RESEARCH PAPER

SCN5A Screening for Cardiac Arrest Risk in Pulmonary Embolism Patients in North Karnataka Population, India

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Highlights:

1. First study to screen 22nd exon of SCN5A in pulmonary embolism patients among North Karnataka population in India.
2. Bidirectional Sanger sequencing of 50 PE cases (stratified by cardiac-arrest status) and 50 matched controls identified no coding or splice-relevant variants.
3. Findings indicate SCN5A exon 22 is unlikely to be a major genetic determinant of cardiac-arrest risk in PE, refining rather than closing the hypothesis.
4. Results support extending screening to additional SCN5A exonic/intronic regions and other candidate genes to identify cardiac arrest risk biomarkers in PE.

Abstract:

Background: The most common and frequently under-diagnosed cause of cardiac arrest is pulmonary embolism (PE). In predisposed PE patients, acute right ventricular pressure overload can cause fatal cardiac arrhythmias, and this arrhythmia risk is known to be associated with the SCN5A gene (sodium voltage-gated channel alpha subunit), which codes for the cardiac voltage-gated sodium channel Nav1.5. It's unclear, though, if variations in this gene have an impact on the Indian population's risk of cardiac arrest in PE. **Methods:** A nested case-control study was conducted to screen exon 22 of the SCN5A gene in the North Karnataka population, which included 50 PE cases, stratified by cardiac-arrest status and 50 age- and sex-matched healthy controls. The DNeasy blood and tissue kit was used to isolate genomic DNA, and the 22nd exon was amplified using (Polymerase chain reaction) PCR and followed by bidirectional Sanger sequencing and sequence analysis using DNA baser. **Results:** No coding or splice-relevant variants were identified in any of the 100 participants in this cohort.

Conclusion: The absence of variation in the 22nd exon of the SCN5A gene in this cohort should be interpreted as hypothesis-refining rather than hypothesis-negating, suggesting that the 22nd exon of SCN5A may not be a major significant factor in the genetic predisposition to cardiac arrest risk in PE cases. These findings highlight the need for further research to explore other exonic and intronic regions of the SCN5A gene or additional genetic factors to develop biomarkers for cardiac arrest risk in the PE cohort.

Keywords: Pulmonary embolism; Cardiac arrest; SCN5A; Arrhythmia; Sanger sequencing

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1. INTRODUCTION:

Pulmonary embolism (PE) continues to be a significant cause of mortality and morbidity globally and the most severe outcome of PE is cardiac arrest which results from right ventricular (RV) pressure overload. Sudden occlusion of the pulmonary vascular tree causes elevation in RV afterload, leading to dilation of the RV, septal shift, and hemodynamic compromise in a proportion of patients; this is the rationale behind right heart failure in patients with high-risk PE, which is responsible for the high mortality [1,2,3]. In addition to hemodynamic failure, there is increasing evidence to suggest that PE may be an under-diagnosed cause of cardiac arrest both out- and in-hospital, being responsible for 2-5% of cases of resuscitated cardiac arrest, as well as up to 3% of all cases of cardiac arrest admitted alive to hospital in population registries, with a disproportionate incidence in those cases with non-shockable rhythms like pulseless electrical activity [4,5]. The case-fatality rate for PE leading to cardiac arrest has been found to be about 90-95%, compared to 30% for massive PE without arrest [5,6].

It is unclear how the specific mechanism that underlies progression from haemodynamic stress to sudden arrhythmia in only a proportion of patients

with PE may occur. RV pressure overload does not merely present as a mechanical challenge but also involves regional hypoxia, acidosis, and changes in intracellular Ca^{+2} and Na^{+} concentrations, which may reduce the threshold for developing ventricular arrhythmias in a structurally normal heart [3,7,8]. The second line of evidence comes from genetically-based ion channel disorders, where a genetically-mediated predisposition to develop an abnormal cardiac electrical substrate, which is expressed only when an additional physiologically-induced stress is applied, has been well documented, and particularly so in the case of catecholaminergic polymorphic ventricular tachycardia (CPVT) due to mutations in Ryanodine receptor (RYR2), Calsequestrin 2 (CASQ2), Triadin (TRDN), or Calmodulin (CALM) 1–3 that result in destabilization of sarcoplasmic reticulum calcium handling, thus causing ventricular arrhythmia under adrenergic or exertional stress. Therefore, genetic variability in ion-channel genes could similarly affect the electrical substrate in acute physiological stress of PE [9,10,11].

