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FULL LENGTH ARTICLE

Maternal vitamin D level and vitamin D receptor gene polymorphism as a risk factor for congenital heart diseases in offspring; An Egyptian case-control study

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Abstract Vitamin D & vitamin D receptor (VDR) signaling play a very crucial role in early embryonic heart development. We construct this case-control study to investigate the association between maternal serum vitamin D level & VDR gene Fok1 polymorphism and risk of congenital heart defects (CHD) in offspring. Fifty mothers who had term neonates with CHD were considered as cases. Fifty age-comparable healthy mothers who had neonates without CHD were contemplated as controls. Maternal serum 25 hydroxyvitamin D [25(OH) D] level was tested using ELISA. Maternal VDR gene Fok1 polymorphism was analyzed using PCR-based RFLP-assay. There was a significant decrease in maternal vitamin D level ($P = 0.002$) and a significant increase in vitamin D deficient status ($P = 0.007$) among cases when compared to controls. VDR gene Fok1 genotypes distribution frequency were in accordance with Hardy Weinberg equilibrium (HW) among controls. A significant increase in VDR gene Fok1 F/f & f/f genotypes and f allele were observed in cases compared to controls with estimated odds ratio (95% confidence interval) & *P*-value of 3 (1–8) & $P = 0.006$, 11 (1–97) & $P = 0.01$ and 3 (2–6) & $P = 0.001$ respectively. There was a significant decrease in maternal vitamin D level in neonates with cyanotic CHD ($P = 0.000$) compared to those with a cyanotic CHD while there was no significant difference in VDR Fok1 genotype ($P = 0.18$) & allele ($P = 0.05$) distribution between two groups. We concluded that maternal vitamin D deficiency and VDR gene Fok1 F/f, f/f genotype and f allele were associated with increased risk of CHD in offspring.

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Introduction

Congenital heart defects (CHD) are assured to be one of the highly crucial & universally allocated types of congenital malformations that exemplify nearly (one-third) of the overall congenital-malformations evoked infant-mortality.[1] The underlying patho-etiological mechanisms of CHD are mostly multifactorial and only 15% of CHD can be related to known cause.[2] It has been formerly actualized that the interactive synergism between diverse genetic propensity and maternal environmental risk factors including (maternal nutritional status and maternal health & lifestyle before conception) implicate an influential role in the consequences of fetal organs development and pathogenesis of nearly almost CHD.[3] Vitamin D; which is one of structurally-related fat-soluble vitamins, possess cardinal role in mineral neutralization and balance, calcium homeostasis and skeletal system function & health.[4] Presently, vitamin D has been settled as a pleiotropic hormone that modulates plentiful biological processes of various organs and body systems including neural, endocrinal, immune and cardiovascular system.[5,6] The biological actions of vitamin D are arbitrated through binding of its active metabolite 1, 25 Dihydroxyvitamin D (1, 25$(OH)_2$D) to vitamin D receptors (VDRs). VDRs are a member of nuclear receptor superfamily that arbitrate the physiological effect of vitamin D via regulation of various genes which have been enclosed in vitamin D metabolic pathway.[7] VDRs gene; which is encoded on chromosome (12q13.11); is an extremely polymorphic gene.[8] A reported number of functional single-nucleotide-polymorphisms (SNPs) of VDRs gene had been genotyped. Interestingly, it has been emphasized that certain SNPs were correlated to impaired concentrations of vitamin D in the circulation.[9] In last decade, numerous reports had been emerged and strongly focused on the probable role of these polymorphisms in a diversity of human diseases and pathologies including the risk of cancer,[10,11] infectious diseases,[12] autoimmune disorders,[13] and cardiovascular diseases.[14,15] Formerly conducted researches actualized that VDRs had been expressed in almost all tissues and individual cells including all cardiovascular cells. Moreover; recent in vivo animal studies disclosed that vitamin D and VDRs signaling are entangled in early embryonic cardiac development.[16,17] Up to our knowledge, the present study was the first study that investigates the association between certain VDR gene polymorphism and risk of CHD. We hypothesized that there may be a probable role of both maternal VDR gene (Fok1) polymorphism and low circulating vitamin D in the risk of having CHD in offspring.

