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RESEARCH ARTICLE

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Association between Maternal *FUT2* 204A>G (rs492602) Genetic Polymorphism and Congenital Heart Disease in the Indian Population: A Study in Maternal-fetal Dyads

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Background: *FUT2* secretor genetic variants are strongly associated with absorption and circulatory levels of vitamin B12, thereby affecting folate metabolism pathway. The aim of this study was to evaluate the association between maternal *FUT2* 204A>G (rs492602) genetic polymorphism and CHD in the Indian population.
Materials and method: One hundred and ten pregnant women who were vitamin B12 deficient with fetuses diagnosed with CHD were included in the case group and an equal number of healthy pregnant women with normal fetuses were selected as the control group. DNA was extracted from blood and umbilical cord tissue samples, and genotyped for *FUT2* rs492602 polymorphism using allele-specific polymerase chain reaction. Hardy–Weinberg equilibrium test was used to calculate allele and genotype frequencies.
Results: Significant increase in the frequency of AG (odds ratio=2.25; 95% CI: 1.25–4.05; *p*=0.009) and GG (odds ratio=3.51; 95% CI: 1.47-8.43; *p*=0.006) genotypes as well as G allele of *FUT2* rs492602 were observed in the maternal case group. Furthermore, in the fetus case group, there was a significantly higher incidence of GG genotype (odds ratio=2.87; 95% CI: 1.26–6.57; *p*=0.018) and G allele (odds ratio=1.70; 95% CI: 1.15–2.53; *p*=0.009).
Conclusion: *FUT2* rs492602 are associated with CHD in the Indian population. Maternal genetic polymorphism that regulates vitamin B12 metabolic pathway might influence fetal cardiac development, thus serving as a predictor for CHD.

Keywords: *congenital heart disease, FUT2, single nucleotide polymorphism (SNP), vitamin B12*

Introduction

Congenital heart disease (CHD) has a global prevalence varying from 4 to 50/1,000 live-births, with prevalence in India is around 0.8 to 26.4/1,000 children.[1,2] Studies suggest that interactions between genetic and environmental factors contribute to increased risk of CHD.[3,4] Maternal folic acid intake has been reported to reduce the risk of CHD.[5,6]

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Several changes in maternal plasma metabolites related to folate metabolism have been observed to be associated with CHD along with other genetic and metabolic risk factors.[7-13] Previous studies have shown that pregnant women with fetuses affected with CHD had higher plasma homocysteine concentrations than those with unaffected pregnancies.[14] Therefore, genes involved in vitamin B12 metabolism alter vitamin B12 levels, which may further disrupt folate metabolism pathway and ultimately contribute to the development of CHD.

In the highly polymorphic *FUT2* gene, three single nucleotide polymorphisms (SNPs), *i.e.* rs492602, rs602662 and rs601338, have been reported to be strongly associated with plasma B12 levels.[15] The present study aimed to evaluate the association between maternal *FUT2* 204A>G (rs492602) genetic polymorphism and CHD in Indian population using maternal blood and cord tissue samples. To the best of our knowledge, this is the first study that analyzed the association of *FUT2* rs492602 with CHD in Indian population using maternal blood and umbilical cord samples.

Materials and methods

Research Subjects

Pregnant women between 16 to 28 weeks of gestation, enrolled at Govt. Modern Maternity Hospital, Care Hospital and Asian Institute of Fetal Medicine, Hyderabad between September 2016 to December 2018 were included in the present study. The entire study group was screened for vitamin B12 deficiency. In addition, 3D/4D ultrasound was performed by a fetal medicine specialist and a pediatric cardiologist to detect CHD in the fetus. One hundred and ten pregnant women who were vitamin B12 deficient with fetuses diagnosed with CHD were included in the case group. Similarly, an equal number of healthy pregnant women with normal fetuses were selected as the control group. The mother and her fetus were considered as a single subject or dyad. Pregnant women who had been habituated to alcohol or tobacco, exposed to teratogenic drugs, and infected with infectious disease such as rubella were excluded. The study was taken up after the approval from the Institutional ethical committee (No. 388/IOU/Ethical committee/certificate). Informed consent was obtained from all study participants. Detailed history with reference to known risk factors for CHD was collected using a standard questionnaire along with clinical examination of all the study subjects. The maternal case and control groups were matched in terms of age, body mass index (BMI), family history of CHD and consanguinity, history of diabetes mellitus and hyperthyroidism, as well as dietary restriction (vegetarianism).[16]

