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Genetic variants in post myocardial infarction patients presenting with electrical storm of unstable ventricular tachycardia

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ABSTRACT

Electrical storm (ES) is a life threatening clinical situation. Though a few clinical pointers exist, the occurrence of ES in a patient with remote myocardial infarction (MI) is generally unpredictable. Genetic markers for this entity have not been studied. In the present study, we carried out genetic screening in patients with remote myocardial infarction presenting with ES by next generation sequencing and identified 25 rare variants in 19 genes predominantly in RYR2, SCN5A, KCNJ11, KCNE1 and KCNH2, CACNA1B, CACNA1C, CACNA1D and desmosomal genes - DSP and DSG2 that could potentially be implicated in electrical storm. These genes have been previously reported to be associated with inherited syndromes of Sudden Cardiac Death. The present study suggests that the genetic architecture in patients with remote MI and ES of unstable ventricular tachycardia may be similar to that of Ion channelopathies. Identification of these variants may identify post MI patients who are predisposed to develop electrical storm and help in risk stratification.
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1. Introduction

Sudden Cardiac Death (SCD) in patients with remote myocardial Infarction (MI) is due to the occurrence of malignant ventricular arrhythmias, the most common being 'Ventricular Tachycardia' (VT). Few patients in this subset during their natural history develop Electrical storm (ES) which is defined as "Three or more distinct episodes of ventricular tachycardia (VT)/ventricular fibrillation (VF) within 24 h, requiring the intervention of the defibrillator (anti-tachycardia pacing or shock)" [1]. The timing and occurrence of ES is unpredictable. It is a life threatening cardiac emergency with a reported incidence of 10–28% and an in-hospital mortality of 60–70% [2]. Current knowledge on genetic markers related to ventricular arrhythmias in post MI patients with LV dysfunction is very limited. This paper summarizes the genetic variations identified in patients with remote myocardial infarction presenting with ES of unstable VT by next generation sequencing.

2. Material and methods

2.1. Patient population

Consecutive patients with Left ventricular dysfunction (LVEF≤ 35%), underlying remote myocardial infarction (>1 year), presented to our institute with electrical storm and hemodynamically unstable monomorphic VT, were included in the study. Patients with ES and other underlying substrates and those with stable VT or VF were not included. Study patients were managed by standard institutional protocol involving mechanical ventilation, hemodynamic support, anti-arrhythmic medications, radiofrequency ablation and stellate ganglionectomy as indicated. The management protocol and clinical outcomes of these patients have been detailed in a separate manuscript [3].

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2.2. Genetic analysis by next generation sequencing

The saliva samples were collected for genetic analysis after taking informed written consent from the patients and genomic DNA (g DNA) was extracted using Q IA amp DNA mini kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. Patient's genomic DNA was sequenced using TruSight Clinical Exome panel (Illumina, San Diego, CA, USA) that contains genes associated with known inherited diseases by Strand Life sciences, Bengaluru, India. Of these, 145 genes associated with arrhythmias and coronary artery disease was assessed. The input DNA was first converted into adaptor tagged index using Nextera DNA library preparation protocol (Illumina, San Diego, CA, USA) followed by adapter ligation and enrichment. Target library was amplified using limited cycles PCR (ABI9700, Life Technologies) steps and sequenced using Miseq platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

The trimmed FASTQ files were generated using MiSeq Reporter from Illumina. The reads were aligned against the whole genome build hg19 using STRAND NGS v1.6 (http://www.strand-ngs.com/) which is an integrated platform that provides analysis, management and visualization tools for NGS data and interpreted using StrandOmics (a proprietary clinical genomics interpretation and reporting platform from Strand Life Sciences). The variants identified were classified according to the ACMG (American Society of Medical Genetics and Genomics) recommendation for standards for interpretation and reporting of sequence variations [4].

3. Results

There were 10 patients (9 males &1female) with a mean age of 59.92 ± 7.6 years. All patients had myocardial infarction, 101.4 ± 78.7 months prior to development of ES. The mean LVEF was $33.17 \pm 9.45\%$. The clinical and demographic profile of these patients is summarised in Table 1. Genetic analysis was performed in all the ten patients by next generation sequencing (NGS) of SCD panel, which screened for genes involved in arrhythmias and sudden cardiac death. (Table 2). Of the ten patients, two did not reveal any variation. In the remaining 8 patients, 25 rare variants were observed in 19 genes, predominantly in RYR2, SCN5A, KCNJ11, KCNE1 and KCNH2, CACNA1B, CACNA1C, CACNA1D and desmosomal genes - DSP and DSG2. These are essentially cardiac ion channel genes and previous studies have established their role in LQT and other arrhythmic disorders. The clinical significance of these rare variants as per ClinVar database ranged from being benign to uncertain clinical significance. However, In-silico tools predict some of these variants to be disease causing (Table 3).