Materials and methods

A case-control study was carried out on fifty mothers who gave birth to an equal number of term neonates with CHD who were diagnosed during the first two weeks of life. They were recruited from our tertiary neonatal-intensive-care unit (NICU) at Zagazig university children hospital, Zagazig University, Egypt in a period from January 2016 to Mai 2018. Fifty age-comparable healthy mothers who gave birth to age & sex harmonized term neonates without congenital heart defects were taken as a control group. Diagnosis of congenital heart diseases was confirmed by the pediatric cardiologist using ECHO. Mothers who had neonate suffered from sepsis, proven congenital infection, genetic syndromes or multiple congenital malformations were excluded from the study. Furthermore; mothers, who disclosed a history of diseases during pregnancy including (diabetes, hypertension, autoimmune disease, renal insufficiency, epilepsy), drug intake (teratogenic drugs or drugs antagonize folate metabolism) and those who were proven to have an infection or congenital infection during pregnancy were excluded from the study. Both cases and control groups were invited to fill a questionnaire including information about maternal age, level of education, residency, history of previous abortion or stillbirth, previous sibling with CHD, exposure to any risk factors during pregnancy, special habits, use of vitamin D or multivitamin supplementation containing vitamin D during pregnancy. Regarding neonates, a thorough history taking and detailed clinical examination were taken for both cases and controls.

We defined pattern of vitamin D supplementation during pregnancy as 1) non user: if mother reported no regular intake of vitamin D during a period of 6 months (3 months before pregnancy and first 3 months after pregnancy) 2) Peri-conception user:if mother reported regular intake of vitamin D during a period of 6 months (3 months before and first 3 months after pregnancy) 3) early post-conception user: if mother reported regular vitamin D intake from the first month to the third month of pregnancy 4) late post-conception user: if mother reported regular vitamin D intake after the first 3 months of pregnancy.[18] We also defined "regular vitamin D intake" as using vitamin D supplements with a dose of more than 600IU/day at least three times per week.[18,19] Neonates with CHD were classified according to main clinical presentation into [Group1] Those with a cyanotic congenital heart defects, [Group 2] Those with cyanotic congenital heart defects. Mothers of both case and control groups were subjected to serum 25 hydroxyvitamin D [25(OH) D] assay which is considered the best circulating marker for evaluation of vitamin D condition and status in the majority of health conditions[20] and also, were genotyped for VDR gene Fok1 polymorphism.

Our study was directed in accordance to the ethical standards of the Helsinki Declaration of 1964 as revised in 2008 and was legalized by the local ethics committee of faculty of medicine, Zagazig University. An informed written consent was taken from all mothers shared in the study.

Methods

Blood sampling

Peripheral venous blood samples with average 2 ml of each sample were collected in EDTA-treated tubes for DNA extraction and serum separation tubes for 25 (OH) D assays. The serum was separated with the help of centrifugation and then stored at −20 °C until further analysis.

Vitamin D assay

Serum 25(OH) D level was measured by direct enzyme-linked immunosorbent assay (ELISA) kit supplied by [DRG International, Inc, New Jersy, United States] according to manufacturer instructions. The coefficients of variations (CV) were (13%—19%). The concentrations suggested for defining vitamin D levels were deficient (less than 25 nmol/L), insufficient (25—49 nmol/L), inadequate (50—74 nmol/L), sufficient (more than 75 nmo/L).[21]

FokI C > T (rs2228570) genotype analysis

Genomic DNA was extracted from whole blood using the commercially available G-spin TM total DNA extraction kit (iNtRON Biotechnology, Seongnam, Korea) as described in the user manual. The quality of the genomic DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm. The purified genomic DNA was stored at −80 °C until use.

FokI C>T polymorphism was analyzed by polymerase chain reaction amplification followed by restriction fragment length polymorphism (RFLP-PCR) analysis after Fok1 digestion using forward primer 5′-AGCTGG CCCTGG CAC TGACTC GCT CT-3′ and reverse primer 5′-ATGGAA ACA CCT TGC TTC TTC TCC CTC-3′.[22]

PCR was carried out with 100 ng of template DNA, 25 pmol of each primer (Biosource Europe SA, Nivelles, Belgium), and 12.5 ul of 2x Dream TaqTM Green PCR Master Mix (MBI Fermentas, St. Leon-Rot, Germany), then they were amplified with cycling parameters as follows: Denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 61 °C for 30 s and 72 °C for 60 s and one final cycle of extension at 72 °C for 7 min. The PCR product with 265 bp was digested with 3.0 units of Fok I restriction enzyme (New England Biolabs) and incubated at 37 °C for 4 h; then the digested products were separated in 2% agarose electrophoresis system and visualized with ethidium bromide staining under ultraviolet transillumination.

