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Exploring the association between the rs2285666 variant of the ACE2 gene and COVID-19 severity in a population from Quito, Ecuador

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ABSTRACT

COVID-19, caused by the SARS-CoV-2 virus, is well known for its wide spectrum of clinical features. The host's genetic makeup may influence the severe form of COVID-19. To evaluate the association between the ACE2 rs2285666 gene variant and the severe form of COVID-19 in subjects who attended the Hospital IESS Quito Sur, Ecuador. Subjects and methods: for this study, 100 Ecuadorian subjects with COVID-19 infection verified by RT-PCR were included. The individuals were divided into two groups: 43 patients with the severe clinical picture (case group) and 57 subjects with the asymptomatic-mild form (control group). Of these individuals, 73 were males, 33 of whom belonged to the case group. DNA was extracted from the peripheral blood lymphocytes, and PCR-RFLP was performed for genotyping analysis. Results: the general sample of individuals was in Hardy-Weinberg Equilibrium. Homozygous AA females in the case group were not detected. No allele or genotype was statistically significantly associated with severe COVID-19, and no appropriate genetic model was found. Conclusion: the Ecuadorian population substructure may have had an impact on this analysis. In addition, males need to be included in the analysis to address HWE at X-chromosome chromosome-linked loci.

Keywords: *ACE2 gene, Complex trait, COVID-19, Genetic association study, Genetic variant, Hardy-Weinberg equilibrium.*

INTRODUCTION

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus (Forchette et al. 2021). Until September 2023, it has caused about 7 million deaths worldwide, with different outbreaks (World Health Organization 2023). COVID-19 can be considered a complex phenotype with a wide phenotypic spectrum that includes asymptomatic patients to severe forms of the disease (Wiersinga et al. 2020). On the one hand, the development of severe form is primarily determined by sex, aging and the pre-existence of comorbidities, including cardiovascular and pulmonary diseases, obesity, diabetes mellitus, and hypertension (Gao et al. 2021). In addition, the genetic constitution of the host has been shown to influence the clinical form of the disease.

The *ACE2* gene is located on the X chromosome; thus, a loss-of-function null mutation results in the complete absence of the enzyme in the hemizygous male. It is known that the *ACE2* gene is expressed in several human tissues and its expression is organ- and cell-specific. In addition, the encoded protein

functions as a receptor for the spike glycoprotein of the SARS-CoV-2. The peptidase domain of *ACE2* interacts with the receptor binding domains of SARS-CoV-2 mainly through polar residues with nanomolar-level binding affinity (Gheblawi et al. 2020). *ACE2* has been associated with the severe phenotype of COVID-19 through the Genetic Wide Association Study (GWAS) (Horowitz et al. 2022) and candidate gene (Choudhary et al. 2021) approaches. The rs2285666 is a single-nucleotide variation (SNV) at position 8790 in intron 3 of the *ACE2* gene, which consists of a transition G→A. This SNV has a broad ethnic distribution with a wide variability reported for the frequency of the minor allele (MAF). It has been associated with comorbidities of COVID-19, such as hypertension, type 2 *diabetes mellitus*, and dyslipidemia (Pan et al. 2018). Studies with European and Asian populations have reported different allele and genotypic frequencies (Augusto et al. 2023) for this gene variant (Gómez et al. 2020; Gupta et al. 2022; Najafi et al. 2022). The risk allele, however, has not been specifically identified in genetic association studies with COVID-19 in populations of Latin America (Angulo-Aguado et al. 2022; Martínez-Gómez et al. 2022).

The Ecuadorian population is a multi-ethnic society, with a large Native American influence as well as Western European and Sub-Saharan ancestries (Nagar et al. 2021). Genetic association with the severe form of COVID-19 may be influenced by ethnicity (Ishak et al. 2022). Afro-American, Latin American, and Native American individuals have had greater rates of hospitalization and death than Western European subjects (Webb Hooper et al. 2020). Individuals from mixed ethnicities, such as Latin-American subjects, have also been associated with the highest rates of infection and death (Zhang et al. 2022). Thus, in a study with participants from different ethnic groups who had comparable socioeconomic situations and access to healthcare, Native American ancestry was found to be a factor associated with morbidity and mortality of COVID-19 (Oda et al. 2021).

