

## **SCN5A (G892A) gene is associated with AV block conduction disorder in north Indian population**

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### **Abstract**

**Objective:** Electrical abnormalities of the heart cause the cardiac arrhythmia with or without essential structural heart disease. Due to genetic variation, cardiac arrhythmia can occur in any age group patients as well as in healthy people. The present study aimed to examine the allele and genotype of *SCN5A* (G892A) gene in cardiac conduction disorder (especially AV block conduction disorder) cases and comparison with healthy controls.

**Method-** A total number of 104 cases and 104 controls were enrolled in this study. DNA was extracted using salting out method followed by polymerase chain reaction amplification and restriction endonuclease digestion (using *AluI* restriction enzyme). Digested PCR products were identified using agarose gel electrophoresis and stained with ethidium bromide.

**Results:** There was a strong association of *SCN5A* G892A polymorphisms (GA vs. GG, OR= 4.01 and AA vs. GG OR= 3.12) with cardiac conduction disorder. Further multivariate logistic regression analysis, after adjustment for age, gender showed that when compared with wild type GG genotype, carriers of the A alleles had an increased risk of cardiac conduction disorder (OR=1.89, 95%CI- 0.78-1.67, p value- <0.0001).

**Conclusion:** In conclusion the results of the present study suggest that the *SCN5A* G892A gene polymorphism carries an increased risk for cardiac conduction disorder in north Indian populations.

**Keywords:** Cardiac conduction disorder, AV block, Gene polymorphism, Polymerase chain reaction, *SCN5A* gene

### **1. Introduction**

Cardiac conduction defect (CCD) is a serious and potentially life-threatening disorder [1]. It belongs to a group of pathologies with an alteration of cardiac conduction through the atrioventricular (AV) node, the His-Purkinje system with right or left bundle branch block, and widening of QRS complexes. CCD can lead to complete AV block and cause syncope and sudden death [1,2] Atrioventricular (AV) block conduction disorder occurs due to impairment of the electrical continuity between the atria and ventricles. Classification of AV block has utilized biophysical characteristics, usually the extent (first, second, or third degree) and site of block (above or below His bundle recording site). The genetic significance of this classification is unknown. Usually the injury or the major cardiac manifestation of neuromuscular disease causes the AV block. However, in some cases, AV block has unknown or idiopathic cause [3].

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By slowing the heart rhythm cardiac conduction disorders cause disability in millions of people worldwide. *SCN5A* gene encodes the human cardiac sodium (Na<sup>+</sup>) channel whose primary function is to initiate cardiac impulse conduction. A number of sudden and life threatening mutations have been attributed to *SCN5A* gene [4-6]. A critical determinant for the generation and propagation of the cardiac action potential is voltage-gated sodium channels. Genetic variations in the major pore-forming sodium channel  $\alpha$  subunit in the heart (Na<sub>v</sub>1.5) encoded by *SCN5A* gene [4,6-8]. A reduction in sodium current leads to cardiac conduction disease, which may be progressive (OMIM 113900) [7,8], and Brugada syndrome (OMIM 601144), characterized by ST segment elevation in the right precordial leads (V1 to V3) of the 12-lead ECG and episodes of ventricular fibrillation [6].

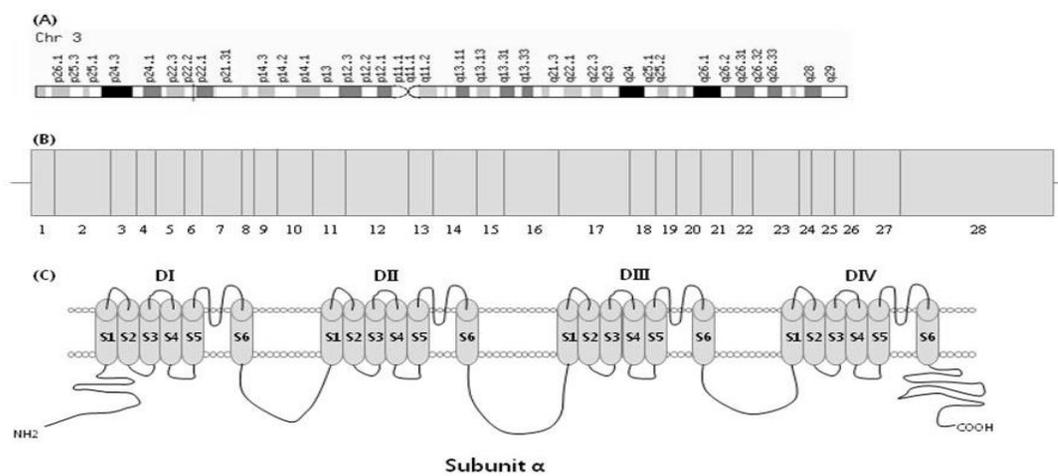
Although expressed in several tissues, *SCN5A* gene is predominantly expressed in the heart where it plays a vital

