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ABCA1 gene R1587K polymorphism could be associated with metabolic syndrome and increased plasma triglyceride concentration in adults from northern Mexico

El polimorfismo R1587K del gen ABCA1 podría estar asociado con el síndrome metabólico y la concentración elevada de triglicéridos en adultos del norte de México

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ABSTRACT

Introduction: the ABCA1 protein plays a key role in reverse cholesterol transport, promoting its clearance and high-density lipoprotein (HDL) biogenesis. The R1587K (rs2230808) single-nucleotide polymorphism (SNP) in the *ABCA1* gene has been associated with dyslipidemia.

Objectives: to investigate the relationship of R1587K genotypes with cardiovascular (CV) risk, metabolic syndrome (MetS), lipid profile, paraoxonase-1 (PON1) activity, and anti-oxLDL titers.

Methods: we performed a cross-sectional study in 57 northern Mexican adults with no reported diseases. The *ABCA1* R1587K SNP was detected by real-time polymerase chain reaction (qPCR) using TaqMan allelic discrimination probes. We evaluated the relationship of R1587K with metabolic syndrome and clinical parameters including lipid profile, glucose and insulin, PON1 activity and concentration, anti-oxLDL antibodies, anthropometry and body-composition parameters, and the atherogenic index of plasma calculation.

Results: our results show higher triglyceride levels in the RK + KK carriers as compared to RR carriers ($p = 0.031$). An association between the RK + KK genotype and the presence of MetS (OR = 4.566, 95% CI = 1.386-14.92, $p = 0.010$) and a tendency towards high CV risk (OR = 3.317, 95% CI = 0.910-8.611, $p = 0.069$) was observed in comparison to RR carriers; however, there were no differences in HDL-C levels, PON1 activity and concentration, and anti-oxLDL titers among the R1587K genotypes.

Conclusions: in the northern Mexican population, the *ABCA1* gene R1587K SNP is present and the RK + KK genotypes are associated with MetS and increased triglyceride concentrations; therefore, it could be a CV risk biomarker. Nevertheless there is a need for further confirmation in longitudinal studies.

Keywords: rs2230808 polymorphism. Cardiovascular diseases. Paraoxonase-1. anti-oxLDL antibodies. Hypertriglyceridemia. R1587K.

RESUMEN

Introducción: la proteína ABCA1 juega un papel principal en el transporte reverso del colesterol, promoviendo su eliminación y la biogénesis de HDL. El polimorfismo de un solo nucleótido (SNP) R1587K (rs2230808) del gen *ABCA1* se ha asociado con dislipidemias.

Objetivo: investigar la relación de los genotipos del SNP R1587K con el riesgo cardiovascular (CV), el síndrome metabólico (SM), el perfil de lípidos, la actividad de paraoxonasa 1 (PON1) y los anticuerpos contra las LDL oxidadas (anti-oxLDL).

Métodos: se realizó un estudio transversal con 57 adultos del norte de México que reportaron no tener enfermedades diagnosticadas. El SNP R1587K del gen *ABCA1* se detectó a través de PCR en tiempo real (qPCR) usando sondas TaqMan para discriminación alélica. Para evaluar la asociación del SNP R1587K con el SM y determinados parámetros clínicos se determinaron el perfil de lípidos, los niveles de glucosa e insulina, la actividad y concentración de PON1, los anticuerpos anti-oxLDL, los parámetros antropométricos y de composición corporal, y el cálculo del índice aterogénico en plasma.

Resultados: los resultados mostraron mayores niveles de triglicéridos en los portadores del genotipo RR + KK que en los portadores de RR ($p = 0,031$). Se observó una asociación entre el genotipo RK + KK y la presencia de SM (OR = 4,566, IC 95% = 1,386-14,92, $p = 0,010$) y una tendencia hacia un mayor riesgo cardiovascular (OR = 3,317, IC 95% = 0,910-8,611, $p = 0,069$) al compararlos con los portadores de RR. No se encontraron diferencias en los niveles de HDL-C, la actividad y concentración de PON1 y los anti-oxLDL entre los genotipos R1587K.