The F/F genotype (homozygote of the common allele) showed one band at 265 bp with an absence of restriction site. The f/f genotype (homozygote of the infrequent allele) showed two fragments at 196 bp and 69 bp. The F/f genotype (heterozygous form) showed three fragments at 265 bp, 196 bp and 69 bp.

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 22.0, IBM Corp, Armonk, NY. Qualitative data were represented as frequencies and relative percentages. Chi square test was used to compare between groups regarding qualitative variables with calculation of odds ratio as a risk estimate of polymorphism. Mann Whitney & Kruskal Wallis tests were used to compare between independent groups regarding quantitative variables that were not normally distributed and represented with median (inter-quartile range). The significance level for all above mentioned statistical tests done and the threshold of significance is fixed at 5% level (P-value). P value of >0.05 indicates non-significant results. P value of <0.05 indicates significant results.

Results

The general demographic characterization of both maternal and neonatal cases (n = 50) and control groups (n = 50) were illustrated in (Table 1). In this table, There was a significant increase in the maternal history of previous abortions ($P = 0.005$) and previous sibling with CHD ($P = 0.01$) in cases when compared to controls. In addition, there was a significant decrease in maternal vitamin D level ($P = 0.002$) and a significant increase in maternal vitamin D deficient status ($P = 0.007$) among cases when compared to controls (Table 2). Regarding VDR gene Fok1 polymorphism, the genotypes frequency distribution was in accordance with Hardy Weinberg equilibrium (HW) among controls. There were significant increase in F/f (48%),f/f (12%) genotypes and f (36%) allele frequency distribution in maternal cases versus 26%, 2% and 15% respectively in controls with estimated OR (95%CI) & P value of 3 (1—8) & $P = 0.006$, 11 (1—97) & $P = 0.01$ and 3 (2—6) & $P = 0.001$ respectively (Table 3). Moreover, a significant decrease on serum vitamin D ($P = 0.000$) and deficient vitamin D status ($P = 0.000$) were observed in maternal cases who carrying f/f genotype followed by F/f genotype when compared to F/F wild genotype (Table 4). Types and frequency distribution of CHD among neonatal cases were presented in (Table 5). Neonates with cyanotic CHD showed significant decrease in maternal vitamin D level ($P = 0.000$) with deficient and insufficient vitamin D state ($P = 0.009$) when compared to neonates with a cyanotic CHD while there was no significant difference in Fok1 F/f, f/f genotypes ($P = 0.18$) and f allele ($P = 0.05$) between cyanotic and a cyanotic CHDs (Table 6).

Discussion

Over the last two decades, it has been currently approved that deficiency of vitamin D is considered a major public health concern in several countries and among people of all ages.[23] Unfortunately, an increased rate of poor vitamin D status were discovered between pregnant mothers all over the world with a high incidence of significant maternal and neonatal complications.[24,25] The process of embryogenesis and particularly "Cardiogenesis" takes place early in gestation; between 2 and 7 weeks of pregnancy.[26] Vitamin D is considered as a crucial element in the embryonic development of the heart. It affects the physiologic cell processes through the active binding of 1,25 Di-

Table 1 General Characteristics of studied groups.