Due to the deteriorating healthcare infrastructure and epidemiologically vulnerable population (Webb Hooper et al. 2020). COVID-19 had a high impact in Latin America. *Alpha*, *Delta*, and *Omicron* (Carrazco-Montalvo et al. 2022) were the most common variants during the pandemic, and Ecuador was one of the most affected countries in the region. However, thanks to vaccination campaigns, this effect was partly mitigated as 86% of the population (15 million) received at least one dose, and 79% of the population (14 million) received two or more doses. Few genetic association studies have reported severity genes for the Latin American populations. For all these reasons, the objective of this study was to evaluate the association of the rs2285666 variant of the *ACE2* gene and the severe form of COVID-19 in an Ecuadorian population. The variant does not seem to directly influence COVID-19 severity.

MATERIALS AND METHODS

Design and Study Subjects

In this observational, analytical, and case-control study, a total of 100 Ecuadorian individuals with COVID-19 were analysed. The current study was carried out in accordance with the guidelines for reporting observational studies of the STROBE statement (TABLE S1). The individuals were split into two groups: 43 patients with the severe clinical form (case group), enrolled from October 2021 to March 2022 and 57 subjects with the asymptomatic-mild form (control group), enrolled in January 2021 at the Quito Sur Hospital of the Ecuadorian Institute of Social Security, Quito, Ecuador. Case group consisted of individuals without regard to sex who had a diagnosis of the severe form of COVID-19 confirmed by a positive RT-qPCR test specific for SARS-CoV-2; a chest computed tomography image showing a pattern of viral pneumonia due to diffuse infiltration of both lungs greater than 50% (CORADS 6); and the presence of respiratory failure and the need for mechanical ventilation (PaO2/FiO2 ≤100 mmHg (with PEEP ≥ 5cm H2O) and SpO2/FiO2 ratio <315). This group had received at least two doses of SARS-CoV-2 vaccines. Control group subjects presented the asymptomatic or mild clinical form of the disease, which was confirmed by a positive RT-qPCR test for SARS-CoV-2. The control group was composed of health

professionals from the same hospital who cared for patients with the severe form of COVID-19 admitted to the intensive care unit. Subjects in the control group had not received any COVID-19 vaccination when they were diagnosed with COVID-19. This subject selection technique was used to find COVID-19-associated alleles. The exclusion criteria for both groups included consanguineous individuals, children, pregnant or nursing women, and refugees or displaced, and people with little or no knowledge of the Spanish language.

TABLE 1. Allele and genotype frequencies for the rs2285666 polymorphism in the *ACE2* gene in the general, case, and control groups.

Sex	Alleles/ Genotypes	Groups					
		General (n)	*p*	Case (n)	*p*	Control (n)	*p*
Male (n=63)	G	0.67 (42)		0.67 (22)		0.63 (19)	
	A	0.33 (21)		0.33 (11)		0.37 (11)	
Female (n=37)	G	0.69 (51)		0.70 (14)		0.69 (37)	
	A	0.31 (23)	0.85	0.30 (6)	1	0.32 (17)	0.63
	GG	0.46 (17)		0.40 (4)		0.48 (13)	
	GA	0.46 (17)		0.6 (6)		0.41 (11)	
	AA	0.08 (3)		0.00 (0)		0.11 (3)	

p-value = *comparison of the allele frequencies between males and females in the general, case or control groups (Fisher's exact test).*

Molecular Analysis

Ten milliliters of peripheral blood were drawn from each subject in an EDTA blood collection tube. To reduce bias in the laboratory phase, each tube was assigned a special code without discriminating to which clinical group it belonged. The Column-Pure Blood Genomic DNA (ABM, Vancouver, Canada) kit was used to extract DNA according to the manufacturer´s instructions. The Qubit dsDNA BR ASSAY Kit (21000 ng 100RX) (Invitrogen, Massachusetts, USA) was then used to quantify the DNA using the Qubit fluorometer (Invitrogen, Massachusetts, USA). The DNA quality was determined by electrophoresis in 1.5% agarose gels at 80V for an hour, with the bands visualized using the Microtek Bio-1000F program scanner (Microtek International Inc., Hsinchu City, Taiwan). The *ACE2* gene was amplified through PCR using the forward primer 5´-CAT-GTG-GTC-AAA-AGG-ATA-TCT-3 the reverse primer 5´-AAA-GTA-AGG-TTG-GCA-GAC-AT-3. (Benjafield et al. 2004). The master mix and thermocycler settings (Applied Biosystems MiniAmp, Thermo Fisher Scientific Inc., Massachusetts, USA) for PCR were performed based on previously published (Wu et al. 2017), with a single change in alignment temperature (56°C). PCR products were examined by electrophoresis on 1.5% agarose gels in the blueGelTM system (48V, 45 minutes) (MiniPCR Bio, Massachusetts, USA). Subsequently, these products were digested with 10 U of *Alu*I at 37°C for 16 hours, in a total volume of 20 µl according to the manufacturer's instructions. The digested products were electrophoresed in 3% agarose gels in the Thermo ScientificTM equipment (120V, 2 hours) (Thermo Fisher Scientific Inc., Massachusetts, USA). Ten percent of all samples were randomly sequenced to control the reproducibility and quality of genotyping of PCR-RFLP, which showed complete matching of results.

