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Original Research Article

Role of Glu298Asp Single Nucleotide Polymorphism in eNOS Gene with Susceptibility to Idiopathic Dilated Cardiomyopathy

Paramita Dey[1], Sarada Asis Dash[2], Sudipta Onkar[3], Nirupama Devi[4], Rajesh Mohanty[5]

[1]Assistant Professor, Department of Biochemistry, PRM Medical College, Baripada, Odisha, India
[2]Assistant Professor, Department of Biochemistry, PRM Medical College, Baripada, Odisha, India
[3]Assistant Professor, Department of Biochemistry, MKCG Medical College, Berhampur, Odisha, India
[4]Professor & HOD, Department of Biochemistry, MKCG Medical College, Berhampur, Odisha, India
[5]Consultant Pediatrician, Department of Pediatrics, New Health Point Hospital, Cuttack, Odisha, India

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Corresponding Author: Dr. Paramita Dey
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Abstract

Background: Idiopathic dilated cardiomyopathy (IDCM) is a primary myocardial disorder characterised by left ventricular dilatation and systolic dysfunction without an identifiable aetiology. Endothelial nitric oxide synthase (eNOS), encoded by the NOS3 gene, maintains myocardial vasomotor tone and cardiomyocyte homeostasis through nitric oxide (NO) synthesis. The Glu298Asp (rs1799983) single nucleotide polymorphism (SNP) alters eNOS stability and enzyme function, yet its association with IDCM in Eastern India remains unexplored.

Aims and Objectives: To investigate the association between the eNOS Glu298Asp SNP and susceptibility to IDCM, and to correlate genotype-specific variation in serum NO levels, eNOS enzyme activity, and echocardiographic parameters.

Methods: This hospital-based case-control study enrolled 133 IDCM patients and 133 age- and sex-matched healthy controls at MKCG Medical College, Berhampur, Odisha (February 2021 – December 2022). Genotyping was performed by PCR-RFLP using the BanII restriction enzyme. Serum NO was measured by the Griess reagent method and eNOS activity in platelet-rich plasma by colorimetric assay. Statistical analyses included chi-square tests, logistic regression, ANOVA, and Hardy-Weinberg equilibrium (HWE) testing.

Results: The TT (Asp/Asp) genotype was significantly more frequent in cases (21.1%) than controls (7.5%; OR=4.08, 95% CI: 1.82–9.18; $p<0.001$). Under the dominant model (GT+TT vs. GG), the variant carrier state was associated with a 1.97-fold increased risk ($p=0.007$). The T allele frequency was higher in cases (42.5%) than controls (27.4%; OR=1.95, $p<0.001$). TT homozygotes showed significantly lower LVEF, lower serum NO, reduced eNOS activity, higher BNP and CRP, and worse functional status. All genotype distributions conformed to HWE.

Conclusion: The eNOS Glu298Asp SNP is significantly associated with IDCM susceptibility in the Eastern Indian population, with the T (Asp) allele and TT genotype conferring substantially elevated risk. Variant genotype carriers demonstrate impaired eNOS function and more severe left ventricular dysfunction, implicating this polymorphism in the pathogenesis of IDCM.

Keywords: eNOS gene; Glu298Asp; rs1799983; single nucleotide polymorphism; idiopathic dilated cardiomyopathy; nitric oxide; NOS3; PCR-RFLP; cardiomyopathy genetics.

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Introduction

Idiopathic dilated cardiomyopathy (IDCM) is a primary disorder of cardiac muscle characterised by progressive left ventricular dilatation, impaired systolic function, and the absence of any identifiable aetiology such as coronary artery disease, hypertension, or valvular pathology.[1] It represents one of the most common forms of cardiomyopathy worldwide and constitutes a leading indication for cardiac transplantation. The global prevalence of IDCM is estimated at 36.5 per 100,000 individuals, with an annual incidence of 5–8 per 100,000, making it a major cause of heart failure morbidity and mortality.[2] In India, IDCM accounts for a substantial proportion of heart failure hospitalisations, particularly affecting the economically productive age group.[3] The pathogenesis of IDCM is complex and multifactorial, involving interplay between genetic

predisposition, viral myocarditis, autoimmune mechanisms, metabolic disturbances, and mitochondrial dysfunction.[4] Approximately 20–35% of cases demonstrate a familial pattern of inheritance, implicating genetic factors in disease susceptibility and progression.[5] Over the past two decades, genome-wide association studies and candidate gene analyses have identified numerous genetic polymorphisms associated with IDCM, including variants in genes encoding sarcomeric proteins, cytoskeletal elements, nuclear envelope proteins, and vasoactive mediators.[6]