Characteristics	Cases (n = 50)	Controls (n = 50)	*P*-value
Maternal general characterization			
Maternal Age; in years[a]			
Median (IQR)	28 (20–35)	28 (20–35)	0.85
Min-Max	17–38	19–37	
Residency; n (%)[b]			
Urban	7 (14)	13 (26)	0.13
Rural	43 (86)	37 (74)	
Educational level; n (%)[c]			
High	3 (6)	6 (12)	0.53
Intermediate	19 (38)	20 (40)	
Low	28 (56)	24 (48)	
Previous abortion; n (%)[b]			
Positive history	15 (30)	4 (8)	0.009*
Negative history	35 (70)	46 (92)	
Previous CHD in sibilings; n (%)[c]			
Positive history	10 (20)	2 (4)	0.02*
Negative history	40 (80)	48 (96)	
Maternal vitamin D intake; n (%)[c]			
Non-user	24 (48)	13 (26)	0.15
Peri-conceptional users	3 (6)	6 (12)	
Early post-conceptional users	6 (12)	9 (18)	
Late post-conceptional users	17 (34)	22 (44)	
Neonatal general characterization			
Gestational Age; in Weeks[a]			
Median (IQR)	38 (37–40)	38 (37–40)	0.14
Min-Max	37–40	37–40	
Birth Weight; Kg[a]			
Median (IQR)	3 (2–4)	3 (3–4)	0.81
Min-Max	2.3–4	2.5–4	
Sex; n (%)[b]			
Male	29 (48)	24 (48)	0.31
Female	21 (42)	26 (52)	
Mode of delivery; n (%)[b]			
C.S	24 (48)	27 (54)	0.54
NVD	26 (52)	23 (46)	

Statistical analysis: a, Mann Whitney test; b, Chi Square test; c, Fisher's exact test.
*Significance at $P \leq 0.05$.
C.S, caesarian section; CHD, congenital heart diseases; NVD, normal vaginal delivery; IQR, inter-quartile range.

Table 2 Serum vitamin D level and vitamin D status among cases and controls.

	Cases (n = 50)	Control (n = 50)	*P*-value
Maternal vitamin D level; nmol/L[a]			
Median (IQR)	26 (10–8)	37 (16–76)	0.002*
Min-Max	6.5–78	13–80	
Maternal vitamin D status; n (%)[b]			
Deficient	24 (48)	8 (16)	0.006*
Insufficient	17 (34)	25 (50)	
Inadequate	6 (12)	11 (22)	
Sufficient	3 (6)	6 (12)	

Statistical analysis: a, Mann Whitney test; b, Fisher's exact test.
*Significance at $P \leq 0.05$.

hydroxyvitamin D to VDRs.[27] Interestingly, it was found that 1,25 Di-hydroxyvitamin D physiologically increased in concentration by 100–200% during the period of early gestation, suggesting an increase in need during early pregnancy.[28] Moreover, in a recent functional study conducted by Kwon[16] who investigated the important role of VDR signaling on heart development using knockdown approach in zebra fish model system, he concluded that VDR signaling plays a very important role in cardiac development. In the present study, we analyzed the association between both maternal vitamin D level and VDR gene Fok1 polymorphism and risk of CHD in offspring. Concerning maternal vitamin D level and status among Egyptian healthy control mothers, we concluded that the percentage of mothers having deficient, insufficient, inadequate and sufficient vitamin D status were 16%, 50%, 22% and 12% respectively. In a recent systematic review about vitamin D deficiency in the Middle East and North Africa region, they

Table 3 VDR gene Fok1 polymorphism genotype and allele distribution among cases and controls.

	Cases (n = 50)	Controls (n = 50)	P-value	OR	95%CI
Fok1 genotypes; n (%)					
F/F	20 (40)	36 (72)	Reference	Reference	Reference
F/f[a]	24 (48)	13 (26)	**0.006***	3	1–8
f/f[b]	6 (12)	1 (2)	**0.03***	10	1–97
Fok1 alleles; n (%)[a]					
F	64 (64)	85 (85)	**0.001***	3	2–6
f	36 (36)	15 (15)			

Statistical analysis: a; chi square test, b; fisher's exact test.
*Significant at $P \leq 0.05$.
95%CI, 95% confidence interval; OR, odds ratio; VDR, vitamin D receptor.

Table 4 Maternal vitamin D level & status in relation to VDR gene Fok1 polymorphism among cases.

	Fok1 genotype			
	F/F (n = 20)	F/f (n = 24)	f/f (n = 6)	P-value
Maternal vitamin D level; nmol/L[a]				
Median (IQR)	31 (20–78)	21 (12–41)	11 (7–17)	0.000*
Min-Max	10–78	10–52	6.5–17	
Maternal Vitamin D status; n (%)[b]				
Deficient (n = 24)	2 (8.3)	16 (66.7)	6 (25)	0.000*
Insufficient (n = 17)	9 (52.9)	8 (47.1)	0 (0)	
Inadequate (n = 6)	6 (100)	0 (0)	0 (0)	
Sufficient (n = 3)	3 (100)	0 (0)	0 (0)	

Statistical analysis: a, Kruskal–Wallis test; b, fisher's exact test.
*Significance at $P \leq 0.05$.