Genotyping

For females, the undigested fragment generated a unique band of 466 bp indicating homozygosity for G, whereas the digested fragment gave rise to two bands of 281- and 135-bp, indicating homozygosity for the allele A. The presence of all three fragments indicated a heterozygous genotype (GA). Males were observed to be hemizygous for G (a single band of 466 bp) or A (two bands, one of 281 bp and the other of 135 bp).

Statistical Analysis

Microsoft Excel 2019, SNPStats (https://www.snpstats.net; Solé et al. 2006), InfoStat, and the Hardy Weinberg statistical package for R Studio were used. Allele and genotype frequencies were calculated by direct counting and expressed in proportions and percentages. Using the exact test, the likelihood ratio test, and the permutation test, allele and genotype frequencies were examined to determine whether the groups were in Hardy-Weinberg equilibrium (HWE). *Fisher* exact test was then used to compare allele frequencies between males and females. Women who could be heterozygous and homozygous for both alleles as well as hemizygous men were included in the calculation of the HWE of the current study under the fulfilment of two conditions: 1) the equality of allele frequencies between males and females; and 2) compliance with the HWE in women. The statistical power of the exact test for HWE, as the mid-p value was calculated using a function from the Hardy Weinberg R package. The estimation of the association between the rs2285666 alleles of *ACE2* and the severe phenotype of COVID-19, including covariates, was carried out considering different inheritance models (codominant, dominant, recessive, overdominant, and logit additive) expressed in frequencies and percentages. The covariates were sex, age, obesity, and the presence of comorbidities. The sex was taken as male or female, and the men were taken as homozygous. Age (≥50 years old), obesity, and other comorbidities (hypertension, *diabetes mellitus*, autoimmune diseases, among others) were dichotomous for absence (0) or presence (1). For each analysis, the odds ratios (OR), 95% confidence intervals (95% CI), and corresponding *p*-values were calculated. An association was considered significant when the *p*-value was <0.05 in all two-tailed statistical tests.

Ethical Considerations

Participants provided a written consent to sample extraction, the use of clinical histories, and the processing of biological samples. Hospital staff members collected the samples and data; they had no interaction with the researchers who conducted the molecular analysis. The data were collected according to the WHO "COVID-19 Case Registration Form", and the information was handled confidentially. This study had the ethical, legal, and methodological endorsement of the Comité de Ética para Revisión Expedita de Investigaciones en COVID-19 of the Ministerio de Salud Pública of Ecuador (MSP-CGDES-2020-0244-O1).

RESULTS

TABLE 1 shows the allele and genotype frequencies discriminated by sex in the whole, case, and control groups. No differences were found in allele frequency between males and females in the general, case or control groups (TABLE 1). For the general, case and control groups reported a MAF of 0.31, 0.30 and 0.32, respectively, considering only homogametic females. Similar MAFs were found for hemizygous males (TABLE1). Alleles and genotypes were found in HWE in the general group (*p*= 0.9424*)*, case group (p= 0.49) and control group (p= 0.92) (TABLE S2). Estimation of the association between the rs2285666 variant of the *ACE2* gene and the severe phenotype showed that the over-dominant model had the highest fit. However, no allele or genotype was significantly associated with the severe phenotype of COVID-19 enough to be considered an associated factor (risk or protective) (TABLE 2).

TABLE 2. Genetic Models of Association of the ACE2 rs2285666 SNV with the Severe Phenotype of COVID-19.

Genetic Model†		Group Control n=57 (%)	Group Case n=43 (%)	*p*	OR (CI 95%)
Co	G/G	34 (59.6)	26 (60.5)	0.41	1.00
	A/A	12 (21.1)	11 (25.6)		0.61 (0.16-2.26)
	A/G	11 (19.3)	6 (13)		2.84 (0.40-20.15)
Do	A/A+A/G	23 (40.4)	17 (39.5)	0.94	0.96 (0.33-2.83)
	G/G	34 (59.6)	26 (60.5)		1.00

Re	A/A+G/G	45 (79.0)	32 (74.4)	0.41	1.00
	A/G	12 (21.1)	11 (25.6)		0.59 (0.16-2.14)
Over-do	A/G+G/G	46 (80.7)	37 (86)	0.27	1.00
	A/A	11 (19.3)	6 (13.9)		0.59 (0.16-2.14)
Ad	-	-	-	0.68	1.18 (0.54-2.58)

†Genetic Models: Co: codominant; Do: dominant; Re: recessive; Over-do: overdominant; and Ad: Logit-additive. (n=100, covariate-adjusted analysis).