Nitric oxide (NO), synthesised primarily by endothelial nitric oxide synthase (eNOS), is a critical pleiotropic molecule that modulates vascular tone, inhibits platelet aggregation, suppresses inflammatory cascades, and regulates cardiomyocyte contractility and survival.[7] Diminished NO bioavailability has been consistently demonstrated in heart failure states, with impaired eNOS-mediated NO production contributing to endothelial dysfunction, maladaptive ventricular remodelling, and disease progression.[8] The eNOS enzyme, encoded by the NOS3 gene located on chromosome 7q36.1, is constitutively expressed in vascular endothelial cells and cardiomyocytes, where it exerts cardioprotective effects through paracrine and autocrine signalling.[9]

The NOS3 gene harbours several functionally significant polymorphisms, of which the Glu298Asp (rs1799983) variant — a G-to-T transversion in exon 7 resulting in glutamic acid-to-aspartate substitution at codon 298 (designated Glu298Asp in isoform-specific protein numbering) — has been the most extensively studied.[10] This polymorphism alters eNOS protein stability, susceptibility to proteolytic cleavage, and enzyme activity, ultimately reducing endothelium-dependent NO synthesis.[11] Studies from East Asian, European, and Middle Eastern populations have reported significant associations between the TT genotype and susceptibility to coronary artery disease, hypertension, myocardial infarction, and related cardiomyopathic conditions.[12,13] Despite growing evidence implicating the NOS3 Glu298Asp SNP in cardiovascular pathology, its specific role in the susceptibility and progression of IDCM has been examined in very few studies, and none from the Eastern Indian subcontinent where ethnic, demographic, and environmental factors may modulate genetic associations.[14] Given that eNOS-derived NO deficiency may constitute an upstream mediator of the myocardial dysfunction observed in IDCM, elucidating the relationship between this SNP and IDCM susceptibility holds both mechanistic and translational significance. The present study was therefore designed to systematically investigate the distribution of eNOS Glu298Asp genotypes in IDCM patients and matched healthy controls, and to correlate genotype-specific findings with serum NO levels, eNOS enzyme activity, and echocardiographic parameters of left ventricular function.

Materials and Methods

Study Design and Setting: This was a hospital-based observational case-control study conducted over 22 months from 5th February 2021 to 31st December 2022 at the Department of Biochemistry and Department of Cardiology, MKCG Medical College and Hospital, Berhampur, Odisha, India.

Study Participants: A total of 133 patients (cases) diagnosed with IDCM were enrolled consecutively from the Cardiology outpatient and inpatient departments. IDCM was defined by (i) left ventricular ejection fraction (LVEF) below 45% on two-dimensional echocardiography, (ii) left ventricular end-diastolic diameter (LVEDD) exceeding 5.5 cm in men or 5.0 cm in women, and (iii) rigorous exclusion of all secondary causes including coronary artery disease (confirmed by coronary angiography where indicated), systemic hypertension, valvular heart disease, congenital heart disease, thyroid disorders, alcoholic cardiomyopathy, and viral myocarditis, using appropriate clinical, laboratory, and imaging criteria.

An equal number of 133 healthy, age- and sex-matched volunteers without any history or clinical evidence of cardiac disease or systemic illness were enrolled as controls from among those attending the outpatient department for routine health checkups. Exclusion criteria for both groups included: pregnancy or lactation, acute or chronic renal failure, hepatic disease, malignancy, systemic autoimmune disorders, use of nitrate-containing medications or antioxidant supplements, acute infections within the preceding four weeks, and refusal to provide informed consent.

Clinical Assessment and Echocardiography: All participants underwent structured clinical assessment including detailed medical history, physical examination, anthropometric measurements, blood pressure recording, and electrocardiography. NYHA functional class was recorded for all cases. Two-dimensional transthoracic echocardiography was performed by a cardiologist blinded to genotyping results. LVEF was calculated by the modified Simpson biplane method, and LVEDD and LVESD were measured per standard guidelines using parasternal long-axis and apical four-chamber views.