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role in the generation and propagation of the cardiac impulse [4]. Spanning 80kb with 28 exons, *SCN5A* maps to the short arm of chromosome 3 [9,10]. In spite of being expressed in several tissues *SCN5A* gene shares a common structure and encodes the  $\alpha$ -subunit of the voltage-gated cardiac sodium channel, thus playing a key role in conduction of cardiac sodium current *I<sub>Na</sub>* [11-14]. Heteromeric assemblies of pore-forming  $\alpha$ -subunit is a characteristic feature of the cardiac sodium channel (Na<sub>v</sub>1.5), which is a member of the voltage-dependent family of sodium channels. With a total of 2016 residues Nav1.5 has an approximate molecular mass of 227kDa.

It consists of 4 homologous domains, known as DI to DIV, joined by so-called linkers. Joined by linkers, *SCN5A* contains 4 homologous domains (DI to DIV) each of which contains 6 transmembrane helices (from S1 to S6) linked by intra or extracellular loops. S5 and S6 are the pore forming helices in each domain (Figure 1).

Mutations in *SCN5A* gene have been related to cardiac conduction system disease which manifests as slower intramyocardial conduction or atrioventricular conduction block (AVB) in some cases [5]. Such genetic variations in AVB patients have been widely studied in Caucasians, Han Chinese, and Japanese, but no study has yet been published in North Indian patients as far we know.



**Figure 1:** Genomic location and exon image of *SCN5A* gene and voltage-gated Na<sup>+</sup> channel  $\alpha$  subunit. (A) The *SCN5A* gene is located on human chromosome 3p21. (B) Structure of *SCN5A* gene: the *SCN5A* gene consists of 28 exons; among these, exon 1 is an untranslated region. (C) The voltage-gated Na<sup>+</sup> channel  $\alpha$  subunit consists of 4 homologous domains (DI-DIV), each containing 6 transmembrane-spanning segments (S1-S6).

Therefore, we carried out a polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) of the *SCN5A* gene, except the untranslated region, in North Indian AVB patients to investigate the *SCN5A* variations associated with AVB and compared them with normal control subjects.

## 2. Methods

### 2.1 Patient enrolment

We enrolled 104 North Indian AVB patients who were previously diagnosed and had permanent pacemakers implanted and 104 normal controls with no cardiac symptoms. All patients and control subjects were recruited from Department of Cardiology, King George's Medical University.

Each of the participants and individuals whose data are included here signed an informed consent form after a complete and clear explanation of the nature and purpose of the study. The study was approved by the Ethics Committee (ethical clearance no. 63ECM-II B/2) of King George's Medical University, Lucknow, India.

### 2.2 Inclusion and exclusion criteria

The patients who were diagnosed with cardiac conduction disorders and had permanent pacemaker implantation *in situ* were enrolled for study. Inclusion criteria of patients were- a) Subjects of both sexes presenting with cardiac conduction disorder. b) Subjects of both sexes having pacemaker implanted previously for cardiac conduction disorder. c) Subjects willing to give written informed consent. Subjects of both sexes, not having indication of pacemaker implantation and those not willing to give written informed consent were excluded from the study.