Table 5 Types of structural CHDs among neonatal cases.

CHDs	n = 50	%
A cyanotic CHDs	**37**	**74**
VSD	14	28
ASD	11	22
PDA	9	18
A-V defect	3	6
Cyanotic CHDs	**13**	**26**
TGA	5	10
TOF	3	6
Truncus arteriosus	2	4
Hypoplastic left heart syndrome	2	4
DORV	1	2

A-V defect, Atrio-ventricular defect; ASD, Atrial septal defect; CDH, congenital heart defects; DORV, double outlet right ventricle; VSD, ventricular septal defect; PDA, Patent ductus arteriosus; TGA, Transposition of great vessels; TOF, Tetralogy of fallout.

concluded that being of a female gender was among the risk factor of having vitamin D deficiency.[29] Regarding Egyptian women, several studies were conducted to investigate the actual vitamin D status in different age groups. In agreement with our results, El Rifai et al[30] who conducted a cross sectional study to investigate vitamin D status immediately before delivery among 135 pregnant Egyptian females at ≥37 weeks gestation. They reported vitamin D insufficiency (vitamin D < 50 nmol/L) in 40% of maternal cases while inadequacy (vitamin D of 50–80 nmol/L) was reported in 28.9% of mothers. Nearly similar results were reported by Botros et al[31] who conducted another cross sectional study on 404 healthy Egyptian females. They measured vitamin D level across different age groups. Among pregnant females, vitamin D was found in a deficient range in 54% of cases and 10% was found in insufficient range. In the current study, there was a significant decrease in vitamin D level with a significant increase in deficient vitamin D status among cases when compared to controls. Few studies were conducted to investigate the association between maternal vitamin D level and risk of CHD in offspring. Similar to our results, Dilli et al[32] conducted a case control study to measure serum level of micronutrients (including vitamin D) in 108 neonates with CHD and their mothers. They found a significant decrease in vitamin D level in both neonates and their mothers compared to controls. Furthermore, another study conducted by Koster et al.[33] They measured vitamin D status in 345 mothers having sibling with CHD, and disclosed that a deficient (>50 nmol/L) and a moderate (50–75 nmol/L) vitamin D status were significantly associated with CHD in offspring ($P = 0.02$). Concerning VDR gene Fok1 polymorphism, it has been well-kwon that VDR gene; which is located on chromosome (12q13.11), contains 11 exons and expand about 75 kb of DNA genome.[8] Several polymorphisms have been found in this gene including FoK1 polymorphism which is located in exon 2 at the 5′ coding

Table 6 Maternal vitamin D level and Fok1 genotype distribution between A cyanotic and Cyanotic CHD.

		A cyanotic CHD n = 37	Cyanotic CHD n = 13	P-value
Maternal vitamin level; nmol/L[a]				
Median (IQR)		31 (15–65)	14 (8–28)	**0.000***
Min-Max		10–78	6.5–30	
Maternal vitamin D status; n (%)[b]				
Deficient n = 24		12 (50)	12 (50)	**0.01***
Insufficient n = 17		15 (88.2)	2 (11.8)	
Inadequate n = 6		6 (100)	0 (0)	
Sufficient n = 3		3 (100)	0 (0)	
Fok1 genotype; n (%)[b]				
F/F	n = 20	12 (60)	8 (40)	**0.18***
F/f	n = 24	20 (83.3)	4 (16.7)	
f/f	n = 6	5 (83.3)	1 (16.7)	
Fok1 allele; n (%)[c]				
F	n = 64	44 (68.7)	20 (31.3)	**0.05***
f	n = 42	36 (85.7)	6 (14.3)	

Statistical analysis: a, Mann–Whitney test; b, Fisher's exact test; c, chi square test.
CHD, congenital heart defects; IQR, interquartile rang.
*Significance at $P \leq 0.05$.