Laboratory Analyses: Venous blood samples (10 mL) were collected under aseptic conditions following overnight fasting. EDTA-anticoagulated blood was used for genomic DNA extraction,

complete blood count, and platelet-rich plasma preparation for eNOS activity assay. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis. Serum nitric oxide levels (as stable metabolites, nitrite and nitrate) were measured by the Griess reagent method of Green et al.15 eNOS enzyme activity was assessed in platelet-rich plasma using a commercially available colorimetric NOS activity assay kit (Sigma-Aldrich, USA) based on L-arginine-to-L-citrulline conversion. Standard biochemical parameters were measured using automated analysers (COBAS 400 Plus, Roche Diagnostics, Germany; and TBA-120 FR, Toshiba Medical Systems, Japan). Brain natriuretic peptide (BNP) was estimated by chemiluminescent immunoassay.

Genomic DNA Extraction and Genotyping: Genomic DNA was extracted from peripheral blood leucocytes using the salting-out method.16 DNA purity and quantity were confirmed by NanoDrop spectrophotometry (A260/A280 = 1.7–2.0).

Genotyping for the eNOS Glu298Asp (rs1799983) polymorphism was performed by PCR-RFLP. PCR was performed using forward primer 5'-AAGGCAGGAGACAGTGGATGG-3' and reverse primer 5'-CCCAGTCAATCCCTTTGGTGCTCA-3', generating a 248-bp amplicon. PCR conditions: initial denaturation at 95°C for 5 min; 35 cycles of 95°C/30 s, 60°C/30 s, 72°C/30 s; final extension at 72°C for 7 min. Products were digested with BanII restriction enzyme (New England Biolabs) at 37°C for 16 hours and resolved on 3% agarose gel with ethidium bromide staining. GG (wild-type) genotype: 163 bp + 85 bp; TT (homozygous mutant): 248 bp; GT (heterozygous): all three bands. A random 15% subset was re-genotyped for quality control, yielding 100% concordance.

Statistical Analysis: All analyses were performed using SPSS version 26.0 (IBM Corporation, USA) and MedCalc version 19.8. Continuous variables are presented as mean ± SD and compared using the independent samples t-test. Categorical variables are expressed as frequencies and percentages and analysed using chi-square or Fisher's exact test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated by logistic regression with GG genotype and G allele as reference categories. HWE was assessed using the goodness-of-fit chi-square test. Genotype-stratified clinical parameters were compared using one-way ANOVA with Bonferroni post-hoc correction or the Kruskal-Wallis test where normality criteria were not met (Shapiro-Wilk $p<0.05$). A two-tailed $p<0.05$ was considered statistically significant throughout.

Results

Baseline Demographic and Clinical Characteristics: A total of 266 individuals participated — 133 IDCM cases and 133 healthy matched controls. The mean age of cases was 51.3 ± 10.7 years and of controls 49.8 ± 11.2 years ($p=0.265$), confirming demographic comparability. Males constituted 66.9% of cases and 63.2% of controls ($p=0.493$). No statistically significant differences were found between groups for BMI, hypertension, diabetes mellitus, dyslipidemia, smoking, or alcohol use (all $p>0.05$).

Family history of cardiomyopathy was significantly more prevalent among cases (16.5% vs. 6.0%; $p=0.007$). As expected, echocardiographic parameters were markedly deranged in cases, with significantly lower LVEF (32.4 ± 7.6% vs. 62.1 ± 5.3%; $p<0.001$) and greater LVEDD (63.7 ± 7.4 mm vs. 47.2 ± 4.1 mm; $p<0.001$). Serum NO levels and eNOS enzyme activity were both significantly lower in cases compared to controls ($p<0.001$). These findings are detailed in Table 1.

