No association between polymorphisms in the \textit{INSIG1} gene and the risk of type 2 diabetes and related traits$^{1-4}$

Magdalena Scopa, Aline Meirhaeghe, Jian'an Luan, Luis A Moreno, Marcela Gonzalez-Gross, Antonio Vidal-Puig, Cyrus Cooper, Rachel Hagen, Philippe Amouyel, Nicholas J Wareham, and Ruth JF Loos

\textbf{ABSTRACT}

\textbf{Background:} The insulin-induced gene 1 (\textit{INSIG1}) encodes a protein that blocks proteolytic activation of sterol regulatory element binding proteins, which are transcription factors that activate genes that regulate cholesterol, fatty acid, and glucose metabolism.

\textbf{Objective:} We tested for associations between 6 \textit{INSIG1} tag single nucleotide polymorphisms (SNPs) (and captured all common variations in \textit{INSIG1}) and the risk of type 2 diabetes (T2D), obesity, and related traits in 10,567 adults and 1155 adolescents from 5 population-based studies, a T2D case-control study, and a T2D case-series.

\textbf{Design:} We genotyped tag SNPs and tested them for associations with the risk of T2D or obesity and with body mass index, waist circumference, systolic and diastolic blood pressure, and concentrations of fasting glucose, 2-h oral-glucose-tolerance test glucose, cholesterol, and triglyceride, with the assumption of an additive effect of the minor allele. Dominant effects were tested for the less-frequent SNPs (minor allele frequency <5%). Summary statistics of each study underwent meta-analysis.

\textbf{Results:} Meta-analyses, which included 1655 T2D cases and 2911 control subjects, showed no association between any of the \textit{INSIG1} SNPs and T2D ($P > 0.08$). Furthermore, none of the SNPs showed an association with obesity in 1666 obese and 5737 nonobese individuals ($P > 0.17$). In agreement, none of the associations between the SNPs and any of the metabolic traits showed convincing associations in the 7562 adults from 4 population-based studies. Although a few nominally significant associations emerged, none of the associations survived multiple-testing correction. We observed no convincing associations with any of the studied traits in 1155 adolescents.

\textbf{Conclusion:} Although our study was sufficiently powered to identify small effects, the results suggest that common variation in \textit{INSIG1} is unlikely to have a major effect on T2D and obesity risk and related traits in white Europeans. \textit{Am J Clin Nutr} 2010;92:252–7.

\textbf{INTRODUCTION}

The prevalence of type 2 diabetes (T2D) is rapidly increasing with $>170$ million afflicted persons worldwide (1). T2D poses a substantial burden on personal health and health-care systems and has been subjected to intensive biomedical research.

T2D involves both environmental and genetic factors. Although recent genome-wide association studies (GWASs) identified $>20$ genetic variants associated with T2D and related traits (2–9), candidate-gene studies have had limited success. So far, only a few candidate genes have been convincingly associated with the risk of T2D, mainly through the use of large-scale studies and meta-analyses. The insulin-induced gene 1 (\textit{INSIG1}) is such a T2D candidate gene that has been explored only sparsely in humans.

\textit{INSIG1} is an endoplasmic reticulum anchor protein that regulates the release of the sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP)–SREBP complex. It prevents the proteolytic processing of the 3 SREBP isoforms into active transcription factors that are known to regulate lipid and cholesterol synthesis. \textit{INSIG1} also binds and accelerates a key enzyme in cholesterol biosynthesis, $\beta$-hydroxy-$\beta$-methylglutaryl coenzyme A reductase. Consequently, \textit{INSIG1}...
plays a fundamental role in the regulation of cholesterol and fatty acid metabolism (10). INSIG1 is a close homolog of INSIG2 (11), which was recently proposed to be associated with a risk of obesity (12, 13). They fulfill complementary roles in the regulation of the SREBP pathway in mice (11) but differ in their expression pattern (14). Insig double-knockout mice display continuous activation of SREBP despite sterol accumulation and an enlarged liver, which is enriched with cholesterol and triglycerides (15). In contrast, down-regulation of lipogenic gene expression and repression of triglyceride accumulation were observed in the liver of Zucker diabetic fatty rats when Insig1 was overexpressed because of the failure of the production of the active SREBP1 protein (16). In addition, overexpression of INSIG1 in preadipocytes leads to the failure of lipid accumulation during adipogenesis, and conversely, a reduction of INSIG1 messenger-RNA expression results in increased triglyceride accumulation and accelerates the differentiation of preadipocytes (17). Finally, INSIG1 may play a role in insulin sensitization because peroxisome proliferator–activated receptor γ agonists induce Insig1 expression in vitro and in white adipose tissue in vivo (18). These studies underline the multifunctional role of INSIG1 and strongly support its candidacy as a T2D candidate gene.