### 2.3 Molecular Genetic Methods

Genomic DNA was isolated from the venous blood of study participants (cases and controls) and the polymerase chain reaction was used to amplify coding region and flanking intronic sequence of *SCN5A*. In this study, 5ml peripheral blood was collected into ethylene di-amine tetra-acetic acid-containing (EDTA) tubes, and genomic deoxyribonucleic acid (DNA) was extracted from whole blood samples using the DNA isolation kit (High Yield Genomic DNA Mini Kit, Real Genomics). The purity of the

DNA was assessed based on the 260/280 nm absorbance ratio.

#### 2.4 Genotyping of human SCN5A gene

SCN5A polymorphism (G892A) was analyzed by the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). DNA was amplified using the following primers: forward primer, 5'-TGACCCCGTGCTTGTCTGCCT- 3'; reverse primer, 5'-GTCTGCGGTCTCACAAAGTCTTC - 3' [15]. PCR was performed using 50 ng of DNA in a volume of 25 µl and the final concentration of reagents was 1 X buffer (20 mmol/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 75mmol/l Tris-HCl, pH 8.8, at 25°C, 0.01% (v/v) Tween 20, 1.5 mmol/l MgCl<sub>2</sub>), dNTPs (0.2 mmol/l each), 0.2 µmol/l of each primer, and 1.25 units Red Hot Taq polymerase. Amplification was carried out with the use of a Touchdown thermal cyler (AB Applied Biosystems). The thermocycling procedure consisted of an initial denaturation at 94°C for 3 min (one cycle); 35 cycles of denaturation (94°C for 1 min), annealing (64°C for 1 min), and extension (72°C for 1 min); and a final extension at 72°C for 10 min. PCR products were first digested by restriction endonuclease *AluI* (Fermentas,UK) in a total volume of 20 µL containing 1 µg of PCR product; 5 U of restriction endonuclease *AluI*; and 2 µL restriction enzyme buffer. Genotypes were then determined to detect the presence of SCN5A polymorphisms (G892A) by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. For G892A polymorphism of SCN5A gene, heterozygous nucleotide change G892A in exon 7 creates an *AluI* restriction enzyme site (G892A) that allowed independent confirmation of the G to A transition mutation resulting in the substitution of serine for glycine (G298S) in the domain I S5-S6 loop.

#### 2.5 Statistical Analysis

Data are shown as mean±SD. Clinical data were compared between North Indian AVB subjects and controls by the unpaired Student's t test or the Mann–Whitney U test. Qualitative data were compared by the  $\chi^2$  test. Tests for association between genotypes and lipid parameters were performed by t test within North Indian AVB subjects and controls and one-way ANOVA between the two groups. Allele frequencies were estimated by the gene-counting method, and the  $\chi^2$  test was used to identify significant departures from Hardy–Weinberg equilibrium. For differences of allele frequencies and genotypes between North Indian AVB subjects and controls, two models were tested for comparing either allele frequencies in 2×2 contingency tables or genotypes in 2×3 contingency tables. We also performed multivariable logistic regression analysis to adjust risk factors. AVB was a dependent variable, whereas independent variables included age, lipid parameters, smoking status (0=non smoker, 1=smoker), metabolic variables (0=no history of hypertension, or no hypercholesterolemia; 1=positive history), and SCN5A genotype or allele. SCN5A genotype was calculated according

to a dominant (CC=CT=1, TT=0) or additive [CC=(1,1), CT=(1,0), TT=(0,0)] genetic model. The OR and 95% CI were also calculated. Statistical significances were considered significant at P<0.05 levels. The SPSS statistical software package version 15.0 was used for statistical analysis.

### 3. Results

#### 3.1 Subject characteristics

A total number of 208 subjects, 104 AVB patients and 104 healthy controls, were enrolled in this study. The anthropometric, clinical and biochemical characteristics of study subjects (case and control), are presented in **Table 1**. Mean age of case and controls subjects were 55.94±11.99 and 38.91±11.10 respectively. The frequency of smokers (p= 0.0005), diabetic (p= 0.0001) and hypertensive patients (p= 0.0002) were significantly higher in case subjects. The level of glucose (p= 0.0001), TC (p=0.0001), TG (p=0.0001), LDL (p= 0.0001), and VLDL (p=0.0001) was significantly higher in case subjects whereas the level of HDL was significantly higher in healthy controls (p= 0.0001) in this study population. In reference to electrolyte estimation the level of serum sodium (p= 0.30), urea (p= 0.002), creatinine (p= 0.0001) was significantly higher in case subjects whereas the level of serum potassium (p= 0.0001) was higher in healthy controls.