Conclusiones: el SNP R1587K del gen *ABCA1* se encuentra presente en la población del norte de México y el genotipo RK + KK se asocia con el SM y concentraciones elevadas de triglicéridos, por lo que este SNP podría ser un biomarcador de riesgo cardiovascular. Sin embargo, se necesita confirmación a través de estudios longitudinales.

Palabras clave: Polimorfismo rs2230808. Enfermedades cardiovasculares. Paraoxonasa 1. Anticuerpos anti-oxLDL. Hipertrigliceridemia. R1587K.

INTRODUCTION

Cardiovascular disease (CVD) is the primary cause of death worldwide. One of the leading disorders that are linked to premature development of CV disease is metabolic syndrome (MetS), a condition characterized by multiple alterations including abdominal obesity, hypertriglyceridemia, elevated fasting glucose, hypertension, and low HDL-C levels (1). A key event that precedes CVD is the formation of the atheroma plaque, caused by the unregulated uptake of oxidized low-density lipoproteins (oxLDLs) by macrophages that progressively turn into foam cells, the main trigger of the atherosclerotic lesion (2). These oxLDLs elicit the formation of immunogenic epitopes in the LDL molecule, and the subsequent production of anti-oxidized low-density lipoprotein antibodies (anti-oxLDL Abs) (3). However, the physiological role of anti-oxLDL Abs remains controversial since these are present in healthy subjects as well as in different diseases. Therefore, it has been suggested that they may have a pro-atherogenic role, an anti-atherogenic function, or no correlation at all (4-6).

The atheroprotective role of HDL particles results from their antioxidant and anti-inflammatory properties, mainly conferred by the paraoxonase-1 (PON1) enzyme, which circulates chemically bound to the apolipoprotein A-1 (Apo A1), the largest protein constituent of HDL particles (7). PON1 is a 345

amino-acid glycoprotein, and its serum activity is inversely associated with oxidative stress, as it can hydrolyze lipid peroxides, cholesteryl esters, and oxidized phospholipids, thus preventing the formation of atherosclerotic lesions (8). The circulating levels of HDLs are mainly mediated by reverse cholesterol transport (RCT), of which several pathways have been described—one of them is HDL particles traveling towards peripheral tissues, where their Apo A1 protein region stimulates cholesterol efflux from ATP-binding cassette transporters, ABCA1 and ABCG1, promoting its clearance and/or HDL biogenesis (9). Furthermore, it has been suggested that the PON1 enzyme enhances RCT by facilitating the union of HDL particles to macrophages and the ABCA1 protein (10).

The ABCA1 transporter protein is coded for by the *ABCA1* gene, located in the long arm of the human chromosome 9 (9q22-31). This protein plays a key role in HDL particle formation and cholesterol efflux from macrophages, thereby precluding the formation of macrophage foam cells (11). Several single-nucleotide polymorphisms (SNPs) in the *ABCA1* gene have been reported—R219K (rs2230806), R1587K (rs2230808), and I883M (rs4149313) are the most extensively studied, and have been associated with CV risk and plasma lipid profile (12). Particularly, the R1587K SNP, which is located in exon 35 and induces a change from arginine to lysine in position 1,587 of the ABCA1 protein, has been associated with increased levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and TGs, and reduced HDL-C levels (12,13).

Nevertheless, the relationships between R1587K genotypes and lipids levels are inconsistent among all populations, and have not been studied in Mexicans, especially in the northern region population, where dyslipidemias are common, mainly hyperalphalipoproteinemia, hypertriglyceridemia, and hypercholesterolemia (14). Therefore, we aimed to evaluate the potential association between the R1587K *ABCA1* polymorphism with CV risk and MetS presence, as well as with plasma lipid profile, PON1 activity and concentration, and anti-oxLDL titers in a northern Mexican population.

MATERIAL AND METHODS

Subjects

A cross-sectional study considering 57 apparently healthy adults from Sonora, Mexico, was performed. The subjects disclosed their not having any diagnosed cardiovascular, hepatic, renal, infectious, or thyroid disease, as well as not being under lipid-lowering medication, all these by self report. The study was approved by the Research Center for Food and Development A.C. Ethics Committee Review Board, and was conducted according to the principles of the Declaration of Helsinki. All volunteers were adults and signed an informed consent prior to their inclusion in the study.