There have been 3 reports in humans that examined the relation between INSIG1 gene polymorphisms and diabetes-associated traits (19–21). These studies, carried out in relatively small size samples, showed associations between INSIG1 single nucleotide polymorphisms (SNPs) and plasma glucose or triglyceride concentrations.

The current study examined the association between 6 INSIG1 tagSNPs and the risk of T2D, obesity, and related traits [body mass index (BMI; in kg/m2), waist circumference, blood pressure, and concentrations of glucose, cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride] in 10,567 adults and 1155 adolescents from 5 population-based studies, a T2D case-control study, and a T2D case series.

METHODS

Cohort description

Detailed descriptions of the samples and associated references are available online (see supplemental Methods and Table 1 under “Supplemental data” in the online issue). In brief, the Hertfordshire Cohort Study is a population-based cohort study that comprises 3000 men and women (60 to 75 y of age) who were born in the English county of Hertfordshire. Genotypic data and data on anthropometric measures, blood pressure, glucose tolerance, and fasting serum cholesterol and triglycerides were available for 2901 individuals. The Fenland Study is an ongoing population-based cohort study of men and women aged 30–55 y who live in the Fenland, Ely, and Cambridge areas of the Cambridgeshire Primary Care Trust in the United Kingdom. Participants attended research facilities after an overnight fast for a detailed clinical examination, and blood samples were collected. For current analyses, data on 1865 individuals were available. The Medical Research Council (MRC) Ely Study is a population-based cohort study of people who live in Ely and surrounding villages (East Anglia, United Kingdom). All participants attended a clinical examination that included standard anthropometric measurements, medical questionnaires, and a 75-g oral-glucose-tolerance test. The current analyses includes 1699 men and women (aged 35–79 y) for whom genotypic and phenotypic data were available from phase 3. The MONICA (MONItoring of trends and determinants in Cardiovascular disease) study includes participants (aged 35–64 y) who were recruited as part of the World Health Organization MONICA population survey conducted in 3 different parts of France. The whole MONICA Lille sample (n = 1195) was used for this study as well as individuals who presented with T2D in the 2 other French centers to obtain 282 individuals with T2D and 854 normoglycemic individuals. For quantitative trait analyses, only participants from the MONICA-Lille study without antidiabetic treatment were included (n = 1097).

The ADDITION study (Anglo-Danish-Dutch study of intensive treatment in people with screen-detected diabetes in primary care) is a T2D case series that, together with nondiabetic individuals of the MRC Ely study, forms a case-control study. Previously undiagnosed prevalent cases of T2D were identified via a population-based stepwise screening strategy among 40–69 y olds participating in the United Kingdom Cambridge arm of the ADDITION study. Current analyses include 800 men and women who had DNA available and information on BMI. Participants of the MRC Ely study were confirmed as control subjects (n = 1607) or classified as cases (n = 891) for the case-control comparison. The Cambridgeshire Case-Control Study consists of 552 patients with T2D (aged 45–76 y) who were randomly sampled from a population-based diabetes register and 552 control subjects recruited from the same population and individually matched for age, sex, and geographical location. For the current analyses, genotypic data were available in 531 control subjects and 538 cases.

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study includes a total of 3865 adolescents (mean ± SD age: 14.8 ± 1.4 y) who were recruited through schools. Clinical biochemistry assays and genetic analyses were performed on one-third of the classes (n = 1155).

Ethical permission

Ethical permission for the all studies was granted by the respective Local Research Ethics Committee, and study participants provided informed consent. In the HELENA cross-sectional study, written informed consent was obtained from each participant and both of his/her parents or legal representatives.

SNP selection

The INSIG1 gene is located on chromosome 7q36. SNPs were selected by using genotype data from the HapMap project that were collected in individuals of European ancestry (CEU) (release 23a; dbSNP b126). Haploview software (version 3.2; Cambridge, MA) was used to assess the linkage disequilibrium (LD) structure between SNPs. Tagger software (Cambridge, MA) was used to select tagSNPs with the pairwise tagging only option (r2 threshold > 0.8). There were 12 SNPs encompassing INSIG1 (including 10 kb upstream and downstream) in HapMap with a minor allele frequency (MAF) ≥5% and a Hardy-Weinberg equilibrium (HWE) P > 0.01, which were captured by 6 tagSNPs (rs10258075, rs1128636, rs9692071, rs9690040, rs10271719, and rs9770066) (see supplemental Figure 1 under “Supplemental data” in the online issue).
Genotyping

The 6 INSIG1 tagSNPs were genotyped by using the Taqman platform (Applied Biosystems, Warrington, United Kingdom) except for the MONICA and HELENA studies (Illumina system; GoldenGate Technology, San Diego, CA). Genotype data were reviewed independently for each SNP; overall call rates were >95%. Genotype distributions of all SNPs respected the HWE (P > 0.05) except for rs9692071 in the Hertfordshire study (P = 0.001), which was excluded from analyses (see supplemental Table 2 under “Supplemental data” in the online issue).