#### 3.2 Distribution of SCN5A G892A genotypes and allele frequencies

**Table-2** shows the distribution of genotype and allele frequencies of AVB subjects and healthy controls for SCN5A G892A genotype. The frequencies of GG, GA and AA genotype in AVB subjects were 37.5, 57.69 and 4.80 % respectively; in the healthy controls, the corresponding frequencies were 70.19, 26.92 and 2.88 % respectively. The frequencies of heterozygous GA genotype (OR= 4.01, 95% CI= 2.21-7.26, p=0.0001) and mutant AA genotype was high in case subjects (OR= 3.12, 95% CI= 0.70-13.75, p= 0.23). The genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium (p= 0.05)

Overall, allele A was found to a significantly greater degree in the AVB as compared to the control group, with a corresponding reduction in the G allele frequency in AVB patients.

#### 3.3 Association between SCN5A G892A polymorphisms and the risk of AVB

Only eight AA homozygotes were found in the total cohort of subjects so, for further analysis, the GA and AA genotypes were combined to form a single group (**Table-3**). As indicated in Table 3, compared with the GG homozygotes, the OR for AVB was OR- 3.92 (95% CI = 2.20–6.99; P = <0.0001) for A allele carriers. Correction for age, gender, smoking status, diabetes and the presence of hypertension did not change this estimate OR- 1.89 (95% CI = 0.78–1.67; P = <0.0001).

### 3.4 Distribution of genotype and allele frequencies in smoker and non smoker groups.

We further divided the case and control study subjects in smoker and non smoker. In case subjects the frequency of GG, GA and AA genotype in the smoker group were 37.31, 58.20 and 4.47% respectively and in non-smoker group the corresponding frequencies were 51.35, 45.94 and 2.70% respectively. On comparison with wild type GG

genotype, the OR for heterozygous GA is 1.74 and for mutant AA the OR is 2.28. Likewise in healthy controls also the frequency of corresponding genotype was also higher in smoker group of healthy control. In healthy controls, the frequency of heterozygous GA (34.14%), mutant AA (4.87%) genotype and minor allele A (21.95%) was higher in smoker group as compared to non-smoker. (Table-4)

**Table-1 Baseline Characteristics of the total study population.**

Characteristics	Case (n=104) (Mean±S.D.)	Controls (n=104) (Mean±S.D.)	p-value
Age (in years)	55.94±11.99	38.91±11.10	< 0.0001* <sup>1</sup>
HR	103.60±41.74	78.76±20.05	< 0.0001* <sup>1</sup>
DBP	132.37±19.00	106.48±48.00	< 0.0001* <sup>1</sup>
SBP	83.19±13.06	78.78±7.34	0.003* <sup>1</sup>
DM	53 (50.96%)	12 (11.53%)	< 0.0001* <sup>2</sup>
HTN	38 (36.53%)	14 (13.46%)	0.0002* <sup>2</sup>
Smoker	67 (64.42%)	41 (39.42%)	0.0005* <sup>2</sup>
Glucose	143.19±54.91	88.59±13.21	< 0.0001* <sup>1</sup>
TC	183.66±44.94	145.89±72.48	< 0.0001* <sup>1</sup>
TG	176.60±40.34	102.95±25.61	< 0.0001* <sup>1</sup>
HDL	35.40±9.85	47.09±7.84	< 0.0001* <sup>1</sup>
LDL	113.69±44.45	78.20±27.81	< 0.0001* <sup>1</sup>
VLDL	34.55±8.90	20.59±5.12	< 0.0001* <sup>1</sup>
Serum sodium	140.25±7.31	136.00±41.46	0.30* <sup>1</sup>
Serum potassium	3.23±0.76	4.45±1.09	< 0.0001* <sup>1</sup>
Serum Urea	39.50±17.18	34.04±5.82	0.002* <sup>1</sup>
Serum creatinine	1.31±0.64	1.02±0.30	< 0.0001* <sup>1</sup>

Data are given as mean ± SD, <sup>1</sup>Unpaired t-test, <sup>2</sup>Chi-square test. \*A value of p<0.05 was considered statistically significant. Abbreviation used- HR- Heart rate, DBP- diastolic blood pressure, SBP- systolic blood pressure, DM, diabetes mellitus, HTN, hypertension, TC total cholesterol, TG- triglycerides, HDL- high density lipoprotein, LDL- low density lipoprotein, VLDL- very low density lipoprotein.

