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Association of *TGFBR2* gene polymorphisms (rs6785358 and rs764522) with congenital heart disease susceptibility in Egyptians

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Abstract

Aim: Transforming growth factor beta (*TGF-β*) receptor II (*TGFBR2*) is a basic constituent of *TGF-β* signalling pathway and is important in heart development. This study investigates the relationship between *TGFBR2* gene variance and congenital heart defects (CHD) among Egyptians.

Methods: The study involved 75 CHD-affected subjects and 100 healthy controls. Genotyping of two selected tag single nucleotide polymorphisms (tagSNPs, rs6785358, rs764522) within the *TGFBR2* gene was conducted using polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) assays.

Results: Significant genotype differences were found for rs764522 and rs6785358 ($P < 0.05$). In the case of rs6785358, the G/G genotype was more prevalent in cases than controls (18.7% *vs.* 4.0%). This significance was observed in both the codominant model [A/A *vs.* A/G *vs.* G/G; odds ratio (OR) = 0.20, 95% confidence interval (CI) = 0.06–0.66, $P = 0.0073$] and the recessive model (A/A + A/G *vs.* G/G; OR = 0.19, 95% CI = 0.06–0.60, $P = 0.0018$). For rs764522, the G/G genotype was more prevalent in cases than controls (21.3% *vs.* 0.0%). Significant associations were observed in the codominant model (C/C *vs.* C/G *vs.* G/G; OR = 0.43, 95% CI = 0.02–0.90, $P < 0.0001$), as well as in the dominant model (C/C *vs.* C/G + G/G) and recessive model (C/C + C/G *vs.* G/G; $P < 0.0001$). Gender-specific analysis indicated that the C/G genotype was less common in male cases compared to females and controls (OR = 0.24, 95% CI = 0.07–0.84). For rs6785358, the G/G genotype frequency was higher in male cases compared to females and controls (OR = 0.10, 95% CI = 0.01–0.88 and OR = 0.22, 95% CI = 0.05–0.94, respectively).

Conclusions: These findings indicate that *TGFBR2* gene SNPs (rs6785358 and rs764522) may be risk factors for CHD in Egyptians.

Keywords

Transforming growth factor beta receptor II (*TGFBR2*), single nucleotide polymorphisms (SNP), congenital heart disease, case-control study

Introduction

Congenital heart defects (CHD) are structural issues with the heart that exist at birth [1]. These abnormalities can affect the heart's chambers, valves, or blood vessels that transport blood to and from the heart. The defects are frequently found alone or in combination with other defects [2, 3]. Physiologically, they are classified as cyanotic and acyanotic heart defects [4]. Cardiac abnormalities can range in complexity from simple that have no symptoms to complex lesions that have significant, life-threatening symptoms [5]. Congenital birth malformations can arise in children for a variety of reasons [6]. According to previous studies, genetic factors may be the primary cause of CHD [7].

Transforming growth factor beta (*TGF-β*) receptor II (*TGFBR2*), a transmembrane protein, is a member of the serine/threonine protein kinase family and the *TGFBR* subfamily with a molecular weight of 70/80 kDa [8]. The *TGFBR2* gene, which is found on chromosome 3p22 and has seven exons and a 567 codon open reading frame, is responsible for encoding the *TGFBR2* protein [9]. The TGF-β ligands bind to the TGFBR2 for the initiation of *TGF-β* signalling leading to the activation of the *TGFBR1*, which then causes the translocation to the nucleus following a series of processes, including phosphorylation of mothers against decapentaplegic homolog 2 (SMAD2) and SMAD3 with the association of SMAD4. SMAD proteins are involved in managing transcription of target genes which can be affected by *TGFBR2* inactivation [10]. The *TGFBR2* gene mutation in aortic pathology has been reported, and during cardiac development, the endothelial cells might be affected by *TGFB* signalling through *TGFBR2* [11]. The valvuloseptal heart defect may occur due to disruptions in the signal transformation of endocardial cushions [12]. *TGFBR2* is expressed during heart development in embryonic myocytes, endothelial cells, and endocardial cushion of mice [13].