Statistics

A likelihood ratio test was performed to assess whether the observed genotype distributions in the population-based studies and in the control subjects of the Cambridgeshire case-control study respected the HWE.

In case-control analyses (Cambridgeshire case-control, ADDITION, and MONICA studies), each SNP was tested for association with the risk of T2D by using logistic regression adjusted for age, sex, and BMI. We also tested for the risk of obesity in the cohort studies (Hertfordshire, MRC Ely, Fenland, and MONICA Lille studies) by comparing obese individuals (BMI ≥ 30) with nonobese individuals (BMI < 30) by using logistic regression analyses adjusted for age and sex.

For the associations between each SNP and the continuous traits, we used general linear regression models adjusted for age and sex and additionally for BMI for biochemical variables and blood pressure levels. The summary statistics of the case-control and sex and additionally for BMI for biochemical variables and blood pressure levels. Values for triglyceride and insulin concentrations were log-transformed to obtain a normal distribution. We applied an additive model for all SNPs and a dominant model for rs10258075, rs9692071, and rs10271719 because of lower minor-allele frequencies for these 3 SNPs.

Statistical analyses were conducted with SAS 9.1.2 software (SAS Institute, Cary, NC), and the meta-analyses were performed with STATA 9.2 software (StataCorp, College Station, TX). Power calculations were performed with the Quanto v1.1.1 program (http://hydra.usc.edu/gxe). Bonferroni correction was applied to take multiple testing into account [by using a stringent approach that assumed complete independence between tests: a P threshold = 0.00069 (ie, 0.05/72 tests [6 SNPs × 12 traits]); accounting for nonindependence between SNPs (r² > 0.20) and traits: P threshold = 0.0042 (ie, 0.05/16 tests [4 SNPs × 4 trait groups])].

RESULTS

The meta-analyses of the 3 T2D case-control series, including 1655 cases and 2911 control subjects, showed no compelling evidence of an association between any of the 6 INSIG1 SNPs and the risk of T2D (Table 1). We also tested for the association between the 6 SNPs and the risk of obesity in the combined

### Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Type 2 diabetes study</th>
<th>Obesity study</th>
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<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Control subjects</td>
</tr>
<tr>
<td>Additive model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10258075</td>
<td>1649</td>
<td>5642</td>
</tr>
<tr>
<td>rs1128636</td>
<td>1537</td>
<td>5256</td>
</tr>
<tr>
<td>rs9692071</td>
<td>1656</td>
<td>5737</td>
</tr>
<tr>
<td>rs9690040</td>
<td>1654</td>
<td>5708</td>
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</tr>
<tr>
<td>rs9770068</td>
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<td>5666</td>
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<tr>
<td>Dominant model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10258075</td>
<td>1649</td>
<td>5642</td>
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</tr>
<tr>
<td>rs10271719</td>
<td>1609</td>
<td>5557</td>
</tr>
</tbody>
</table>

1 OR, odds ratio.
2 Adjusted for age and sex.
3 Adjusted for BMI, age, and sex.
4 Values represent the increase or decrease in the odds of type 2 diabetes or obesity for each additional minor allele (under the additive model) or for minor allele carriers compared with noncarriers (under the dominant model).
DISCUSSION

On the basis of in vitro and animal studies, INSIG1 seemed to be an obvious candidate gene to test for associations with T2D, obesity, and associated traits in humans. However, in the current study we were unable to detect significant association between polymorphisms in the INSIG1 gene and risks of T2D and obesity in either adults or adolescents, despite the fact that our study was sufficiently powered to identify effect sizes of >0.075-SD score, which is comparable with the 30% for T2D risk and 20% for obesity risk (9).

In the HELENA study (n = 1155 adolescents), we observed no significant associations between any of the 6 INSIG1 SNPs and anthropometric (waist circumference and BMI) or biochemical traits (concentrations of glucose, insulin, triglycerides, cholesterol, LDL cholesterol, and HDL cholesterol), whereas there was a weak association between rs9770068 and systolic blood pressure (P = 0.04) (see supplemental Table 3 under “Supplemental data” in the online issue).