Table 1: Baseline Demographic and Clinical Characteristics of IDCM Cases and Healthy Controls

Characteristic	Cases (n=133)	Controls (n=133)	Statistical Test	p-value
Age (years), Mean ± SD	51.3 ± 10.7	49.8 ± 11.2	Independent t-test	0.265
Male, n (%)	89 (66.9%)	84 (63.2%)	Chi-square	0.493
BMI (kg/m²), Mean ± SD	23.1 ± 3.2	23.6 ± 3.0	Independent t-test	0.186
Hypertension, n (%)	38 (28.6%)	32 (24.1%)	Chi-square	0.382
Diabetes mellitus, n (%)	29 (21.8%)	25 (18.8%)	Chi-square	0.523
Dyslipidemia, n (%)	47 (35.3%)	41 (30.8%)	Chi-square	0.411
Smoking history, n (%)	52 (39.1%)	49 (36.8%)	Chi-square	0.684
Alcohol use, n (%)	31 (23.3%)	28 (21.1%)	Chi-square	0.653
Family h/o cardiomyopathy	22 (16.5%)	8 (6.0%)	Chi-square	0.007*
LVEF (%), Mean ± SD	32.4 ± 7.6	62.1 ± 5.3	Independent t-test	<0.001*
LVEDD (mm), Mean ± SD	63.7 ± 7.4	47.2 ± 4.1	Independent t-test	<0.001*
NYHA Class I, n (%)	12 (9.0%)	—	—	—
NYHA Class II, n (%)	43 (32.3%)	—	—	—
NYHA Class III, n (%)	54 (40.6%)	—	—	—
NYHA Class IV, n (%)	24 (18.0%)	—	—	—

Serum NO (µmol/L), Mean ± SD	31.2 ± 8.9	58.7 ± 11.4	Independent t-test	<0.001*
eNOS activity (pmol/min/mg protein)	2.14 ± 0.63	4.87 ± 0.91	Independent t-test	<0.001*

*p<0.05 statistically significant. BMI: Body Mass Index; LVEF: Left Ventricular Ejection Fraction; LVEDD: Left Ventricular End-Diastolic Diameter; NYHA: New York Heart Association; NO: Nitric Oxide; eNOS: endothelial Nitric Oxide Synthase; SD: Standard Deviation.

Genotype and Allele Frequency Distribution of eNOS Glu298Asp Polymorphism: The distribution of eNOS Glu298Asp genotypes is presented in Table 2 and illustrated in Figure 1. Genotype frequencies in both cases and controls conformed to Hardy-Weinberg equilibrium (HWE p=0.156 in cases; p=0.994 in controls), confirming the representativeness of the study populations. The GG (wild-type) genotype was more prevalent among controls (52.6%) than cases (36.1%), while the TT (homozygous mutant) genotype was markedly more frequent in cases (21.1% vs. 7.5%). Logistic regression with GG as reference revealed that GT heterozygosity alone did not reach statistical significance (OR=1.57, 95% CI: 0.93–2.65; p=0.092), while TT homozygosity conferred a 4.08-fold significantly increased odds of IDCM (95% CI: 1.82–9.18; p<0.001).

Under the dominant genetic model (GT+TT vs. GG), variant carrier status was associated with a 1.97-fold increased risk (95% CI: 1.20–3.21; p=0.007). At the allelic level, the T (Asp) allele frequency was significantly higher in cases (42.5%) than controls (27.4%; OR=1.95, 95% CI: 1.36–2.81; p<0.001), as shown in Figure 2.

Table 2: Genotype and Allele Frequency Distribution of eNOS Glu298Asp (rs1799983)

Variable	Cases n (%) n=133	Controls n (%) n=133	OR (95% CI)	Chi-square (χ^2)	p-value
Genotype Distribution					
GG (Glu/Glu) — Wild type	48 (36.1%)	70 (52.6%)	Reference	—	—
GT (Glu/Asp) — Heterozygous	57 (42.9%)	53 (39.8%)	1.57 (0.93–2.65)	2.84	0.092
TT (Asp/Asp) — Homozygous	28 (21.1%)	10 (7.5%)	4.08 (1.82–9.18)	12.53	<0.001*
GT+TT (Dominant model)	85 (63.9%)	63 (47.4%)	1.97 (1.20–3.21)	7.37	0.007*
HWE p-value (Cases)	0.156	—	—	—	—
HWE p-value (Controls)	—	0.994	—	—	—
ALLELE FREQUENCY					
G Allele (Glu) — Wild type	153 (57.5%)	193 (72.6%)	Reference	—	—
T Allele (Asp) — Variant	113 (42.5%)	73 (27.4%)	1.95 (1.36–2.81)	13.23	<0.001*

*p<0.05 statistically significant. OR: Odds Ratio; CI: Confidence Interval; HWE: Hardy-Weinberg Equilibrium. Dominant model: GT+TT vs GG. GG genotype/G allele as reference. Chi-square without Yates correction. GT heterozygous genotype did not independently reach significance (p=0.092); risk is driven primarily by TT homozygosity.