It is known that genetic polymorphisms may have an impact on the susceptibility or resistance to certain infections [14] or have a key role in the contribution of the susceptibility to some diseases [15, 16] and personalized nutrition [17]. The relation between *TGFBR2* gene polymorphisms (rs6785358 and rs764522), the 5' upstream were also named -3779A/G and -1444C/G, respectively, and some disorders, such as Marfan syndrome and cardiac arrest, were reported [18, 19].

This study aims to explore the link between *TGFBR2* gene polymorphisms (rs6785358 and rs764522) and CHD in Egyptians. To our knowledge, among the studied population, this is the first report focusing on this issue.

Materials and methods

This study involved 75 cases affected by CHD, 21 had atrial septal defect (ASD), 43 had ventricular septal defect (VSD), and 11 had tetralogy of Fallot (TOF), from the Department of Cardiology, Internal Medicine Specialized Hospital, Mansoura University, Egypt. While normal controls were enrolled via invitations.

Samples collection

Peripheral blood samples were taken in sterile EDTA tubes (Kemico Vacutainer, Egypt) for genotyping and hematological evaluations. Additionally, blood aliquots were taken in serum tubes for other laboratory tests. Sera were stored at –20°C until use. All samples were identified and given matched numbers that corresponded to all investigations.

Laboratory investigations

Physical inspections and routine questionnaires were done for all patients. Hematological parameters, such as hemoglobin, white blood cells (WBCs), and platelets, were investigated using Sysmex KX21 Hematology

Analyzer (Sysmex Corporation, Japan). Other standard biochemical analyses, such as fasting blood sugar, aspartate transaminase (AST), alanine transaminase (ALT), urea, and creatinine (Biosystem, Spain), were performed.

Single nucleotide polymorphisms selection

Single nucleotide polymorphisms (SNPs) under investigation were selected according to PubMed published data (SNP database). To see if the promoter region harboured any genetic variants susceptible to CHD, we chose two tagSNPs, 5' upstream, of the *TGFBR2* gene (rs6785358 and rs764522).

Isolation of total genomic DNA

In an EDTA-containing tube, approximately 4 mL of venous blood samples were collected. Genomic DNA extraction was done from samples using the phenol-chloroform method in the presence of proteinase K digestion (Sigma-Aldrich, Germany). Using a UV spectrophotometer (Spectronic 1201, Milton Roy, USA), the absorbance at 260 nm was used for DNA concentration, however, the A_{260}/A_{280} nm was used for purity determination.

Genotype assessment

To examine the allelic variance, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was done. The primer sequences of the rs6785358 were 5'-GAACTGCAAACAAGAGAATGGAT-3' (forward) and 5'-TTAGAATTCTACCCTAATGATTGTAAGG-3' (reverse), however, the rs764522 primers were 5'-GAGTGAAAGAGCCCAGAACG-3' (forward) and 5'-GGGCTAGGCATCTTCTTTCC-3' (reverse) [13]. A total volume of 10 µL of PCR reaction containing 10 ng of DNA, 0.5 pmol of each primer, and 1 × PCR master mix was prepared. Using an GeneAmp™ PCR System 9700 (Thermo Fisher, USA), the amplifications were accomplished. The reaction program proceeded as follows: one cycle for 5 min at 95°C, 30 cycles at 95°C for 30 s, 30 s for annealing at 61°C (rs6785358)/63°C (rs764522), and 30 s at 72°C.

A final extension cycle at 72°C for 10 min. The PCR products were digested using the restriction enzymes BsuRI and MvaI (Thermo Fisher, USA), for rs6785358 and rs764522, respectively [13].

Statistical analysis

Fisher's exact χ^2 test was used for Hardy-Weinberg equilibrium assessment (HWE 14 program in SPSS). The SPSS for Windows version 13.0 (SPSS Inc, USA) was used for performing statistical analyses. The Chi-square (χ^2) test was done for the analysis of allelic variation among studied subject groups. The relation was illustrated as odds ratio (OR) with 95% confidence interval (95% CI) of the risk. The level of statistical significance was set at 0.05 (two tails). Also, Binary logistic regression was done for the association between different variables and the disease status.