**Table-2 Genotype distribution between cases and controls**

Genotype frequency	Cases n= 104 (%)	Controls n= 104 (%)	OR (95%CI)	p-value <sup>1</sup>
GG	39 (37.5%)	73 (70.19%)	Reference	Reference
GA	60 (57.69%)	28 (26.92%)	4.01 (2.21-7.26),	<0.0001*
AA	5 (4.80%)	3 (2.88%)	3.12 (0.70-13.75)	0.23
<b>Allele frequency</b>				
G Allele	138 (66.4%)	174 (83.65%)	Reference	Reference
A Allele	70 (33.65%)	34 (16.34%)	2.59 (1.62-4.14)	<0.0001*

<sup>1</sup>Binary logistic regression. \*A value of p< 0.05 was considered statistically significant.  
OR-Odds ratio, CI-Confidence interval.

**Table-3 Association of the presence of minor allele and risk of cardiac conduction disorders using binary logistic regression analysis.**

Genotype (G892A)	Unadjusted OR 95% CI	p-value	Adjusted OR 95% CI	p- value <sup>1</sup>
GG	Reference	-	Reference	-
GA+AA	3.92 (2.20-6.99)	<0.0001	1.89 (0.78- 1.67)	<0.0001*

<sup>1</sup>Binary logistic regression. \*A value of p< 0.05 was considered statistically significant.  
OR-Odds ratio, CI-Confidence interval.

**Table-4 Distribution of genotype and allele frequencies in smoker and non smoker case and control subjects.**

Genotype	CASE			CONTROL		
	Smoker n= 67	Non-smoker n= 37	OR (95%CI), p-value	Smoker n= 41	Non-smoker n= 63	OR (95%CI), p-value
GG	25 (37.31%)	19 (51.35%)	Reference	25 (60.97%)	48 (76.19%)	Reference
GA	39 (58.20%)	17 (45.94%)	1.74 (0.76-3.97), 0.2	14 (34.14%)	14 (22.22%)	6.72 (2.00-22.58), 0.002*
AA	3 (4.47%)	1 (2.70%)	2.28 (0.21-23.69), 0.85	2 (4.87%)	1 (1.58%)	3.84 (0.33-44.46), 0.59
G	89 (66.41%)	55 (74.32%)	Reference	64 (78.04%)	110 (87.30 %)	Reference
A	45 (33.58%)	19 (25.67%)	1.46 (0.92-4.05), 0.11	18 (21.95%)	16 (12.69%)	1.93 (0.92-4.05), 0.11

\*A value of  $p < 0.05$  was considered statistically significant. (Post-hoc comparison tests)

#### 4. Discussion

To the best of our knowledge this is the first case report with genetic variations of *SCN5A* in north Indian patients with AVB. To date, a number of *SCN5A* variations associated with various cardiac diseases, such as LQT, BrS, progressive cardiac conduction defect, AF, and overlapping syndromes, have been reported. Although *SCN5A* genetic variations associated with AVB have been well studied in Caucasians, Han Chinese, and Japanese, no study has yet been published in Indians as far we know. In previous studies, it has been shown that in some cases, progressive AVB has been linked to *SCN5A* gene variations [16].

In this study we present clinical and genetic characterizations of one new allele (G892A) associated with AV block. The clinical and molecular genetic characterizations of these diseases are providing refined genotype-phenotype characterizations that lead to improved understanding of the pathophysiology of specific *SCN5A* mutations. Such distinctions may ultimately provide the basis for improved diagnosis and treatment. Functional characterization of G892A revealed a distinct pattern of abnormalities not previously observed for any other single *SCN5A* allele. The polymorphic study we report here shows that gene variation in *SCN5A* (G892A) is associated with AVB.

