*J Women's Health Dev 2020; 3 (4): 432-436* *DOI: 10.26502/fjwhd.2644-28840051*

Research Article

# Maternal TCN1 (rs526934) G>A Gene Polymorphism and its Association with Congenital Heart Disease (CHD)

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**Received:** 05 October 2020; **Accepted:** 13 October 2020; **Published:** 28 October 2020

**Citation:** Sunitha Tella, Shehnaz Sultana, Venkateshwari Ananthapur, Nageshwar Rao K, Jyothy Akka, Satyanarayana U. Maternal TCN1 (rs526934) G>A Gene Polymorphism and its Association with Congenital Heart Disease (CHD). Journal of Women's Health and Development 3 (2020): 432-436.

## Abstract

**Background:** Congenital heart diseases (CHDs) represent the most common birth defect, affecting 0.6–9.1 per 1000 live births, and the leading cause of infant deaths worldwide. Genes involved in vitamin B12 metabolism such as TCN1 may affect the balance of folate metabolism and ultimately contribute to the development of CHD through the disrupted folate metabolism pathway.

**Methods:** A total of 100 pregnant women carrying foetus with congenital heart defects are considered as case group and an equal number of healthy pregnant women carrying healthy foetus devoid of any defects are considered as control group for the present study. Blood samples were collected from all the study subjects. DNA was extracted and genotyping of TCN1 intron 372 G>A (rs526934) polymorphism was carried out using allele specific PCR, followed by agarose gel electrophoresis.

**Results:** Pregnant women in patient group, with GA genotype has 1.95 times more risk of having foetus with CHD compared to controls. Whereas, GG genotype played a protective role. 'A' allele was 2.14 times susceptible towards the disease, while 'G' allele showed protective effect.

**Conclusion:** Understanding the possible underlying genetic factors of vitamin B12 metabolism will lead to an increased understanding of the biological mechanisms underlying the maternal TCN1 gene polymorphism effect on CHD. This helps in early diagnosis, management and provide appropriate therapeutic strategies.

**Keywords:** Congenital Heart Disease; Transcobalamin; Single Nucleotide Polymorphism; Vitamin B12

## 1. Introduction

Congenital heart diseases (CHDs) represent the most common birth defect, affecting 0.6–9.1 per 1000 live births, and the leading cause of infant deaths worldwide [1]. Several studies suggested that the interaction of genetic and environmental factors contributed to the increasing risk of CHDs [2-4]. Earlier studies showed that the folate metabolism pathway is involved in the development of CHD. Peri-conceptual folic acid supplementation could prevent fetal CHD, and folate deficiency in a pregnant woman potentially contributes to CHD in the developing embryo [5, 6]. Vitamin B12, or cobalamin, plays an important role in folate metabolism, in the form of methyl cobalamin, it participates in the folate-dependent methylation of homocysteine to form methionine in the presence of the enzyme methionine synthase. Therefore, vitamin B12 levels and the genes involved in vitamin B12 metabolism may affect the balance of folate metabolism and ultimately contribute to the development of CHD through the disrupted folate metabolism pathway. The transcobalamin 1 (TCN1) gene is located on chromosome 11 and codes for the vitamin B12 binding protein, transcobalamin I (TCI) also known as haptocorrin (HC) or R binder [7-9]. TCI is involved in facilitating the entry of vitamin B12 into the cells, via receptor mediated endocytosis [10]. Several studies have reported associations between variants within the TCN1 gene and circulating vitamin B12 concentrations [11-16]. In the present study, we hypothesized that the maternal TCN1 gene polymorphism may play a role in the aetiology of congenital heart disease (CHD).

## 2. Materials and Methods

### 2.1 Subjects

The present study consists of a total number of 200 samples. 100 pregnant women carrying foetus with congenital heart defects are considered as case group, whereas, 100 healthy pregnant women carrying healthy foetus devoid of any defects are considered as control group. The study subjects were enrolled from Government Modern Maternity Hospital, Care Hospital and Asian Institute of Fetal Medicine Hyderabad. The study subjects were screened for the foetal anomalies by a fetal medicine specialist and paediatric cardiologist by 3D/4D ultrasound. Prior informed consent was obtained from all the study subjects. Institutional ethical committee has approved the present study.