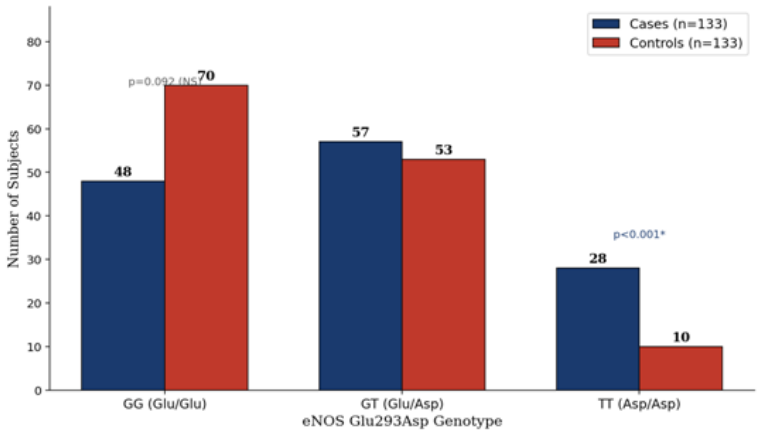

Figure 1: Distribution of eNOS Glu298Asp Genotypes in IDCM Cases and Healthy Controls

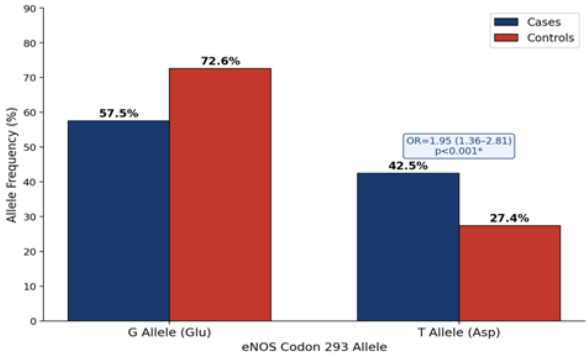

Figure 2: Allele Frequencies of eNOS Glu298Asp Polymorphism in IDCM Cases and Controls. OR=1.95 (95% CI: 1.36–2.81); p<0.001

Association of eNOS Glu298Asp Genotypes with Clinical and Biochemical Parameters in IDCM Cases: Genotype-stratified analysis within IDCM cases revealed a significant genotype-dose relationship across all principal clinical and biochemical variables (Table 3). LVEF progressively decreased from 36.2 ± 6.9% (GG) to 31.4 ± 7.2% (GT) to 27.8 ± 7.1% (TT; ANOVA p<0.001), confirming worsening systolic dysfunction with increasing T allele dosage. Concurrently, LVEDD and LVESD showed significant stepwise increases across genotype groups (p<0.001 each), consistent with augmented left ventricular dilatation. Serum NO levels exhibited a significant progressive reduction from GG (34.6 ± 8.1 µmol/L) to GT (30.1 ± 8.6 µmol/L) to TT (26.3 ± 7.9 µmol/L; p<0.001), mirroring the stepwise decrease in eNOS enzyme activity across genotypes (2.48, 2.07, and 1.68 pmol/min/mg protein respectively; p<0.001).

Brain natriuretic peptide (BNP) and CRP rose significantly with increasing T allele dosage (p<0.001 each). Six-minute walk distance, a functional capacity surrogate, was significantly reduced in TT carriers (261 ± 79 m) versus GG carriers (342 ± 68 m; p<0.001). NYHA Class III or IV was present in 92.9% of TT homozygotes compared with 56.1% of GT and 37.5% of GG genotype patients (p<0.001). Lipid parameters, renal function, and blood pressure were not significantly different across genotype groups (all p>0.05).