region of the gene and considered one of the commonly studied functional VDR polymorphisms. The presence of Fok1 gene polymorphism results in presence of different translation initiations sites due to substitution of thiamine (T) to cytosine (C).[34] Thus, two proteins variants can exist as a result of the two available initiation sites: f allele form which is a long version of VDR protein of (427 aa) and F allele form which is protein shortened by three amino acids (424 aa).[35] Some Functional Reports stated that the changed (424 aa) VDR protein variant seems to be more active than long (427 aa) variant in terms of transactivation capacity as being a transcription factor.[36,37] Consequently, the increased frequency of f allele might leads to production of less functional VDR protein that impairs transcription and vitamin D mediated response. In this study, the frequency distribution of Fok1 polymorphism F/F, F/f and f/f genotypes among Egyptian healthy females were 72%, 26% and 2% respectively. In agreement with our results, Elsoud et al[38] reported that VDR gene Fok1 F/F, F/f and f/f genotypes distribution among Egyptian healthy females were 67%, 33% and 0% respectively. Another study conducted by Zaki et al[39] on Egyptian obsess females, they concluded that the frequency distribution of VDR gene Fok1 polymorphism among controls for F/F, F/f and f/f genotypes were 70.28%, 20.08% and 9.63 respectively which is nearly similar to our results. In contrary, Hamed et al[40] who investigated VDR gene Fok1 polymorphism among Egyptian adults (both male and females) with type 1 diabetes, they concluded that the frequency of Fok1 F/F,F/f and f/f genotypes among healthy controls were 20%,70% and 10% respectively. This reported difference in genotype distribution might be due to the difference in gender distribution. Furthermore, in this study, we found a significant increase in the frequency distribution of Fok1 F/f & f/f genotypes and f allele in cases compared to controls with significant increased risk of congenital heart defects among offspring. Maternal VDR gene Fok1 polymorphism had been formally studied as a risk factor for various neonatal complications. Patel et al[41] conducted a case-control study to investigate the association between maternal vitamin D level and VDR gene Fok1 & Taq1 polymorphisms and premature birth among Indian women, they found a significant increase in f/f genotype and f allele frequency with deficient vitamin D level in women with preterm delivery. Another similar study was conducted in the Brazilian population and disclosed near similar results.[42] Further, VDR gene Fok1 polymorphism had been studied in relation to broncho-pulmonary-dysplasia (BPD) development in preterm neonates. They concluded that VDR gene F/f and f/f genotypes were associated with increased risk for BPD in preterm babies.[43] Regards the relation between maternal vitamin D level and Fok1 polymorphism, we reported that there were significantly lower vitamin D level with f/f genotype followed by F/f genotype when compared to F/F wild genotype. In agreement with our results, Tayel et al[44] also found significantly lower level of vitamin D in neonates with f/f genotype. In contrary, In a study conducted by AparmA et al[45] on Indian population in order to detect the frequency distribution of VDR gene Fok1 and Taq1 polymorphisms and its relation to vitamin D level, they found no association between the Fok1 polymorphism and vitamin D level. In this study, we classified neonates with CHD into 2 groups, a cyanotic CHD and cyanotic CHD. We found a significant decrease in maternal vitamin D level with deficient and insufficient vitamin D status in cases with cyanotic CHD when compared to those with a cyanotic CHD. Few studies reported association between vitamin D level and specific CHD phenotype. Despite the difference in CHD phenotype classification, Dilli et al[32] found that maternal and neonatal vitamin D level were lower in truncal anomalies including truncus arteriosus, tetralogy of Fallot, and D-transposition of great arteries. In addition, Koster et al[33] reported that there was a significant association between maternal vitamin D status (deficient and moderate) and isolated group of congenital heart disease but not a complex one. On the other hand and due to the heterogeneity of CHD

phenotype, we found no significant difference in the frequency distribution of FOk1 F/F, F/f and f/f genotype nor f allele between non cyanotic and cyanotic CHD groups. There were some limitations in our study, the small sample size of the study group and the heterogeneity of congenital heart defects types, so we were not able to detect the association between vitamin D level and VDR gene Fok1 polymorphism and each separate type of CHD an increasing rates.

Conclusion

In this study, we concluded that maternal vitamin D deficiency and VDR gene Fok1 F/f & f/f genotypes and f allele were significantly associated with increased risk of CHD in offspring. Further larger sample studies were recommended to find the association between each type of CHD and previously studied parameters.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.gendis.2018.08.001.

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