### 2.2 Genotyping

2ml of venous blood was collected from all the study subjects. Genomic DNA was isolated following the method of Lahiri et al [17] and genotyping of TCN1 intron 372 G>A (rs526934) polymorphism was carried out using allele

specific PCR. The primers used for genotyping are WRP (TCATGCATTGAATTTCAGGG), MRP (TCATGCATTGAATTTCAGGA) and CFP (ATAGATTGTGTATTCACTTGCC). The amplified 198bp product was detected using agarose gel electrophoresis. Statistical analysis of the data was done by using Epi info online software. P value >0.05 was considered significant.

| **TCN1 rs526934 G>A** | **Patients** | | **Controls** | | **OR (95% CI)** | **P-value** |
|---|---|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** | | |
| **Genotype** | | | | | | |
| GG | 36 | 33.03 | 58 | 58 | 0.35(0.20-0.62) | 0.0002 |
| GA | 51 | 46.79 | 31 | 31 | 1.95(1.10-3.44) | 0.0195 |
| AA | 22 | 20.18 | 11 | 11 | 2.04(0.93-4.47) | 0.0689 |
| **Allele** | | | | | | |
| G | 123 | 56.42 | 147 | 73.5 | 0.46(0.30-0.70) | 0.0002 |
| A | 95 | 43.58 | 53 | 26.5 | 2.14(1.41-3.23) | 0.0002 |

**Table 1:** Genotyping of TCN1 (rs526934) in patients and controls group.

## 3. Results

Genotyping of TCN1 (rs526934) polymorphism in case and control women were shown in Table 1. Pregnant women in patient group, with GA genotype has 1.95 times more risk of having foetus with CHD compared to controls. Whereas, GG genotype played a protective role. 'A' allele was 2.14 times susceptible towards the disease, while 'G' allele showed protective effect.

## 4. Discussion

Genetic studies carried out on vitamin B12, suggest that it is a multifactorial trait, where several single-nucleotide polymorphisms (SNPs)in multiple genes interact with the environment to cause the altered B12 status [18]. Most of the SNPs related to vitamin B12 status have been examined using a candidate gene approach [18]. Vitamin B12, also known as cobalamin (Cbl), is an essential water-soluble micronutrient required to be ingested by pregnant women to maintain a healthy foetus. It is important to maintain adequate vitamin B12 levels during pregnancy, for which pregnant women must ingest sufficient dietary vitamin B12 and retain the ability to absorb vitamin B12. The absorption, transport and cellular uptake of vitamin B12 is dependent upon the co-ordinated action of the binding proteins such as haptocorrin (HC), intrinsic factor (IF), transcobalamin II (TC) and other specific cell receptors. Initially vitamin B12 binds to HC in the stomach and IF in the duodenum, then it binds to TC within the enterocyte and is then released into the blood stream. The vitamin B12-TC complex then binds to the transcobalamin receptor (TC-R) and is taken up by cells through endocytosis [19]. Transcobalamin I (TCI) also known as haptocorrin is one of the cobalamin binding protein in humans. TCN1 gene is located on chromosome11q11-q12.3 [7], has 9 exons of 59 to 191 bp and 8 introns of 160 bp to 3.2 kb, and encodes a protein of 433amino acids [9]. Variants of the transcobalamin 1 (TCN1) gene (vitamin B12 binding protein, transcobalamin I (TCI)) have been associated with circulating B12 concentrations [8, 20]. Proteins involved in vitamin B12 absorption, cellular uptake and intracellular

metabolism are affected by genetic variants, which in turn alters the vitamin B12 status [21]. In spite of a number of genome-wide association studies and candidate gene analyses, the exact relationship between an individual's genotype and their vitamin B12 status remains poorly understood.

A Genome wide association study (GWAS) comprising 534 healthy children from Mysore, India, investigated the association between several nucleotide variations within the TCN1gene and vitamin B12 levels. Carriers of the'G' allele of the rs526934 variant were found to have lower circulating vitamin B12 concentrations ($\beta = -0.16$ pmol/l,$P = 0.02$) compared to 'A' allele carriers [16], which were in consistent with the studies carried out in Chinese, Icelandic, Italian and individuals residing in the US (predominantly non-Hispanic white) [13-15, 17]. Although no functional data are available to confirm the functional effect of these SNPs on vitamin B12concentrations, the results from these studies suggest that the SNPs may have important role of the TCN1 protein in maintaining vitamin B12 levels. The present study showed that pregnant women in patient group, with GA genotype has 1.95 times more risk of having foetus with CHD compared to controls. While, GG genotype played a protective role. 'A' allele was 2.14 times susceptible towards the disease, and 'G' allele showed protective effect. The study results indicate that more studies are needed to elucidate the mechanism of maternal genetic factors in CHDs.

## 5. Conclusion

Understanding the possible underlying genetic factors of vitamin B12 metabolism will lead to an increased understanding of the biological mechanisms underlying the maternal TCN1 gene polymorphism effect on CHD. The study may also help in early diagnosis, management and provide appropriate therapeutic strategies.

## Conflict of Interest

None to declare

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