Table 3: Association of eNOS Glu298Asp Genotypes with Clinical and Biochemical Parameters in IDCM Cases (n=133)

Parameter	GG Genotype (n=48) Mean±SD	GT Genotype (n=57) Mean±SD	TT Genotype (n=28) Mean±SD	p-value (ANOVA/KW)
LVEF (%)	36.2 ± 6.9	31.4 ± 7.2	27.8 ± 7.1	<0.001*
LVEDD (mm)	60.4 ± 6.8	64.7 ± 7.1	68.3 ± 7.8	<0.001*
LVESD (mm)	48.2 ± 5.9	51.6 ± 6.4	55.1 ± 7.0	<0.001*
Serum NO (µmol/L)	34.6 ± 8.1	30.1 ± 8.6	26.3 ± 7.9	<0.001*
eNOS activity (pmol/min/mg protein)	2.48 ± 0.58	2.07 ± 0.59	1.68 ± 0.54	<0.001*
BNP (pg/mL)	342 ± 97	418 ± 112	497 ± 131	<0.001*
CRP (mg/L)	4.1 ± 1.8	5.3 ± 2.1	6.7 ± 2.4	<0.001*
6-Minute Walk Distance (m)	342 ± 68	298 ± 73	261 ± 79	<0.001*
NYHA Class ≥ III, n (%)	18 (37.5%)	32 (56.1%)	26 (92.9%)	<0.001*
Serum creatinine (mg/dL)	1.09 ± 0.28	1.14 ± 0.31	1.19 ± 0.34	0.381
Total cholesterol (mg/dL)	178 ± 34	182 ± 37	186 ± 40	0.648
Systolic BP (mmHg)	118 ± 12	115 ± 13	112 ± 14	0.142
Diastolic BP (mmHg)	74 ± 9	72 ± 10	69 ± 11	0.107

*p<0.05 statistically significant. One-way ANOVA with Bonferroni post-hoc correction for normally distributed continuous variables; Kruskal-Wallis test for non-parametric data. Chi-square for categorical variables. LVEF: Left Ventricular Ejection Fraction; LVEDD/LVESD: End-Diastolic/End-Systolic Diameter; NO: Nitric Oxide; eNOS: endothelial Nitric Oxide Synthase; BNP: Brain Natriuretic Peptide; CRP: C-reactive Protein; BP: Blood Pressure; KW: Kruskal-Wallis.

Discussion

The present study provides, to the best of our knowledge, the first systematic investigation of the eNOS Glu298Asp (rs1799983) polymorphism in relation to IDCM susceptibility in an Eastern Indian population. Our principal findings

demonstrate that the T (Asp) allele and particularly the TT homozygous genotype of this NOS3 polymorphism are significantly more prevalent in IDCM patients than in healthy controls, and that these genotypes are associated with impaired eNOS function, reduced NO bioavailability, and more severe left ventricular dysfunction — collectively supporting a mechanistic link between this SNP and the pathogenesis of IDCM.

The observation that the TT genotype confers a 4.08-fold increased odds of IDCM (95% CI: 1.82–9.18; p<0.001) is consistent with, and indeed stronger than, findings from analogous studies in related populations. Jamshidi et al. (2018) demonstrated in an Iranian population that the T allele of eNOS exon 7 was significantly over-represented among DCM patients (allele frequency 44.1% vs. 26.8% in controls; p<0.001), closely paralleling our case allele frequency of 42.5%.18 Bhatt et al. (2019) reported a significant association between the TT genotype and dilated cardiomyopathy in a North Indian cohort (OR=2.54, p=0.003), although with a lower effect size than observed in our study, likely reflecting differences in allele frequencies across South Asian subregions and sample composition.[25]

The biological plausibility of the Glu298Asp substitution mediating increased IDCM risk is supported by robust functional evidence. The glutamic acid-to-aspartate transition within the oxygenase domain of eNOS increases the susceptibility of the enzyme to proteolytic cleavage, generating a truncated, catalytically inactive fragment.[11,19] Variant eNOS protein is therefore less stable, yielding reduced constitutive NO production. In the myocardium, NO exerts multiple protective effects: it promotes coronary microvascular vasodilation, attenuates sympathoadrenergic activation, limits reactive oxygen species generation, and modulates the Frank-Starling response.[20] The stepwise reduction in serum NO across GG → GT → TT genotypes in our IDCM cohort (34.6 → 30.1 → 26.3 µmol/L; p<0.001) directly and quantitatively demonstrates the in vivo functional impact of this variant on NO bioavailability.