The SCN5A (Sodium voltage-gated channel alpha subunit), encoding the α-subunit of the cardiac sodium channel Nav1.5 which forms the channel pore. The cardiac sodium channel Nav1.5 provides the fast-depolarizing phase of the cardiac action potential and is necessary for normal impulse conduction. Loss-of-function and gain-of-function variants in SCN5A are known etiologies of long QT syndrome type 3, Brugada syndrome, cardiac conduction disease, sick sinus syndrome, atrial fibrillation, and cardiomyopathy and, taken together, represent a well-recognized genetic cause of cardiac death [12,13,14,15]. Hypoxia-induced modulation of the expression and function of Nav1.5 channel and thus possible triggering of ventricular arrhythmias is especially interesting because, in this case, the channel is also modulated acutely in response to myocardial hypoxia and ischemia: chronic hypoxic exposure leads to the down-regulation of Nav1.5 current through transcript and trafficking mechanisms, whereas acute ischemic stress results in rapid SUMOylation of Nav1.5 protein on the lysine 442 residue, leading to increased late sodium current and prolonged potential duration [4,16]. MicroRNA-448 up-regulation in response to myocardial ischemia is another example of hypoxia-induced regulation of Nav1.5 expression leading to arrhythmic vulnerability after myocardial infarction; this process can be reversed by miR-448 inhibition [5]. The above findings suggest a likely pathway by which acute RV hypoxia during PE can lead to dysfunction of Nav1.5 in such a way that the presence of a constitutively expressed SCN5A variant that acts on the same channel can act synergistically with it in reducing the arrhythmogenic threshold. exon 22 of SCN5A encodes the S4 voltage sensing region of the third homologous domain (DIIS4) of the Nav1.5 protein, which is situated immediately adjacent to the DIII-DIV inter-domain linker region, a region that has been found to be among the most common mutational hot spots for Brugada Syndrome and long QT syndrome type 3. It has been demonstrated through studies carried out in a French pedigree cohort that deletion of the DIIIS4 region through abnormal splicing of the exon leads to progressive cardiac conduction disorder. Thus, exon 22 represents a biologically rational and practical starting point for candidate-region screening in a cohort [15,17,18].

From a clinical perspective, risk stratification of acute PE is defined using the 2019 European Society of Cardiology (ESC) guidelines, which have four categories of patients based on their haemodynamic stability, the (simplified) Pulmonary Embolism Severity Index (PESI/PESI-S), the presence of RV dysfunction on imaging, and biomarkers such as troponin and BNP, which are indicators of RV myocardial damage rather than any arrhythmic substrate [19,20]. However, these biomarkers have been well established to predict short-term mortality but are incapable of predicting a person's inherent electrical instability, and there is no genetic marker included in risk stratification of PE. Therefore, determining the role of commonly occurring genes such as SCN5A in the process of development of PE to cardiac arrest becomes a necessary step towards the development of clinical-genetic risk stratification.

2. RESEARCH METHODS:

2.1 Sample collection:

Approval for the ethical clearance of the present study was obtained from the Institutional Ethics Committee of the Karnataka Cancer Therapy and Research Institute (KCTRI), Hubli (Reference: RCTRI/EC-01-A/2024). This study was conducted as a nested case-control study. According to the prevalence of PE in North Karnataka population, 50 patients of PE and 50 healthy controls (n = 100) were selected. These patients of PE were further stratified as PE with cardiac arrest and PE without cardiac arrest. Patients older than 18 years with confirmed diagnosis of PE by CTPA (computed tomography pulmonary angiogram), D-Dimer, and other clinical evaluations in accordance with ESC/ESR 2019 guidelines were considered. Patients with severe co-morbidities like active malignancy, metabolic syndrome or neurological disorders were excluded in order to rule out the presence of any other competing determinants of thrombotic risks and mortality. Controls consisted of healthy individuals with no history of venous thromboembolism or cardiac diseases. Informed consent was taken from the participants (next of kin, if any) before collection of samples. Demographic profile, family medical history, and

pedigree including information about consanguinity were collected from all participants using a proforma. Approximately 4 mL of peripheral venous blood was collected from each participant in a K2-EDTA vacutainer and was stored at 4°C until DNA extraction.

2.2 gDNA (genomic DNA) Extraction:

gDNA was extracted from all 100 samples using the DNeasy Blood and Tissue Kit (QIAGEN). The quality and integrity of the DNA samples were evaluated via agarose gel electrophoresis (0.8%), while their concentration and purity were determined spectrophotometrically using NanoDrop UV spectrophotometer.