Results

Demographic and clinical characteristics

The characteristics of the cases (n = 75), and control (n = 100) groups are listed in Table 1. Regarding age or sex, the demographic results revealed a non-significant difference between the studied groups (P > 0.05). However, non-significant differences among studied groups regarding all laboratory investigations (P > 0.05) were noticed, except AST, hemoglobin, and platelets (P < 0.05).

Genotyping and agarose gel electrophoresis for rs764522

The MvaI-digested PCR fragments were separated using the agarose gel electrophoresis method. The PCR amplicon was seen at 192 bp. For rs764522, the C allele was not digested and appeared as a single band at 192 bp. However, the G allele strand was digested into 41 bp and 151 bp fragments.

Table 1. Demographic and laboratory data of the studied groups

Parameter	Controls (*n* = 100)	CHD (*n* = 75)	*P*-value
Age (years)	4.59 ± 2.34	4.56 ± 2.30	0.934 (*t*-test)
Sex			0.300 (χ^2)
Female	55 (55%)	35 (47%)	
Male	45 (45%)	40 (53%)	
Hb (mg/dL)	11.91 ± 0.60	86.01 ± 5.00	0.001*
WBCs × 10^3	6.28 ± 0.62	6.40 ± 0.62	0.212
Platelets × 10^3	246.70 ± 31.96	261.38 ± 45.98	0.014*
FBS (mg/dL)	86.07 ± 5.66	86.01 ± 5.00	0.945
ALT (U/L)	23.03 ± 2.90	23.92 ± 3.32	0.061
AST (U/L)	25.27 ± 2.91	27.82 ± 6.72	0.001*
Urea (mg/dL)	24.59 ± 3.47	24.09 ± 3.61	0.359
Creatinine (mg/dL)	0.53 ± 0.09	0.50 ± 0.11	0.050

FBS: fasting blood sugar; Hb: hemoglobin; *: *P*-value significant < 0.05. Data were illustrated as mean ± standard deviation (SD)

In Table 2, there are different models for testing the association of *TGFBR2* rs764522 gene polymorphism with CHD. Herein, the G/G genotype frequency is much higher among cases compared to controls (21.3% *vs.* 0.0%). This might imply that the G/G genotype is a predisposing factor to the occurrence of CHD. A positive significance in the codominant model (C/C *vs.* C/G *vs.* G/G; OR = 0.43, 95% CI = 0.02–0.90, $P < 0.0001$) was noticed, as well as, in the dominant model (C/C *vs.* C/G + G/G) and recessive one (C/C + C/G *vs.* G/G; $P < 0.0001$).

Table 2. Testing genetic association of *TGFBR2* (rs764522) gene polymorphism with CHD (*n* = 175, adjusted by sex)

Genotypic states	Genotype	Cases	Controls	OR (95% CI)	*P*-value
Codominant	C/C	14 (18.7%)	40 (40.0%)	1.00	< 0.0001*
	C/G	45 (60%)	60 (60%)	0.43 (0.20–0.90)	
	G/G	16 (21.3%)	0 (0.0%)	0.00 (0.00–NA)	
Dominant	C/C	14 (18.7%)	40 (40.0%)	1.00	0.001*
	C/G + G/G	61 (81.3%)	60 (60.0%)	0.31 (0.15–0.65)	
Recessive	C/C + C/G	59 (78.7%)	100 (100.0%)	1.00	< 0.0001*
	G/G	16 (21.3%)	0 (0.0%)	0.00 (0.00–NA)	
Overdominant	C/C + G/G	30 (40%)	40 (40%)	1.00	0.86
	C/G	45 (60%)	60 (60%)	0.94 (0.51–1.76)	

NA: not applicable; *: *P*-value significant < 0.05

The *TGFBR2* (rs764522) gene polymorphism cross interaction with gender of cases and controls is displayed in Table 3. The genotype C/G frequency was noticed to be lower in male cases than in females and controls (OR = 0.24, 95% CI = 0.07–0.84).