We investigated the effect of SNP G892A in the proximal promoter region of the *SCN5A* gene in relation to AVB. We compared the frequency of polymorphism of the *SCN5A* gene between two groups of north Indian population, an AVB patients group and a control group. The distribution of *SCN5A* genotype in the AVB group was significantly different from that of the control group. The frequency of allele A was higher in AVB patients than in the control group and was found to have an association with increased risk of AVB (OR= 2.59, 95% CI= 1.62-4.14,  $p = 0.0001$ ). Heterozygous GA (OR= 4.01, 95% CI= 2.21-7.26) and mutant AA (OR= 3.12, 95% CI= 0.70-13.75) genotype were significantly associated with AVB.

Familial AV conduction block, characterized by progressive “degree of block” in association with variable apparent “site of block,” may be transmitted as an autosomal dominant trait. Two genetically distinct forms of AV conduction block have been identified [17] Brink et al [18] established a genetic link between AV block and a genetic

locus at chromosome 19q13. Schott et al [7] mapped AV block to chromosome 3p21, where the cardiac sodium channel, *SCN5A*, is encoded and identified 2 *SCN5A* mutations. Recently, Tan et al [8] characterized an *SCN5A* mutation (G514C) that resulted in an isolated cardiac conduction defect. In contrast to *SCN5A* mutants resulting in long-QT or Brugada syndrome, G514C exhibited opposing gating effects, including a depolarizing shift in the voltage-dependence of activation and enhanced fast inactivation, which are predicted to result in isolated conduction slowing.

In the present study we also correlate the smoking habit to *SCN5A* genetic variation in AV block patients. To date, no detailed studies on the interactions between cigarette smoking and *SCN5A* genetic variation have been published. Use of cigarettes and smokeless tobacco is a considerable public health problem. Conversely, nicotine also has the potential to be a valuable pharmacological agent [19]. Nicotine is known to increase the risk of cardiovascular disease, sudden coronary death, hypertension, and stroke [20–23]. It is believed that nicotine promotes sudden cardiac death by provoking lethal ventricular arrhythmias [24–26]. Indeed, nicotine is implicated in a wide spectrum of cardiac rhythmic disorders, including transient sinus arrest and/or bradycardia, sinus tachycardia, atrial fibrillation, sinoatrial block, AV block, and ventricular tachyarrhythmias [21,24–27]. Nicotine binds to the nicotinic cholinergic gating site on cation channels in receptors (nAChRs) throughout the body, stimulating the release of neurotransmitters, including catecholamines from the adrenal medulla. The cardiac effects of nicotine have been ascribed to this enhanced release of catecholamines [27].

In this study we divided the study subjects (case and controls) into two groups of smoker and non-smokers. The frequency of minor allele A was higher in AVB patients with smoking than the non-smoker and was also found to have an association with increased risk of AVB (OR= 1.46, 95% CI= 0.92-4.05). Heterozygous GA (OR= 1.74, 95% CI= 0.76-3.97) and mutant AA (OR= 2.28, 95% CI= 0.21-23.69) genotype were also associated with AVB.

Although smoking and smokeless tobacco cause many health problems including cancers of oral cavity, oral soft tissue lesions, gum recession and nicotine addiction but in one study there were association between tobacco use and cardiovascular disease, [28] although this has not been a

consistent finding. The adverse effects of smoking on vascular function have been examined in human subjects. These studies have shown that acute and chronic cigarette smoking impairs nitric oxide synthase mediated relaxation of large blood vessels. However, a consensus of the causal relationship between electro physical disorders of the heart and consumption of smoking and smokeless tobacco has not yet been reached. This correlation requires further research.

## 5. Conclusion

From the study it has been found that *SCN5A* G892A gene polymorphism is significantly associated with cardiac conduction disorder risk in northern Indian population and finally, the results suggest that evaluation of the above mentioned gene may prove a useful addition to measurement of standard risk factors in prediction the risk of cardiac conduction disorder.

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