2.3 Amplification of Target gene and Sanger sequencing:

Primers were designed for exon 22 of SCN5A using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Primer3 (https://primer3.ut.ee/) software. The resulting primers were as follows: Forward primer 5′-GGCTTCATGTCCACCTTGTC-3′ Reverse primer 5′-TCTCCTTCCTGTTCCCTTCG-3′ An optimal annealing temperature was determined experimentally via gradient Polymerase chain reaction (PCR) among different candidate temperatures. A final annealing temperature of 57.7°C was selected for all amplifications. 22nd exon of SCN5A gene was amplified using cycling conditions: 95ºC for 1 min, followed by 35 cycles of 95ºC for 0.5 min, 57.7ºC for 1 min, 68ºC for 1 min and final elongation of 68ºC for 5 min and PCR was carried out for a total 25uL reaction mixture containing 9µl Molecular biology grade water, 0.5µL Forward primer (10pM), 0.5µL Reverse primer (10pM), 2.5µL Template DNA. PCR products were run on 1.5% agarose gel electrophoresis using 100 bp DNA ladder to verify amplicon size and specificity before sequencing. Sanger sequencing was carried out using both directions of the amplicon to be able to reciprocally confirm the identified variants. Cycle sequencing was carried out by using Big Dye terminator kit v3.1 (Applied Biosystem, USA) as per manufacturer guidelines. The product was sequenced using ABI 3500 Sanger Sequencing platform. Electropherograms from sequencing were analysed using DNA Baser sequence analysis software. Statistical analysis was carried out using SPSS (Statistical package for social sciences) v15 tool.

3. RESULTS:

Out of 50 clinically confirmed PE cases that were included in the study, the mean age was 47.3 ± 15 (15: standard deviation), and age ranged from 20 to 84. The male cases were 78% (n=39) and female cases were 22% (n=11). 20% (n=10) of consanguinity was seen when family history was taken, and 38% (n=19) of patients had previous thrombosis history. Genomic DNA was successfully isolated from all 100 samples and Agarose gel electrophoresis showed intact, high-molecular-weight bands for all samples, and NanoDrop spectrophotometry confirmed purity, with A260/280 ratios in the range of 1.8–2.0. Gradient PCR optimisation identified 57.7 °C as the annealing temperature yielding the most specific amplification, and PCR performed at this temperature produced intense dark bands of the expected size (244bp) for exon 22 in all 100 samples, with no evidence of non-specific amplification on 1.5% agarose gel electrophoresis (Fig. 1).

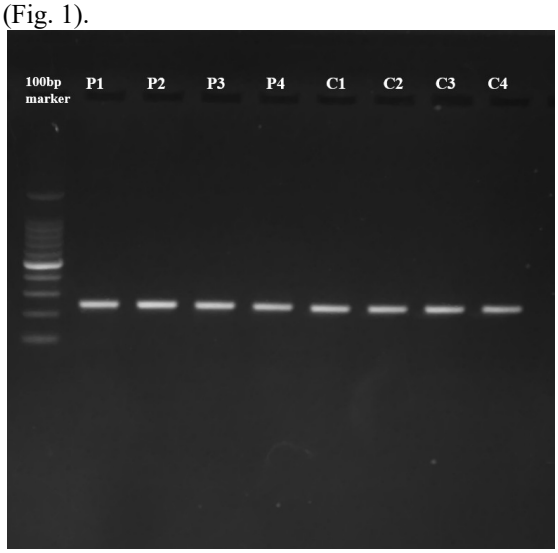

Fig. 1. Visualization of PCR products of the SCN5A gene on a gel documentation system under UV illumination. Lane 1: 100 bp DNA marker; Lanes 2–5: PCR products from PE samples (P1–P4); Lanes 6–9: PCR products from healthy controls (C1–C4).

Bidirectional Sanger sequencing yielded electropherograms with clear peak morphology and unambiguous base-calling in all 100 samples. Analysis using DNA Baser identified no coding sequence in any of the 50 PE or in any of the 50 healthy controls (Fig. 2, 3, 4).