DNA Isolation and Genotyping

Three mL of blood was collected from the antecubital vein in EDTA vacutainer tubes. Umbilical cord tissue sample was collected from the newborn. DNA extraction from the blood and cord tissue samples were performed using sucrose method and QIAamp Fast DNA Tissue Kit (QIAGEN, Hilden, Germany), respectively. *FUT2* rs492602 was genotyped using allele specific polymerase chain reaction (AS-PCR) modified method.[17] Common forward primer (CFP) (5'-CAGTCGTTCAGGTGGTAGTT-3'), wild reverse primer (WRP) (5'-GGATGTGGACGATCAATGAG-3'), and mutant reverse primer (MRP) (5'-GGATGTGGACGATCAATGAA-3') were used for amplification. Two PCR reactions were set up for each sample. One reaction was amplified with CFP and MRP, and the other one was amplified with CFP and WRP primer pairs. The size of the amplified products were 211 bp. The PCR products were visualized using 2% agarose gel.

Statistical Analysis

Hardy–Weinberg equilibrium test was used to calculate allele and genotype frequencies of *FUT2* rs492602 polymorphism. Any deviations between the observed and expected frequencies were statistically evaluated for significance using odds ratio (OR) with 95% confidence interval (CI) and chi-square test.

Results

The mean age of the subjects in the control and case groups were 24.29±3.8 and 25.10±3.7 years old, respectively. The mean BMI of the subjects in the control and case groups were 25.35± 5.1 and 25.41±4.9 kg/m^2, respectively. Most of the subjects in both groups were non-vegetarian and healthy without diabetes mellitus, hyperthyroidism, as well as history of CHD and consanguinity.[16] Electrophoresis results of AS-PCR products were shown in Figure 1. The genotypic distribution and allelic frequency of *FUT2* rs492602 polymorphism in case and control groups of maternal-offspring dyads were given in Table 1 and Table 2, respectively. The frequency of AA, AG, and GG genotypes

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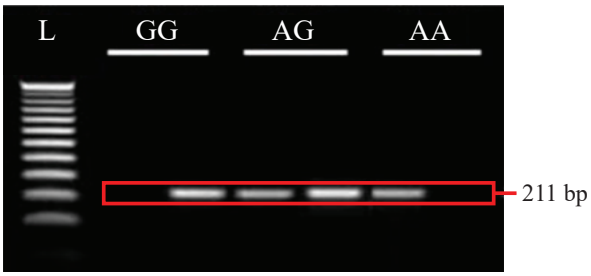

Figure 1. Representation of *FUT2* rs492602 genotyping results. L: 100 bp ladder; GG: sample with GG genotype; AG: sample with AG genotype; AA: sample with AA genotype.

in the maternal case group were 39%, 43%, and 18%, while 62%, 30%, and 8% in the maternal control group, respectively. There were significant differences in the genotype distribution and allele frequency between maternal case and control groups. The comparison between AA and GG genotypes, AA and (AG+GG) genotypes, and A and G alleles had OR of 3.51 (95% CI: 1.47–8.43; p=0.006); 2.52 (95% CI: 1.47–4.34; p=0.001), and 2.16 (95% CI: 1.43–3.27; p<0.001), respectively. Furthermore, the frequency of AA, AG, and GG genotypes in the fetal case group were 36%, 43%, and 21%, while 50%, 40%, and 10% in the fetal control group, respectively. There were significant differences in the genotype distribution and allele frequency between fetal case and control groups, except for AA *vs.* AG, and AA *vs.* (AG+GG) comparisons. The comparison between AA and GG genotypes, AA and (AG+GG) genotypes, and A and G alleles had OR of 2.87 (95% CI: 1.26–6.57; p=0.018), 1.75 (95% CI: 1.02–3.00; p=0.056) and 1.70 (95% CI: 1.15–2.53; p=0.009), respectively. An increase in the G allele frequency was observed in both the maternal and fetal case groups as compared to the control groups, thereby indicating it as a susceptibility allele and its possible role in the etiology of CHD.

