nominal associations of the variants may suggest that subsequent verifications are needed to prove relationships. In this case, approaches using higher sample sizes or the use of functional studies using pharmacokinetic approaches may be helpful. It would be prudent to test the associations of these variants in a population with a larger sample size to see whether statistical significance will be retained then; ideally, there should be at least 62 poor responders to at least 124 normal responders to assure that statistical significance is achieved in a single-variant study. For a multivariant follow-up study, sample sizes should be adjusted based on the total number of variants to be included in the analysis. These insights are some of the important outputs of this preliminary investigation, looking at metformin response association with genetic variants among Filipinos.

Interindividual variations in treatment response are one of the drivers in conducting pharmacogenetics studies, and this study revealed SNPs that were not previously associated with metformin response. The variants identified in this study may serve as markers of interest for future research in a large-scale study of patients with T2DM. This may be used to evaluate the clinical relevance of these identified SNPs on treatment response. Future research may also include the search for variants associated with hyperresponse to drugs among Filipinos; this will help drug developers to understand adverse effects caused by high concentrations of the active drug metabolites in the body. Specific adverse drug reactions may also be explored as well as genetic association studies such as gastric complications in metformin. Once these SNPs are verified, point-of-care test kits appropriate to the local setting may be developed. Evidence-based research is essential in the development of beneficial health policies. The results of this study will build the foundation for future research on risk-benefit analysis on complications and the use of point-of-care test kits to guide clinical practice.

## Acknowledgments

The authors would like to acknowledge the following health, research, and administrative professionals who worked on this project over the years: Dr. Christine Hazel Y. Casanova, Dr. Maria Elizabeth P. Mercado, Dr. Frances Anne A. Catipay, Dr. Andionne Celeste A. Parlade, Dr. Gemmy P. David, Ms. Maria Jaymie L. Jarin, Mr. Mark Joseph G. Mitra, Ms. Rhea D. Conol, Ms. Lennor S. Tabanera, Ms. Ma. Kristina T. Gooco, Dr. Natasha Julienne M. Delgado, Dr. Angela Roberta G. Ong, Dr. Grace Faith U. Salacup, Dr. Cristina A. Dorado, Mr. McDanell A. Araña, Ms. Christine F. Luna, Mr. Jess L. Balahadia, Ms. Rouzle Katherine L. Ocampo, Mr. Jejomar O. Custodio, Mr. Christian Marc S. Ballecer, Mr. Jan Michael M. Evangelista, Mr. Dan Louie Renz P. Tating, Mr. Romer J. Guerbo, Mr. Mark John G. Daoas, Mr. Ramonito B. Nuique, Mr. Nick Paolo G. Tomines, Ms. Kathlyn Sharmaine Valdez, Mr. Kelvin Keith C. Holasca, Mr. Ralph Michael Dennis E. Torres, and Ms. Ma. Angela N. Borcelis. We also thank Chembie Almazar, Roemel Bueno, Reynand Canoy, Mae Belle Lacson, and Kate Wad-Asen of the Microarray Unit of the Institute of Human Genetics, National Institutes of Health, for assistance in genotyping; Prof. Cynthia Cordero and Prof. Kim Cochon for help with statistical analysis; and Ms. Cheenee Calantoc for copyediting the manuscript.

## Financial Support

This research was supported through Grants-in-Aid of the Philippine Council for Health Research and Development, Department of Science and Technology, Taguig City, Philippines.

## Disclosures

E.P.P. serves as member of the advisory board for Sanofi-Aventis and received honoraria as speaker for Sanofi-Aventis and Novo-Nordisk. G.V.J. is a member of the speakers' bureau of Merck, Sharp & Dohme, Sanofi Aventis, Novo Nordisk, Astra Zeneca, Abbott Nutrition, Boehringer-Ingelheim, LRI-Therapharma, Woerwag Philippines, and UMed. M.V.G. received honoraria as speaker for Boehringer Ingelheim, Astra Zeneca, Merck, and MSD. M.A-C. serves as member of the advisory board for Zuellig Pharma-Lilly for Dulaglutide; received research grant on behalf of the Philippine Society

of Endocrinology, Diabetes and Metabolism from Servier Philippines; and received lecture fees from Servier Philippines, Merck, MSD, AstraZeneca, Sanofi, Novo Nordisk and Zuellig Pharma-Lilly. A.B.P-M. received honoraria as speaker for Novartis, Sanofi-Aventis, Otsuka, LRI-Therapharma, and Cathay Pacific. A.U.C. received honoraria as speaker for Boehringer Ingelheim, Astra Zeneca, and Novartis. A.M.L. received lecture fees from Boehringer Ingelheim, Astra Zeneca, MSD, Merck and Novo-Nordisk. D.C.B. serves as member of the advisory board for Zuellig Pharma-Lilly and received lecture fees from Zuellig Pharma-Lilly, Novonordisk, GX International, Astrazeneca, Aspen, and Getz Pharma. N.M.M. gave lectures for GX International, Inc., LRI-Therapharma, and Servier Philippines, Inc. M.D.F. received a lecture fee from Astrazeneca. The remaining authors have no conflicts of interest to disclose.

## Data Availability

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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