Biochemical assays

Blood samples were obtained after overnight fasting and then separated into serum and plasma. Plasma was collected in tubes containing 0.15 g/100 g EDTA and centrifugated at 2400 rpm for 20 min at 4 °C, then placed into vials containing phenyl-methyl-sulfonyl-fluoride (0.015 g/100 g), sodium azide (0.01 g/100 g), and aprotinin (0.01 g/100 g). The sera were collected in tubes containing separator gel, and centrifuged at 3,000 rpm for 20 min at 4 °C.

Plasma total cholesterol (TC), triglycerides (TGs), and glucose were determined using enzymatic colorimetric methods (CHOD-PAP, GPO-PAP and GOD-PAP, respectively), all through commercial reagent sets (Roche Diagnostics, Mannheim, Germany). HDL-C was measured in the supernatant after precipitation of the apo B-containing lipoproteins, and low-density lipoprotein (LDL-C) was determined using the Friedwald equation (15).

Fasting plasma insulin concentration was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (Grupo Mexlab, Jalisco, Mexico). The homeostasis model of assessment (HOMA) was used to calculate insulin resistance (IR) according to the following equation: IR (HOMA IR) – fasting

insulin ($\mu\text{U}/\text{mL}$) fasting glucose (mmol/L) \div 22.5 (16). To measure autoantibodies against oxidized LDLs an indirect ELISA oLAB kit (ALPCO, Salem, NH, USA) was used according to the manufacturer's protocol. The activity of the PON1 enzyme was measured by the arylesterase/paraoxonase assay kit (ZeptoMetrix Corporation, NY, USA). PON1 concentration was quantified through a quantitative sandwich ELISA technique using the Human Serum Paraoxonase-1 (PON1) ELISA kit (Aviscera Bioscience, Inc., CA, USA) according to the manufacturer's instructions.

DNA extraction and genotyping of the R1587K polymorphism in the *ABCA1* gene

A sample of peripheral blood was processed for genomic DNA (gDNA) extraction using the phenol-chloroform and proteinase K method (17). DNA quality and integrity were assessed by visualization on 1% agarose gel and SYBR Safe® DNA staining (Life Technologies, USA). The concentration and purity of gDNA were measured on a spectrophotometer (NanoDrop™ 1000, Thermo Fisher Scientific, USA). gDNA samples were stored at $-20\text{ }^{\circ}\text{C}$ until the genotyping assay was performed.

The genotypes of the R1587K SNP (rs2230808) were obtained by qPCR (StepOne™, Applied Biosystems, Foster City CA, USA) using TaqMan allelic discrimination probes. Amplification assays were performed according to the standard procedures, using 5 μL of TaqMan Genotyping Mix 2X (Applied Biosystem, Foster City, CA, USA), 0.25 μL of each TaqMan genotyping probe (20x), 20 ng of gDNA, and the corresponding volume of deionized sterile water to complete a total reaction volume of 25 μL . The analysis of PCR data and the genotyping was performed with the StepOne software (version 2.3, Life Technologies Corporation, USA).

Clinical data

Body composition parameters were measured using a bioelectrical impedance equipment (BIA-101A, RJL Systems, Inc., MI, USA). Body mass

index (BMI) was calculated by dividing the weight in kg by the square of height in m. Waist circumference (WC) was measured midway between the lower rib and the iliac crest. Blood pressure was monitored with an automatic blood pressure monitor (HEM-7220, OMRON, USA). Physical activity level (PAL) was evaluated employing the three day record method, and classified into levels according to multiples of basal metabolic rate (18).

MetS diagnosis and atherogenic index of plasma (AIP)

The MetS diagnosis was carried out as stated by the American Heart Association and the National Heart, Lung, and Blood Institute (19). To evaluate CV risk the AIP was calculated as the logarithmic function of the ratio of plasma TG concentration to HDL-C as Log (TG / HDL-C) , and the value of AIP was classified as low CV risk (< 0.11 mmol/L), moderate CV risk (0.11-0.21 mmol/L), and high CV risk (> 0.21 mmol/L) (20).