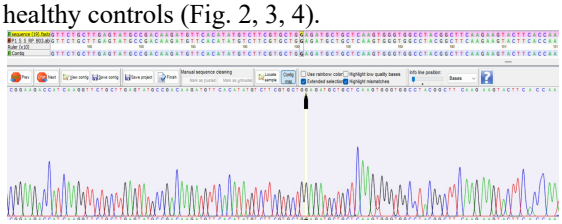

Fig. 2: Representative electropherogram showing exon 22 of the SCN5A gene in PE patient (PE with cardiac arrest)

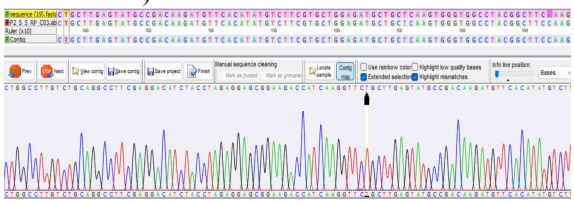

Fig. 3: Representative electropherogram showing exon 22 of the SCN5A gene in PE patient (PE without cardiac arrest)

Fig. 4: Representative electropherogram showing exon 22 of the SCN5A gene in healthy control

4. DISCUSSION:

The investigation of exon 22 of SCN5A, which encodes the DIIIS4 voltage-sensor region of the cardiac sodium channel Nav1.5, was performed on a prospectively gathered group of 100 PE patients from North Karnataka based on their cardiac arrest status as well as matched healthy controls and did not reveal any coding or splice site variant in any of them.

exon 22 is only one of the 27–28 translated exons of SCN5A, and mutational hotspot analysis in both Brugada syndrome and long QT syndrome type 3 patient populations has consistently demonstrated that the majority of disease-causing and possibly disease-causing mutations occur in multiple regions of the protein, including pore loops, the S4 voltage sensors from all four domains, the DI-DII, DII-DIII, and DIII-DIV interdomain linkers, and the C-terminal cytoplasmic region [1,17,18].

The study has only 50 patients with PE, divided into those having had a cardiac arrest (n=8; 16%) and those without (n=42;84%), along with 50 controls, and the experiment was properly powered to detect a genetic variation only if it is of relatively high frequency and significant effect; as evidenced from the population-specific allele frequency databases like IndiGenomes and SAGE, there are many arrhythmia-related SCN5A variations which, if present, occur individually at a low frequency even in the South Asian population [21,22]; hence the sample size of the study does not allow for their detection or elimination of a mild genetic effect.

Taken in combination, these arguments argue for redefining this exon 22 screen in the context of a broader, mutation-independent and multigenic approach to predicting arrhythmic risk in PE, instead of as a test of the SCN5A hypothesis per se. A comprehensive model should at least include: (i) sequencing of SCN5A and other arrhythmia-susceptibility genes in addition to the existing mutational hotspots of the gene; (ii) functional or expression analysis of Nav1.5 protein dysregulation induced by hypoxia, e.g., late sodium currents and SUMOylation as a mutation-independent mechanism of arrhythmias occurring in acute PE [16]; (iii) analysis of calcium-handling genes such as RYR2, CASQ2, TRDN and CALM1-3, because of the well-documented association between calcium-handling dysregulation and stress/exercise-induced ventricular arrhythmia in CPVT and other similar syndromes [9,10,11]; and (iv) incorporation of this genetic information with existing clinical markers of RV strain and injury, such as cardiac troponins and NT-proBNP, which continue to be the most well-validated markers of acute PE but do not provide information about the electrical vulnerability per se [19,20]. This study has a number of limitations that should be considered. Firstly, the sample size, although reflecting the actual prevalence of PE presentations at this centre, is relatively small for a genetic association study, and the lack of any identified variant could indicate either the actual lack of involvement of exon-22 in this population or the lack of power to identify a rare variant – the two hypotheses cannot be differentiated based on this study alone. The screening was performed on one specific exon only, and not the entire coding region of SCN5A gene and present study focused on small population, and the applicability of its results to the wider population of Karnataka population or South India remains unknown.

5. CONCLUSION:

The absence of variation in the 22nd exon of the SCN5A gene in this cohort should be interpreted as hypothesis-refining rather than hypothesis-negating, suggesting that 22nd exon of SCN5A may not be a major significant factor in the genetic predisposition to cardiac arrest risk in PE cases. These findings highlight for further research to explore other exonic and intronic regions of SCN5A gene or additional genetic factors to develop biomarkers for cardiac arrest risk in PE cohort.

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Conflict of Interest:

The authors declared no conflicts of interest concerning the research, authorship, funding, and publication of this article.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI tool in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Authorship contributions:

Pooja Joshi: Study conception, design, research work, data acquisition and analysis, manuscript drafting, and critical revision.

Santoshkumar Gataraddi: Research work, Data analysis, manuscript drafting, and critical revision.

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