Table 3. *TGFBR2* (rs764522) gene polymorphism cross interaction with gender of cases and controls (*n* = 175, crude analysis)

TGFBR2 (rs764522) genotype	Female			Male		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
C/C	4	18	1.00	10	22	0.49 (0.13–1.82)
C/G	24	37	0.34 (0.10–1.14)	21	23	0.24 (0.07–0.84)
G/G	7	0	0.00	9	0	0.00
P-value	$P < 0.0001$*			$P = 0.0013$*		

*: *P*-value significant < 0.05

In Table 4, the *TGFBR2* (rs764522) gene polymorphic genotypes are revealed among cases of different types of CHDs compared to controls. The C/C genotype frequency in the VSD cases was much lower compared to controls (14% *vs.* 40%). On the other hand, the G/G genotype frequency was higher in ASD cases compared to controls (20.9% *vs.* 0.0%) with a significant statistical difference ($P < 0.0001$).

Table 4. *TGFBR2* (rs764522) gene polymorphic genotypes among different types of CHD cases compared to controls

***TGFBR2* (rs764522) genotype**	**Controls**	**VSD**	**ASD**	**TOF**	**Total**
C/C	40	6	4	4	54
	40.0%	14.0%	19.0%	36.4%	30.9%
G/G	0	9	5	2	16
	0.0%	20.9%	23.8%	18.2%	9.1%
C/G	60	28	12	5	105
	60.0%	65.1%	57.1%	45.5%	60.0%
Fisher's exact test – *P*-value	34.143 – $P < 0.0001$*				

*: *P*-value significant < 0.05

Genotyping and agarose gel electrophoresis for rs6785358

For rs6785358, the BsuRI-digested PCR products were resolved on agarose electrophoresis. The results revealed that the A allele remained intact as a single 176 bp band. However, the G allele strand was digested into 147 bp and 29 bp fragments.

The different models for testing the association of *TGFBR2* (rs6785358) gene polymorphism with CHD are given in Table 5. The results revealed that the G/G genotype frequency is much higher among cases compared to controls (18.7% *vs.* 4.0%, respectively). This might imply that this genotype is a predisposing factor to the occurrence of CHDs. Moreover, a positive significance in the codominant model (A/A *vs.* A/G *vs.* G/G; OR = 0.20, 95% CI = 0.06–0.66, $P = 0.0073$) was noticed, as well as in the recessive model (A/A + A/G *vs.* G/G; OR = 0.19, 95% CI = 0.06–0.60, $P = 0.0018$).

Table 5. Testing genetic association of *TGFBR2* (rs6785358) gene polymorphism with CHD ($n = 175$, adjusted by sex)

Genotypic states	**Genotype**	**Cases**	**Controls**	**OR (95% CI)**	***P*-value**
Codominant	A/A	31 (41.3%)	46 (46.0%)	1.00	0.0073*
	A/G	30 (40%)	50 (50%)	1.13 (0.59–2.14)	
	G/G	14 (18.7%)	4 (4.0%)	0.20 (0.06–0.66)	
Dominant	A/A	31 (41.3%)	46 (46.0%)	1.00	0.56
	A/G + G/G	44 (58.7%)	54 (54.0%)	0.83 (0.46–1.53)	
Recessive	A/A + A/G	61 (81.3%)	96 (96.0%)	1.00	0.0018*
	G/G	14 (18.7%)	4 (4.0%)	0.19 (0.06–0.60)	
Overdominant	A/A + G/G	45 (60%)	50 (50%)	1.00	0.19
	A/G	30 (40%)	50 (50%)	1.49 (0.81–2.74)	

*: *P*-value significant < 0.05

The *TGFBR2* (rs6785358) gene polymorphism cross interaction with gender of subjects is shown in Table 6. The results indicated that the genotype G/G frequency was noticed to be higher in male than female cases and controls (OR = 0.10, 95% CI = 0.01–0.88 and OR = 0.22, 95% CI = 0.05–0.94, respectively).

The *TGFBR2* (rs6785358) gene polymorphic genotypes among cases of different types of CHD compared to controls revealed that the G/G allelic frequency in the VSD cases was much higher in cases compared to controls (25.6% *vs.* 4.0%) with a significant statistical difference ($P = 0.002$, Table 7).

The Hardy-Weinberg genetic equilibrium analysis for the *TGFBR2* gene variant (rs764522) showed a negative equilibrium in both the studied subjects and control samples, with significant *P*-values of 0.0008 and 0.0001, respectively. This deviation is likely due to the very low frequency of the G/G genotype among the controls. However, in the cases group, a positive Hardy-Weinberg equilibrium ($P = 0.11$) was observed.