Of the 25 rare variants, pVal125Leu of SCN5A was found to be pathogenic while p.Val30Met of DSP and p.Thr1107Met of RYR2 revealed mixed interpretations of pathogenicity.

In addition, variants of unknown significance were found in JUP,

JPH2, VCL, MYPN, NPPA, APOB genes with possible, but not definite implications in the risk for life threatening arrhythmia events in coronary artery diseases, Brugada syndrome and atrial fibrillation respectively. Table 3 gives the list of all the variants identified.

4. Discussion

This report summarizes our findings of genetic analysis in ten patients with post myocardial infarction having LV dysfunction presenting with electrical storm of unstable VT. This critically ill cohort comprised of patients with an uncommon but clinically relevant entity and the genetics of such a patient cohort have not been studied earlier. To ensure homogeneity of the phenotype, we selected patients with a specific substrate presenting only with monomorphic unstable VT. We used next generation sequencing (NGS) which allows for large-scale and rapid assessment of genes, though it also carries the disadvantage of revealing several variants of unknown significance, a difficult task to decode clinically. 145 genes of the sudden cardiac death panel were screened by NGS which identified 25 variations in 19 genes.

4.1. Genetic variants of pathological significance

Of the 25 rare variants, a pathogenic missense variant p.Val125Leu in SCN5A was observed in a 69 year old male. This heterozygous missense substitution lies in the cytoplasmic topological domain (1—126 residues) and alters a conserved residue of the protein. It has been reported as a rare variant with an allele frequency of 0.2% in the South Asian population. ClinVar database reports the clinical significance of this variant as 'pathogenic' (RCV000058596.2) with respect to congenital long QT syndrome. Three other variants viz His558Arg, c.1141-3C>A, Asp819Asp of SCN5A gene were observed in a male patient aged 60 years who showed recurrent VT. These were earlier reported as a haplotype in affected members of brugada family [5]. His558Arg alters a conserved residue in the sodium channel inter-domain cytoplasmic linker and has been reported to modulate the effect of arrhythmia-causing SCN5A variants. These polymorphisms may be used as genetic markers within a haplotype block in which they are linked to a functionally relevant gene variant [6].

SCN5A gene encodes the alpha subunit of the cardiac voltage-gated sodium channel which plays a crucial role in cardiac excitability and conduction velocity of the electrical impulse within the heart. SCN5A mutations so far described have been linked to sudden cardiac death associated with a number of inherited arrhythmic syndromes such as Brugada syndrome (BrS) and other cardiac arrhythmias like isolated cardiac conduction defects, atrial fibrillation, long QT syndrome (LQT3), left ventricular non-compaction (LVNC) and with a risk of pro-arrhythmia following usage of sodium channel blockers [7]. SCN5A mutations accounts for approximately 10% of LQTS cases with the triggering factors associated with

Table 1
Clinical profile of patients.

Patient no	Age	Gender	LVEF	Clinical presentation	No of VT morphologies
1	58	M	35	Recurrent VT	2
2	62	M	30	Recurrent ICD shocks	4
3	68	M	25	Recurrent ICD shocks	2
4	71	M	30	Recurrent VT	4
5	60	M	30	Recurrent ICD shocks	
6	64	M	30	Recurrent ICD shocks	6
7	63	F	18	Recurrent VT	5
8	60	M	25	Recurrent VT	2
9	71	M	32	Recurrent ICD shocks	4
10	69	M	38	Recurrent ICD shocks	3

Table 2
Genetic variations identified in the patients by Next Generation sequencing.

Patient no	Gene	Variation	Pathogenic status	Implicated in other inherited syndromes of SCD
1	JPH2	Lys33Arg	Benign	HCM
2	RYR2	p.Thr1107Met	Conflicting reports on Pathogenicity	CPVT, LQTS, SCD, coronary heart disease
	KCNJ11	Ser333Phe;	Benign	
		Ser385Cys		
3	VCL	pGlu525Asp	VUS	DCM
4	No mutations			
5	SCN5A,	His558Arg,	Benign	Brugada syndrome, LQT, cardiomyopathy
	KCNE1,	c.1141-3C>A		
	MYH6,	Asp1819Asp;		
	ADRA2B	Ser38Gly;		
	KCNJ11	Val1101Ala;		
		Glu304-Glu306dup;		
		Ala190Ala		
6	DSP	Val30Met	Conflicting interpretation for pathogenicity	ARVC
7	MYPN	p.Arg27Gly;	VUS	DCM, VT, LQT
	KCNH2	Pro728His	VUS	
		Thr353Ser		
8	DSG2	Ala969Val	VUS	ARVD
9	No mutations			
10	SCN5A	Val125Leu	Pathogenic	hypercholesterolemia, LQT3
	APOB	Ile2950Thr	VUS	

Table 3
Variations identified in the study.