Power calculations showed that our samples were sufficiently powered (>99%) to identify effect sizes of a 1.35 OR, which is equivalent to the effects of TCF7L2 variants on T2D risk or of FTO variants on obesity risk (see supplemental Figure 2, A and B, under “Supplemental data” in the online issue). Our samples had ≥80% power to identify effects of 1.15 OR, which is equivalent to the recently identified T2D loci (6, 7) and the near MC4R locus (22) when MAFs were ≥30% for T2D risk and 20% for obesity risk (see supplemental Figure 2, A and B, under “Supplemental data” in the online issue). For the continuous-trait analyses, our sample of 7562 adults had sufficient power (>80%) to identify effect sizes of ≥0.075-SD score, which is comparable with the MTNR1B variant on glucose (9) or the FTO variant on BMI (23) (see supplemental Figure 2C under “Supplemental data” in the online issue). Smaller effect sizes (ie, 0.05-SD score) could be identified with ≥80% power when MAF was ≥30%.

Meta-analysis of the association between INSIG1 single nucleotide polymorphisms (SNPs) and metabolic traits [BMI, fasting glucose, 2-h oral-glucose-tolerance test (OGTT), systolic and diastolic blood pressure, cholesterol, HDL cholesterol, and triglyceride concentrations, and systolic and diastolic blood pressure] in 7562 individuals from the combined 4 population-based studies. We observed nominally significant associations between the rs9692071 SNP and fasting glucose concentrations (P = 0.02) and diastolic blood pressure (P = 0.02) (Tables 2 and 3). However, when taking into account multiple testing, these associations did not reach the required P cutoff of 0.00069 to be considered significant.

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4 adult population–based studies (including 1666 obese and 5737 nonobese individuals), but none of the INSIG1 SNPs showed a significant association (Table 1).

Next, we examined the associations between the 6 SNPs and metabolic traits (BMI, waist circumference, fasting glucose, 2-h oral-glucose-tolerance test, cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride concentrations, and systolic and diastolic blood pressure) in 7562 individuals from the combined 4 population-based studies. We observed nominally significant associations between the rs9692071 SNP and fasting glucose concentrations (P = 0.02) and diastolic blood pressure (P = 0.02) (Tables 2 and 3). However, when taking into account multiple testing, these associations did not reach the required P cutoff of 0.00069 to be considered significant.

In the HELENA study (n = 1155 adolescents), we observed no significant associations between any of the 6 INSIG1 SNPs and anthropometric (waist circumference and BMI) or biochemical traits (concentrations of glucose, insulin, triglycerides, cholesterol, LDL cholesterol, and HDL cholesterol), whereas there was a weak association between rs9770068 and systolic blood pressure (P = 0.04) (see supplemental Table 3 under “Supplemental data” in the online issue). Smaller effect sizes (ie, 0.05-SD score) could be identified with ≥80% power when MAF was ≥30%.

### Table 2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Additive model</th>
<th>Dominant model</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10258075</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
<tr>
<td>rs10271719</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
<tr>
<td>rs9692071</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
<tr>
<td>rs9808668</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
<tr>
<td>rs10271719</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
<tr>
<td>rs10258075</td>
<td>0.01 (-0.23, 0.94)</td>
<td>0.01 (-0.23, 0.94)</td>
</tr>
</tbody>
</table>

Values were adjusted for age and sex.

1. Values were adjusted for age, sex, and BMI.

2. Values were adjusted for age, sex, and baseline value of the variable of interest (absolute values) for each additional minor allele (under the additive model) or for minor allele carriers compared with noncarriers (under the dominant model).
TABLE 3