DISCUSSION

The overall objective of the present study was to investigate whether the *ACE2* rs2285666 gene variant could have a significance in relation to clinical phenotype in Ecuadorian patients with COVID-19. Several studies have speculated about the function of the *ACE2* gene variants in the severity of COVID-19 ever since the pandemic outbreak. The genetic differences in populations could contribute to ethnic differences in the phenotype observed for COVID-19. The MAF of the rs2285666 variant of the *ACE2* gene in Western European populations (Spain and Italy) was reported to be less than 0.20 and close to 0.30 in Asian populations (Iran and Iraq). No association with COVID-19 severity was found in these studies, but they had small sample sizes (Gómez et al. 2020; Mahmood et al. 2020; Strafella et al. 2020; Alimoradi et al. 2022; Najafi et al. 2022). Similar frequencies are reported in the present study. Furthermore, the frequency of the A allele was reported to be 0.32 and 0.38 in populations composed of Latin American individuals with mostly European and Native American ancestry (Strafella et al. 2020; National Center for Biotechnology Information 2022), so our admixed sample would resemble the data with this ancestry. Likewise, similar frequencies were reported for the A allele of rs2285666 of the *ACE2* gene for the Colombian (Angulo-Aguado et al. 2022) and Mexican (Martínez-Gómez et al. 2022) populations. Also, a low frequency was reported for the AA genotype latter three studies (TABLE 3).

TABLE 3. Allele and genotype frequencies reported of *ACE2* rs2285666 SNV andCOVID-19.

First author	Year	Country	Cases (n)	Genotype frequencies			Allele frequencies	
				GG (n)	GA (n)	AA (n)	G (n)	A (n)
Gómez et al.	2020	Spain§	53	NA	NA	NA	0.81 (43)	0.19 (10)
Strafella et al.	2020	Italy	122	NA	NA	NA	0.86 (209)	0.14 (35)
Mahmood et al.	2022	Iraq	49	0.12 (6)	0.35 (17)	0.53 (26)	0.70 (69)	0.30 (29)
Angulo-Aguado et al.	2022	Colombia	71	NA	NA	NA	0.65 (92)	0.35 (50)
Martínez-Gómez et al.	2022	Mexico	125	0.34 (42)	0.16 (20)	0.50 (63)	0.58 (146)	0.42 (104)
Alimoradi et al.	2022	Iran	79	0.30	0.14	0.83	0.90 (143)	0.10 (15)
Najafi et al.	2023	Iran	44	0.25 (11)	0.18 (8)	0.57 (25)	0.66 (58)	0.34 (30)
Current study	2023	Ecuador	100	0.46(17)	0.46(17)	0.08(3)	0.69(51)	0.31(23)

§ only males; NA=Not applicable

None of the alleles of the rs2285666 gene variant of *ACE2* were associated with the severe COVID-19 phenotype in a covariate-adjusted model in the present study. Furthermore, no AA homozygous females were found in our study. This is probably due to a reduced sample size. This is the main limitation of our study. Although the HWE was not altered, it may have influenced in the results of the genotype-phenotype association in our study. The small sample size limits the statistical interpretation of the association analysis reported in our study (Iniesta et al. 2005). A type II error (false negative) may be present in the present study. Thus, we cannot rule out an association between the rs2285666 variant of the *ACE2* gene and the

severe phenotype in COVID-19 in the Ecuadorian population. However, in Spanish (Gómez et al. 2020) and Colombian (Angulo-Aguado et al. 2022) populations using a similar methodology to the present study, the A allele also showed no significant differences between severe and non-severe COVID-19 patients.

In the context of COVID-19, inconsistent results have been described for the A allele. For example, a significant association with lower infection and mortality rates in an Indian population was reported (Srivastava et al. 2020); but an increased association between the A Allele and the severe phenotype of COVID-19 was shown in smokers and obese males under 50 years of age in French-Canadian and British populations (Hamet et al. 2021). Also, this positive association was found in the Iranian (Najafi et al. 2022), Spanish (Sabater Molina et al. 2022) and Mexican (Martínez-Gómez et al. 2022) populations, independently of the presence of comorbidities. By contrast, the G allele was identified as a risk allele for the severity of COVID-19 in a German population, after adjusting for cardiovascular disease (Möhlendick et al. 2021). For all these reasons, it is necessary to point out that the association of these alleles with the phenotype may be given by a high genetic heterogeneity, which is significantly conditioned by the geographical area, ethnicity, and sex (Srivastava et al. 2020; Aziz and Islam 2022; Gupta et al. 2022).