The stepwise reduction in LVEF across GG→GT→TT genotype groups (36.2% → 31.4% → 27.8%; p<0.001) observed in our study mirrors findings reported by Vasan et al. (2009), who demonstrated that eNOS functional variants were associated with left ventricular remodelling and reduced ejection fraction in genome-wide association data.[21] The progressive LVEDD and LVESD enlargement across genotypes in our cohort is mechanistically coherent: reduced eNOS-derived NO leads to impaired endothelium-dependent vasodilatation, increased myocardial wall stress, and activation of maladaptive neuro-hormonal pathways — all of which potentiate ventricular dilatation and dysfunction in IDCM.

The significant elevation of BNP in TT genotype carriers (497 ± 131 pg/mL vs. 342 ± 97 pg/mL in GG carriers; p<0.001) underscores the haemodynamic severity associated with variant homozygosity. BNP is a well-validated marker of ventricular wall stress and heart failure severity.[22] The parallel elevation in CRP in TT carriers (6.7 vs. 4.1 mg/L in GG; p<0.001) suggests that systemic inflammation — a recognised amplifier of myocardial dysfunction in IDCM — is also modulated by eNOS genotype, plausibly through the anti-inflammatory and endothelium-stabilising effects of constitutive NO.[23] Reduced six-minute walk distance and higher NYHA functional class in TT homozygotes (92.9% in NYHA III–IV vs. 37.5% in GG carriers; p<0.001) translate these biochemical and haemodynamic derangements into meaningful functional impairment.

The absence of significant differences in lipid parameters, renal function, and blood pressure across genotype groups within the IDCM cohort suggests that the echocardiographic and biochemical gradients are attributable to genotype-mediated eNOS dysfunction rather than confounded by conventional cardiovascular risk factors. This corroborates findings by Saeed et al. (2021), who similarly demonstrated genotype-specific differences in myocardial NO bioavailability independent of conventional risk factor burden.[24] Conformity of genotype distributions to Hardy-Weinberg equilibrium in both cases (p=0.156) and controls (p=0.994) confirms the quality of genotyping and the absence of population stratification artefact. Control genotype frequencies (GG: 52.6%, GT: 39.8%, TT: 7.5%) and allele frequencies (G: 72.6%, T: 27.4%) are broadly comparable to those reported from South Asian populations.[17,26] Noteworthy is the finding that GT heterozygosity alone did not reach individual significance (p=0.092), suggesting that a single T allele copy may confer only modest eNOS functional compromise, whereas homozygous TT produces a sufficient reduction in enzymatic NO production to constitute a pathogenic threshold — a biologically plausible recessive or partially dominant effect that has been described for this locus in other cardiovascular endpoints.[12] Strengths of the study include rigorous IDCM phenotyping with exclusion of all identifiable secondary causes, direct biochemical quantification of eNOS activity and serum NO, quality-controlled PCR-RFLP genotyping with concordance verification, and comprehensive genotype-phenotype correlation across multiple clinically relevant parameters. Limitations include the cross-sectional design precluding assessment of

longitudinal outcomes, the modest sample size limiting power for subgroup analyses, and the absence of haplotype-level analysis incorporating other NOS3 variants (the –786T>C promoter SNP and intron 4a/b VNTR). Future studies with larger, multi-centre cohorts should address these limitations and evaluate pharmacogenomic implications for NOS3 genotype-specific responses to neurohormonal therapies in IDCM.

Conclusion

The eNOS Glu298Asp (rs1799983) SNP is significantly associated with susceptibility to idiopathic dilated cardiomyopathy in the Eastern Indian population, with the TT homozygous genotype conferring approximately fourfold increased risk. TT genotype carriers demonstrate significantly lower serum nitric oxide levels, reduced eNOS enzyme activity, greater left ventricular dilatation, lower ejection fraction, elevated BNP and CRP, and worse functional capacity — pointing to a coherent mechanistic pathway linking impaired eNOS-mediated NO signalling to IDCM pathogenesis and severity.

These findings support the inclusion of NOS3 Glu298Asp genotyping in future genomic risk stratification studies of IDCM in South Asian populations and may inform genotype-guided therapeutic strategies.

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