Discussion

Incidence of several human diseases is often associated with specific genetic variants.[18-20] Genetic variants related to folate metabolism and their association with congenital heart defects have been studied extensively.[21] Vitamin B12 plays an important role in folate and homocysteine metabolism. Elevated maternal homocysteine levels have been hypothesized to be a potential risk factor for congenital heart disease.[10] Variation in the genes involved in vitamin B12 metabolism alters the circulating vitamin B12 levels as evidenced by genome-wide association study (GWAS) and candidate gene studies.[7,22-24] Absorption, transport, cellular uptake and intracellular metabolism of vitamin B12 is dependent upon the coordinated action of the binding proteins. Therefore, genetic variants coding for these proteins have been suggested to play a role in regulating vitamin B12 levels.[25] One such gene is *FUT2,* which is located on chromosome 19: 48703160 (GRCh38.p13). This gene codes for a secretor enzyme α-1,2-fucosyltransferase and is responsible for the transfer of fucose to form a terminal H type 1 structure.[26] The H-antigen synthesized along with Lewis ABO antigens has been reported to mediate *Helicobacter pylori* attachment to human gastric mucosa.[21,27] Secretors with active FUT2 enzymes are at a greater risk of infections from pathogens such as *H. pylori,* which affects the absorption of vitamin B12.[21,27] A study suggests potential mechanisms by which vitamin

Table 1. Genotype distribution and allelic frequencies of *FUT2* rs492602 polymorphism in the maternal case and control groups.

FUT2 rs492602	Maternal Case Group	Maternal Control Group	OR (95% CI)	*p* -value[a]
Genotype, n (%)				
AA	43 (39)	68 (62)	1.00 (ref)	
AG	47 (43)	33 (30)	2.25 (1.25-4.05)	0.009**
GG	20 (18)	9 (8)	3.51 (1.47-8.43)	0.006**
AG+GG	67 (61)	42 (38)	2.52 (1.47-4.34)	0.001**
Allele, n (frequency)				
A	133 (0.6)	169 (0.8)	2.16 (1.43-3.27)	<0.001**
G	87 (0.4)	51 (0.2)		

ref: reference; *p<0.05; **p<0.01, [a]cases *vs* control.

Table 2. Genotype distribution and allelic frequencies of *FUT2* rs492602 polymorphism in the fetal case and control groups.

FUT2 rs492602	Fetal Case Group	Fetal Control Group	OR (95% CI)	*p* -value[a]
Genotype, n (%)				
AA	40 (36)	55 (50)	1.00 (ref)	
AG	47 (43)	44 (40)	1.47 (0.82-2.62)	0.248
GG	23 (21)	11 (10)	2.87 (1.26-6.57)	0.018*
AG+GG	70(64)	55 (50)	1.75 (1.02-3.00)	0.056
Allele, n (frequency)				
A	127 (0.6)	154 (0.7)	1.70 (1.15-2.53)	0.009**
G	93 (0.4)	66 (0.3)		

ref: reference; *p<0.05; **p<0.01, [a]cases *vs* control.

B12 absorption may be reduced in carriers of the secretor genotype as compared to individuals with the non-secretor genotype.[26] Another study shows that vitamin B12 levels are associated with *FUT2* genetic variants, particularly rs602662, rs492602 and rs601338, which affects the secretory status of FUT2 and determines mediate host-microbe interactions, thereby increasing susceptibility to infection and further affecting vitamin B12 absorption.[28] Furthermore, studies have reported an increase in circulating vitamin B12 levels in homozygotes for non-secretor alleles W143X, rs601338.[23,29] Indians are more prone to *H. pylori* infection due to poor socioeconomic conditions.[30-32] It has also been observed that genetic variants related to vitamin B12 metabolism are associated with CHD in Chinese population.[32] Therefore, folate supplementation in vitamin B12 deficient mothers may be detrimental for the fetus instead of being beneficial.[33] This emphasizes that genetic association with vitamin B12 metabolism pathways are still needed to be studied further.

Conclusion

In summary, our study supports the notion that common *FUT2* rs492602 are associated with CHD in Indian population. Maternal genetic polymorphism that regulates vitamin B12 metabolic pathway might influence fetal cardiac development, thus serving as a predictor for CHD.

Authors Contribution

ST was involved in concepting and planning the research. SGC performed the data acquisition/collection. ST and SGC calculated the experimental data and performed the analysis and prepared figures, as well as interpreted the results. JA prepared the manuscript draft. SU performed critical revision of manuscript.

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