Statistical analysis

Data normality was verified by the D'Agostino-Pearson test, and variables with normal distribution were described with their mean \pm SD values, whereas the median and 25-75th percentiles were used for variables with non-parametrical distribution. Categorical variables were expressed as percentage and absolute frequency. The relationship between clinical and parametric variables with the R1587K SNP was assessed by differences between groups with Student's t-test or Mann-Whitney U-test for independent variables, according to the normality distribution. To evaluate the association between *ABCA1* R1587K polymorphism genotypes and CV risk, the presence of metabolic syndrome a chi-squared test was performed to estimate the odds ratio (OR) and the 95% CI. Analyses were carried out using the IBM SPSS Statistics 25 (IBM Corporation, USA) package, and the significance level was set at $p < 0.05$.

RESULTS

Participant characteristics and differences by R1587K genotype

The study population was integrated by 57 participants (29 men and 28 women) with an average age of 38 ± 10 years. Genotypes were distributed as follows: 63.2% had the RR genotype, 26.3% the RK genotype, and 10.5% the KK genotype. Therefore, for statistical purposes we decided to group the RK and KK genotypes (34.1% had RK + KK) for comparison with RR carriers, assuming that the presence of one or two K alleles would modify the risk for the assessed variables.

BMI showed that both study groups were overweight, had an excess of body fat percentage, elevated LDL-C levels, and were also sedentary according to their physical activity level (21). The RR and the RK + KK genotypes carriers had elevated LDL-C and decreased HDL-C levels as compared to the NCEP ATP III guidelines (22). RR carriers showed a moderate CV risk, whereas the RK + KK group had a high CV risk according to the AIP.

As seen in table I, triglyceride levels were significantly higher among the RK/KK genotype carriers than among the RR genotype carriers. Also, the RK/KK carriers showed a tendency to greater waist circumference, TC levels, anti-oxLDL Abs, and atherogenic index of plasma when compared to RR carriers. However, no significant relationship was detected between *ABCA1* R1587K genotypes (RR and RK + KK) with BMI, fat-free mass, fat mass, blood pressure, physical activity, smoking, alcohol consumption, fasting glucose, fasting insulin, HOMA-IR index, LDL-C levels, HDL-C levels, and PON1 concentration and activity (Table I).

Association of R1587K genotypes with CV risk and MetS

The RK + KK genotypes were associated with MetS presence (OR = 4.566, 95% CI = 1.386-14.92, $p = 0.010$). Also, the RK + KK genotypes showed a tendency to association with higher CV risk based on the AIP score (OR = 2.800, 95% CI = 0.910-8.611, $p = 0.069$).

DISCUSSION

The major pathway in HDL particle biogenesis is the RCT, in which the ABCA1 protein plays a pivotal role by stimulating the cholesterol efflux, and precluding the foam cell formation, that induce the atherosclerotic lesion. Several SNPs in the *ABCA1* gene have been described. In particular, the R1587K polymorphism has been associated with dyslipidemias and CV risk, but no information exists regarding its relationship with a highly related predecessor of these diseases such as the MetS, and other non-common metabolic and cardiovascular parameters such as lipid-related enzymes and antibodies involved in these pathways. Therefore, we decided to analyze the R1587K SNP in the *ABCA1* gene and to evaluate its association with CV risk and MetS presence, as well as its possible role in the lipid profile, PON1 activity and concentration, and anti-oxLDL titers of a northern Mexican population. We found an association between the RK + KK genotype and the presence of MetS, as well as higher TG levels and a tendency towards high CV risk among the RK + KK genotype carriers. However, this polymorphism showed no association with HDL-C levels, PON1, and anti-oxLDL titers.

The frequency of R187K genotypes in *ABCA1* observed in our study was 63.2% for RR and 36.8% for the RK + KK genotypes; to our knowledge, this is the first time that R1587K genotypes have been related to MetS in a Mexican population, having previously been mainly described in Caucasians. These frequencies are similar to that previously reported in children and adolescents from Poland in remission of nephrotic syndrome, reporting a frequency of 58% of the RR genotype and 42% of the RK + KK genotype (23). A similar distribution of these genotypes was reported in a Dutch population of men with proven coronary artery disease, as well as in the Danish general population (24,25). However, these frequencies differ from those in a group of young Greek nurses, showing a reduced frequency of the RR genotype (47.08 %) in comparison to the RR + KK genotypes (52.92%) (26). These differences are likely explained by the genetic heterogeneity extant among populations, which translates into different phenotypes,

highlighting the importance of replicating these genetic studies in different populations.