Meta-analysis of the association between single nucleotide polymorphisms (SNPs) and metabolic traits (waist circumference; total, HDL, and LDL cholesterol; triglycerides) in 7562 individuals from 4 adult population-based GWAS-identified loci.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>rs10258075</th>
<th>rs9692071</th>
<th>rs9770068</th>
<th>rs10271719</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P</td>
<td>β (95% CI)</td>
<td>P</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>Additive model</td>
<td>0.21 (0.08, 0.34)</td>
<td>0.003</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td>0.14 (0.03, 0.24)</td>
<td>0.005</td>
<td>0.02 (0.01, 0.03)</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>Additive model</td>
<td>0.04 (0.00, 0.08)</td>
<td>0.000</td>
<td>0.02 (0.01, 0.03)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td>0.03 (0.01, 0.06)</td>
<td>0.002</td>
<td>0.01 (0.00, 0.02)</td>
<td>0.000</td>
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<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>Additive model</td>
<td>0.02 (0.00, 0.04)</td>
<td>0.000</td>
<td>0.001 (0.00, 0.002)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td>0.02 (0.00, 0.04)</td>
<td>0.001</td>
<td>0.001 (0.00, 0.002)</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>Additive model</td>
<td>0.05 (0.03, 0.07)</td>
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<td>0.03 (0.01, 0.05)</td>
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<tr>
<td></td>
<td>Dominant model</td>
<td>0.03 (0.01, 0.05)</td>
<td>0.001</td>
<td>0.01 (0.00, 0.02)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values were adjusted for age, sex, and BMI. Values represent the change in the variable of interest (absolute values) for each additional minor allele (under the additive model) or for minor allele carriers compared with noncarriers (under the dominant model).

Values were adjusted for age, sex, and BMI. Values express the change in the variable of interest (absolute values) for each additional minor allele (under the additive model) or for minor allele carriers compared with noncarriers (under the dominant model).

SNP values were adjusted for age and sex.

SNP [ie, CC homozygotes for the −169C→T polymorphism had significantly (P < 0.01) higher postload plasma-glucose concentrations]. In a case-control study for risk of ischemic heart disease (IHD) that included 1801 Chinese Hans, Liu et al (21) showed that a haplotype that consisted of 3 INSIG1 SNPs was associated with a risk of CHD (OR: 1.59; 95% CI: 1.22, 2.06). In addition, the G minor allele of rs9769826 was associated with higher plasma-glucose concentrations (P = 0.015) in 948 control subjects (21). The rs9769826 SNP is in high LD (r²CEU = 0.96) with the rs9770068 SNP that was genotyped in our study. However, in our population-based samples of 7562 adults and 1155 adolescents, we observed no evidence of an association with glucose (P = 0.22 and P = 0.34, respectively) for this SNP. The differences observed between our study and that of Liu et al (21) may have been because of differences in study design (population-based compared with control subjects of a CHD study) or differences in ethnicity (white Europeans compared with Chinese Hans). Recently, Smith et al (20) described that the rs2721 SNP in the INSIG1 promoter was associated with higher triglyceride concentrations and a 2-fold lower expression of INSIG1 in surgical liver biopsy samples from obese individuals. The rs2721 SNP was not included in our study because it was not reported in the HapMap database. According to Smith et al, the rs2721 SNP is, on the basis of D’ values, in high LD with almost all INSIG1 SNPs, but the r values are not given, and thus the exact extent of LD is not known, and a comparison with our results is not possible.

Data for the genome-wide meta-analyses for T2D by the DIAGRAM (Diabetes Genetics Replication and Meta-analysis) consortium, which included 4549 T2D cases and 5579 control subjects, on 4 INSIG1 SNPs (rs10258075, rs1128636, rs9770068, and rs9767875) that were also genotyped in the current study did not show an association with T2D on their own (P > 0.68) (7) or when meta-analyzed with the current data (P > 0.46).

In conclusion, to our knowledge, this is the largest study to date that examined the association between INSIG1 SNPs and T2D, obesity, and associated traits. Our sample was sufficiently powered to identify effect sizes similar to those reported for GWAS-identified loci. We provided evidence that INSIG1 SNPs are unlikely to play a major role in the development of T2D or obesity, at least in white adult and adolescent Europeans. In the future, samples of other ethnicities should be looked at, and rare variants should also be considered.

We thank all of the volunteers for their time and help and the general practitioners and practice staff for help with recruitment. We also thank the study coordination teams and the field teams of all research units involved for recruitment and clinical testing. Biochemical assays for the Fenland Study and MRC Ely Study were performed by the National Institute for Health Research, Cambridge Biomedical Research Centre, Core Biochemistry Assay Laboratory.

The authors’ responsibilities were as follows—MS: performed statistical analyses, interpreted data, and wrote the first draft of the manuscript; AM: participated in study planning, performed statistical analyses, interpreted data, and participated in writing the manuscript; JL: performed statistical analyses, interpreted data, and approved the final manuscript; LAM, MG, CC, NW, and PA: participated in subject recruitment and approved the final manuscript; AV-P and RH: interpreted data and approved the final manuscript; and RFJL: participated in study planning, supervised and
helped interpret data, participated in writing the manuscript, and approved the final manuscript. None of the other authors had a financial conflict of interest.

REFERENCES