Table 6. *TGFBR2* (rs6785358) gene polymorphism cross interaction with gender of cases and controls (*n* = 175, crude analysis)

***TGFBR2* (rs6785358) genotype**	**Female**			**Male**		
	Cases	**Controls**	**OR (95% CI)**	**Cases**	**Controls**	**OR (95% CI)**
A/A	15	26	1.00	16	20	0.72 (0.29–1.80)
A/G	14	28	1.15 (0.47–2.85)	16	22	0.79 (0.32–1.96)
G/G	6	1	0.10 (0.01–0.88)	8	3	0.22 (0.05–0.94)
P-value	*P* = 0.0287*			*P* = 0.1845		

*: *P*-value significant < 0.05

Table 7. *TGFBR2* (rs6785358) gene polymorphic genotypes among cases of different types of CHD compared to control

***TGFBR2* (rs6785358) genotype**	**Controls**	**VSD**	**ASD**	**TOF**	**Total**
A/A	46	18	9	4	77
	46.0%	41.9%	42.9%	36.4%	44.0%
G/G	4	11	0	3	18
	4.0%	25.6%	0.0%	27.3%	10.3%
A/G	50	14	12	4	80
	50.0%	32.6%	57.1%	36.4%	45.7%
Fisher's exact test – *P*-value	19.690 – *P* = 0.002*				

*: *P*-value significant < 0.05

Regarding another gene variant (rs6785358) within *TGFBR2*, the analysis revealed a positive equilibrium in both the studied patients and controls, with *P*-values greater than or equal to 0.05, indicating no significant deviation from the equilibrium.

Regression modeling is a statistical method employed to examine the connections between variables. In the context of assessing the correlation between genetic markers and disease status, logistic regression is commonly used. The analysis involved evaluating the association of each SNP. In cases of binary responses, the outcomes of logistic regression analysis are succinctly presented in Table 8, encompassing significance levels, OR, and 95% CI. The findings indicate a noteworthy association between the GG genotype of rs6785358, as opposed to the CC genotype, and cardiovascular disease in children. Additionally, the CC genotype of rs764522 exhibited a significant association with the disease. Conversely, the variable of sex displayed a lack of significant association with the disease.

Table 8. Binary logistic regression between each SNP and the incidence of CHD

Variable	**B**	**Chi-square**	***P*-value**	**OR**	**95% CI**	
					Lower	**Upper**
Sex	0.423	1.332	0.248	1.526	0.745	3.127
rs6785358 (total)		8.528	0.014			
rs6785358 (AA)	–0.022	0.003	0.955	0.979	0.460	2.083
rs6785358 (GG)	1.836	7.802	0.005	6.272	1.729	22.747
rs764522 (total)		4.951	0.084			
rs764522 (CC)	–0.934	4.951	0.026	0.393	0.172	0.895
rs764522 (GG)	21.342	0.000	0.998		0.000	
Constant	–0.718	3.294	0.070	0.488		

B: binary logistic regression coefficient. Blank cells represent "not detected". rs6785358 (AG), rs764522 (CG) are used as reference for each related SNP

Discussion

A set of structural and functional deficiencies called CHD occurs during the development of the heart. Furthermore, CHD is the most common cause of child death which is linked to birth defects, and it accounts for one-third of all serious congenital malformations [20]. Since 2000, the prevalence of CHD has increased by more than 50% worldwide. There are notable geographic variances, with Asia reporting the greatest CHD birth frequency while Europe was much higher than North America [21–23].