The R1587K SNP, specifically the K allele, in the *ABCA1* gene has been previously related with dyslipidemias, specifically with high TC, LDL-C, and TG levels, as well as with lower HDL-C levels in both men and women of the general population or with proven CVD of different age groups (12,13). Although we observed low HDL-C levels among the participants, the concentrations of this lipid fraction showed no relationship with the R1587K SNP; however, TG level turned out to be related with this SNP, being higher among RK + KK carriers. Also, we observed that carriers of the RK + KK genotypes tended to have higher TC and anti-oxLDL Ab levels, abdominal obesity, and high CV risk (as evaluated by the AIP), although not reaching statistical significance. This difference in plasmatic TG levels between the RK + KK genotype carriers as compared to RR genotype carriers is consistent with that reported by Kolovou et al. in Greek women without a history of coronary artery disease (26), and with the tendency described by Ksiazek et al. in children and adolescents undergoing nephrotic syndrome remission (23). Conversely, Jensen et al. reported a lower concentration of TGs in women without CV disease who were carriers of the K allele of this SNP (27). Even though the direct connection between the ABCA1 protein and TG levels is not fully understood, there is a direct relationship between HDL-C levels and TGs, seen through HDL modeling, where several processes are involved. One of these is mediated by the cholesteryl ester transfer protein (CETP), where an exchange of cholesteryl esters (CE) from HDL to TGs in apoB-containing lipoproteins (LDL/VLDL) occurs (28). This exchange ends [in apoB-containing lipoproteins enriched with CEs and drained of TGs, and in HDL particles depleted of CEs and high in TGs](#). The clearance of these HDL particles enriched with TGs is faster than that of those enriched with CEs, thus an increase in TG levels triggers a decrease in HDL-C levels (29). This reduction in HDL-C levels may also result in changes in the contents and activities of related proteins. Nevertheless, whether the low HDL-C levels

observed in our study are due to an increase in TG levels or to the presence of a R1587K SNP remains unknown. Therefore, further and larger-scale studies including different genetic variants and proteins involved in lipid metabolism are required.

Another interesting finding was the association between the RK + KK genotypes and the presence of MetS, in addition to the marginal association detected between the RK + KK genotypes and high CV risk, according to the AIP. This is consistent with the fact that hypertriglyceridemia, alone or in combination with low HDL-C concentrations, has been described as a major factor related to MetS (30). Even though LDL-C has been widely known as one of the major atherogenic lipids, the association between hypertriglyceridemia and risk of CVD has been extensively described (31). Nevertheless, this high risk cannot be completely attributed to the presence of the R1587K SNP, since this population presents with other CV risks including elevated LDL-C and low HDL-C levels, low physical activity, and altered body composition (elevated body fat percentage, abdominal obesity, and high BMI), all of which contribute to increased CV risk.

A limitation of this study was its relatively small number of participants, so it is necessary to further perform wider studies where these associations with the R1587K genotypes may be corroborated. However, this study adds new information regarding the role of this SNP in the northern Mexican population. Also, we included a novel and common cardiovascular risk biomarker to help elucidate the findings.

In summary, the R1587K SNP of the *ABCA1* gene was related to TG level; specifically, RK + KK genotype carriers had higher TG concentrations than RR genotype carriers, while HDL-C levels, PON1 activity and concentration, and anti-oxLDL levels did not differ between genotypes. Further, an association was found between the RK + KK genotypes and the presence of MetS, as well as a tendency towards high CV risk, indicating that RK + KK carriers are more likely to develop CVD. These findings provide novel information about the presence and implications of the R1587K SNP of the *ABCA1* gene in lipid

metabolism and CV risk, and could contribute to understand and devise further interventions to prevent the clinical alterations commonly observed in this population.