Similar discrepancies were found for genetic models that explain genotype-phenotype associations. The systematic reviews and meta-analyses currently available to explain the association between the rs2285666 variant of the *ACE2* gene and the severe form of COVID-19 were conducted mostly with European and Asian studies (Aziz and Islam 2022; Gupta et al. 2022; Keikha and Karbalaei 2022). The overdominant model of the current study does not coincide with the recessive model of the Spanish study (Sabater Molina et al. 2022) or with the codominant model of the Mexican study (Martínez-Gómez et al. 2022). However, we acknowledge that the study sample of our study may limit the statistical power to detect models of genotype-phenotype associations of the alleles for the rs2285666 variant of the *ACE2* gene and the severe form of COVID-19 (Honardoost et al. 2018). Furthermore, including males as homozygotes because they did not present differences with the allele and genotype frequencies of females, could be a confounding factor in the present analysis, since there were differences in the sex proportions between cases and controls (Su et al. 2022).

Another limitation of our study is that individuals of both sexes and different ages who were "naïve" to SARS-CoV-2 infection and any vaccines were selected for the control group. They contracted SARS-CoV-2 infection during the first wave of the pandemic. Despite direct exposure to individuals with a severe phenotype of COVID-19, subjects of the control group did not develop the severe phenotype. The case group was represented by subjects who became infected during the dominant *Omicron* VOC wave, and they presented the severe phenotype of the disease. We have used this method of subject selection to identify COVID-19-related alleles. This approach could more easily unmask differences in the genetic influence for the presence of the severe form of COVID-19 between the case and control groups. This selection method, however, adds a selection bias that could skew the genetic composition of the groups under study. On the other hand, the *Omicron* variant of the virus has been associated with increased transmissibility and risk of reinfection but not with the severity of SARS-CoV-2 infection (Fernandes et al. 2022).

In the current study, the conventional method for calculating HWE for an X-linked locus was not used because it subtracts male alleles (non-informative), so it decreases the power to detect an effect or association (Graffelman and Weir 2016). However, although we did report a high MAF (greater than 0.30), statistical power was exceptionally low, which could further limit the ability to detect associations for X-chromosome-linked markers (Graffelman and Moreno 2013). On the other hand, to reduce the likelihood of a type II error, strict statistical tests were used in our study (Graffelman 2015). Nevertheless, the statistical interpretation of the associations is restricted by a limited number of individuals in the analysed population in our study, which raises a certain level of doubt (see above).

To the best of our knowledge, this study is the first effort to identify an association between rs2285666 of the *ACE2* gene and COVID-19 in mixed Latin American individuals since previous studies involving genotyping of this genetic variant did not evaluate this association. Therefore, the use of new strategies in the future is suggested to evaluate the genetic components involved in emerging virus infections in Latin American populations within the framework of existing ethnic heterogeneity and the complex interaction of vaccines and the different variants of COVID-19.

Supplementary material
TABLE S1. STROBE Statement— Checklist of items for study of case-control studies. *Give information separately for cases and controls.

TABLE S2. Tests for Hardy-Weinberg Equilibrium. Only female frequencies included.

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Conflict of interest
None declared.

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TABLE S1. STROBE Statement—Checklist of items that should be included in reports of case-control studies.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
		Introduction	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	6
		Methods	
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	6-7
		(*b*) For matched studies, give matching criteria and the number of controls per case	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	11-12
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Not applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how matching of cases and controls was addressed	8
		(*e*) Describe any sensitivity analyses	8
		Results	
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9-10
		(b) Give reasons for non-participation at each stage	9-10
		(c) Consider use of a flow diagram	No
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-10
		(b) Indicate number of participants with missing data for each variable of interest	No

aOutcome data	15*	Report numbers in each exposure category, or summary measures of exposure	9

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	No
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	No
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24 (lines 547-549)

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

TABLE S2. Tests for Hardy-Weinberg Equilibrium.

	Exact Test (p)	*Likelihood ratio (p)*	Permutation test (p)
General group	0.9424851	0.9057122	0.8867647
Case group	0.4929909	0.2649309	0.3758824
Control group	0.9279199	0.9576681	1.0000000

only female frequencies included.