The *TGFB* signalling pathway plays an important role in the generation of the heart. The signalling of *TGF-β* regulates a variety of biological roles, including cell growth, differentiation, matrix production, and apoptosis in a wide range of cell types [10, 24]. The lethal aortic and cardiac defects were reported due to the inactivation of *TGFBR2* in smooth muscle cells and epicardium [25]. Moreover, the *TGFB* is required during *in vivo* cardiac development [26]. Numerous human congenital illnesses, such as Marfan syndrome, Loeys-Dietz syndrome, neoplasms, aortic aneurysms and dissections, nonsegmental vitiligo, intracerebral hemorrhage, and sudden cardiac arrest in coronary artery disease patients are associated with genetic changes of the *TGFBR2* gene [18, 19], which can be detected during the generation of the heart. Several studies propped up to determine the role of *TGFBR2* in heart development based on mouse models of depleting *TGFBR2* in special-cells. The endocardial depletion of *TGFBR2* caused defects in the ventricular septal and double-inlet left ventricle [26]. The conditional deletion of *TGFBR2* gene in smooth muscle cell-specific protein-expressing mice cells caused death during the last third of gestation, heart defects such as hypoplasia of the compact zone of the myocardium, ventricular, and atrial abnormalities were noticed in about half of mice [26]. *TGFBR2* was essential for the development of the heart's endothelial cells, and when its expression was inhibited, the ventricular septum was not properly formed [11]. The substantial reduction in transcriptional activities and loss of *TGFBR2* gene expression may result in promoter mutation [27].

In the present study, it is revealed that the G/G genotype frequencies of *TGFBR2* (rs764522) were much higher among cases compared to controls (21.3% *vs.* 0.0%, respectively). This might imply that this genotype could be a predisposing factor to the occurrence of CHD. Moreover, a positive significance within the codominant and dominant model ($P \leq 0.001$) was evidenced. Our results were consistent with the previous study on *TGFBR2* (rs6785358) which revealed a significant association between the carrier of the A/G + G/G genotype and the risk of congenital heart defects compared to A/A genotype in the Chinese population [13]. Similarly, in Han Chinese population, the study of Li et al. [28] proved the association of *TGFBR2* (rs6785358) SNP, A/G + G/G variant, with the susceptibility to congenital ventricular septal heart defects. An increased risk of CHD in males was noticed among G allele-carrying individuals (A/G + G/G genotypes, rs6785358) but not in females [13]. Moreover, the allelic variants in rs6785358 were significantly different between the male and female subgroups in cases and controls [28]. In the current study, the cross interaction with gender revealed that the C/G frequency (rs764522) was lower in male than female cases and controls. However, the genotype G/G frequency (rs6785358) was noticed to be higher in male than female cases and controls. Previously, gender variation was confirmed to be significant in specific CHD subgroups [29]. Hormone modulation controls the activation of the TGF-signalling pathway [30], and this may help to explain why sex influences the relationship between the *TGFBR2* gene and the risk of congenital cardiac abnormalities. Additionally, mutations in sex and autosomal chromosomes may have an impact on the risk for CHD [31]. We should draw attention to the possible limitations in the present study, including its small sample size and pilot-study design among just two tagSNPs in the *TGFBR2* gene. In order to determine whether tagSNPs or functional SNPs covering the *TGFBR2* gene possess any mutations that are sensitive to congenital cardiac abnormalities in Egyptian patients, we advise doing further, larger, and multicenter investigations with more inclusion of other CHD medical manifestations.

Conclusions

This study showed that SNP rs6785358 and rs764522 of *TGFBR2* gene were associated with an elevated risk of CHD in the Egyptian population. In the future, the results offer an opportunity for the development of a novel early genetic detection of CHD risk.

Abbreviations

ASD: atrial septal defect

AST: aspartate transaminase

CHD: congenital heart defects

CI: confidence interval

OR: odds ratio

PCR: polymerase chain reaction

SMAD2: mothers against decapentaplegic homolog 2

SNPs: single nucleotide polymorphisms

tagSNPs: tag single nucleotide polymorphisms

TGFBR2: transforming growth factor beta receptor II

TGF-β: transforming growth factor beta

TOF: tetralogy of Fallot

VSD: ventricular septal defect

Declarations

Author contributions

ND: Formal analysis, Investigation, Writing—original draft. ESS and AAHE: Formal analysis, Investigation, Writing—original draft, Writing—review & editing. FRA, IE, and RE: Conceptualization, Writing—original draft, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Ethical approval

The Ethical Committee at the Faculty of Medicine, Menoufia University approved the study (No. 11.2/2020 INTM2). The study is in accordance with the ethical standards of institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Consent to participate

An informed consent was obtained from the parents of all participating subjects of this study.

Consent to publication

Not applicable.

Availability of data and materials

The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

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