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Table I. Demographic, body composition, clinical and lifestyle characteristics of the study population, stratified by genotype

Parameter	Total (n = 57)	RR (n = 36)	RK + KK (n = 21)	p
Age (years)	38 ± 10	37 ± 11	40 ± 10	0.233
Sex (male/female) (n)	29/28	16/20	13/8	-
BMI (kg/m ²)	28.16 ± 4.76	27.39 ± 4.08	29.48 ± 5.60	0.111
WC (cm)	91 [84.25-98.55]	89.9 [82.25-96]	94.20 [88-104.6]	0.064
FFM (kg)	53.33 ± 13.10	51.17 ± 12.22	57.03 ± 14.03	0.104
Fat mass (kg)	25.55 [20.63-30.29]	24.28 [19.58-28.79]	26.64 [21.93-32.94]	0.219
Fat mass (%)	31.72	31.59	33.24	0.951

	[25.67-41.45]	[25.12-41.77]	[25.82-41.32]	
SBP (mmHg)	114.3 ± 15.67	111.7 ± 15.97	118.8 ± 14.41	0.100
DBP (mmHg)	76.50 [70.75-84]	76.25 [70.63-81.75]	81.50 [71-85.75]	0.169
Physical activity (multiples of BMR)	1.65 [1.54-1.77]	1.64 [1.54-1.73]	1.68 [1.55-1.80]	0.405
Smoker (cigarettes/day)	0 [0-0]	0 [0-0]	0 [0-0.85]	0.140
Alcohol (mL/day)	66.66 [0-253.6]	44.37 [0-152.1]	71.42 [0-304.3]	0.386
Glucose (mg/dL)	89.78 ± 10.74	88.51 ± 9.99	91.95 ± 11.87	0.246
Insulin (μU/mL)	4.80 [3.95-5.91]	4.78 [3.81-4.78]	4.98 [4.22-6.16]	0.562
HOMA-IR index	1.11 [0.84-1.37]	1.08 [0.82-1.36]	1.14 [0.91-1.43]	0.380
TC (mg/dL)	178.23 ± 32.78	172.05 ± 32.50	188.81 ± 31.19	0.061
LDL-C (mg/dL)	105.34 ± 27.28	103.62 ± 28.49	108.29 ± 5.56	0.537
HDL-C (mg/dL)	39.44 ± 7.35	39.56 ± 6.68	39.23 ± 8.55	0.872
TG (mg/dL)	139.77 [107.70-202.57]	128.43 [100.27-192.71]	176.65 [127-227.2]	0.032
Anti-OxLDL Abs (mU/mL)	1,156 [934.20-1,317]	1,139 [933.60-1,278]	1,314 [905.60-1,319]	0.093
PON1 concentration (ng/mL)	21.16 ± 11.69	21.06 ± 11.44	21.38 ± 12.67	0.931

PON1 activity (KU/L)	56 [46-64.50]	53.63 ± 12.17	55.95 ± 8.69	0.448
AIP (mmol/L)	0.22 ± 0.24	0.178 ± 0.238	0.300 ± 0.241	0.069

Data are given as mean ± standard deviation or median [25-75 interquartile range]. BMI: body mass index; WC: waist circumference; FFM: fat-free mass; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMR: basal metabolic rate; HOMA-IR: homeostatic model assessment-insulin resistance; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; anti-OxLDL Abs: anti-oxidized low-density lipoprotein autoantibodies; PON1: paraoxonase-1; AIP: atherogenic index of plasma.

Table II. Association of *ABCA1* R1587K genotypes with CV risk and metabolic syndrome presence

	Total n (%)	RR n (%)	RK + KK n (%)	Odds ratio (95% CI)	χ^2	<i>p</i>
AIP						
Low/moderate risk	28 (49.1)	21 (58.3)	7 (33.3)	1.0 ^a	3.317	0.069
High risk	29 (50.9)	15 (41.7)	14 (66.7)	2.800 (0.910-8.611)		
MetS						
Without MetS	39 (68.4)	29 (80.6)	10 (47.6)	1.0 ^a	6.659	0.010
MetS	18 (31.6)	7 (19.4)	11 (52.4)	4.566 (1.386-14.92)		

Data are represented in counts (percentages within parentheses). AIP: atherogenic index of plasma; MetS: metabolic syndrome; p-values and ORs with 95% CIs were calculated using Pearson's χ^2 test; $p < 0.05$ was considered significant.