Gene	Variant	Clinical significance	dbSNP	Allelic frequency
RYR2	p.Thr1107Met	VUS	rs200236750	0.0003
JPH2	p.Lys33Arg	Benign	rs573848816	Single report
RYR2	p.Ser333Phe	VUS	rs397516552	Single report
KCNJ11	p.Ser385Cys	Benign	rs41282930	0.011
ABCC6	p.Leu946Ile	polymorphism	rs61340537	0.22
DSP	p.Val30Met	Pathogenic/Benign	rs121912998	0.003
KCNH2	p.Thr353Ser	Benign	unknown	Unknown
MYPN	p.Arg27Gly	Benign	unknown	0.01
MYPN	p.Pro728His	benign	unknown	0.01
DSG2	p.Ala969Val	VUS	rs373598034	0.0005
SCN5A	p.Val125Leu	Pathogenic	rs199473059	0.2
APOB	p.Ile2950Thr	VUS	rs141591543	0.02
VCL	p.Glu525Asp	Benign	rs548487697	0.03
SCN5A	p.His558Arg	Benign	rs1805124	0.22
SCN5A	c.1141-3C>A	Benign	rs41312433	0.17
SCN5A	p.Asp1819=	Benign	rs1805126	0.38
KCNE1	p.Ser38Gly	Benign	rs1805127	0.32
ADRA2B	p.Glu304_Glu306dup	no info	rs29000568	0.087
MYH6	p.Val1101Ala	Likely Benign	rs365990	0.37
KCNJ11	p.Ala190=	Benign	rs5218	0.25
CACNA1C	p.Arg2009Gln	VUS	unknown	Unknown
NPPA	p.Val32Met	VUS	rs5063	0.114
JUP	p.Arg142His	Benign	rs41283425	0.048
CACNA1B	p.Asn167Lys	VUS	rs4422842	Unknown
CACNA1D	p.Asn566Ser	VUS	rs55797424	Unknown

arrhythmic events being different among the genetic subsets of LQTS.

The genetic contribution to SCD, especially in association with acute MI, is supported by various studies. Genome wide association studies (GWAS), revealed a stronger association of SNP 'rs2824292' at locus 21q21 with ventricular fibrillation after acute myocardial infarction, a major cause of SCD, of which very little is known [8]. Dan Hu et al. (2007) studied Electrical storm with acute myocardial Infarction in a cohort of 19 patients and reported a missense mutation G400A in SCN5A in only one patient aged 70 years old, who apparently developed 6 episodes of VT/VF resulting arrhythmic electrical storm for the first time. This variant has been reported to cause a loss of function in sodium channel current due to reduced current density, impaired recovery from inactivation, and shift in the voltage dependence of inactivation to hyperpolarized potentials. They also reported a H558R polymorphism on the same allele

and functional analysis demonstrated a loss of function of sodium channel activity [9,10]. G400A in SCN5A is reported to be subclinical serving as a modulating factor in this acquired arrhythmic syndrome resulting in a loss of function. Secondly, family history of sudden death can increase the risk for ventricular fibrillation in patients experiencing an acute myocardial infarction (AMI) and may predispose to life-threatening arrhythmias during acute ischemia [11,12].

4.2. Genetic variants of benign or uncertain significance

These variants were found in other ion channel genes and since their pathogenicity in causing electrical storm is not known, they are classified as benign or as a 'VUS'. The missense variant p.Val30Met observed in Desmoplakin gene (DSP) showed conflicting reports regarding its pathogenicity, it has been reported as

'pathogenic' (RCV000018340) as well as 'likely benign'/'uncertain significance' (RCV000029685) with respect to ARVC. The other rare variants such as p.Val1101Ala in *MYH6*, p.Ser38Gly in *KCNE1,* a duplication (p.Glu304_Glu306dup) in the *ADRA2B* gene, Lys33Arg in *JPH2* [13–15], Ser333Phe and Thr1107Met in *RYR2* [16,17] have all been reported in arrhythmic disorders, atrial fibrillation, and tachyarrhythmia and significantly increase the risk for myocardial infarction and for sudden cardiac death.

Based on our observations, we hypothesize that variations in genes coding for cardiac ion channels (SCN5A, KCNE1) that are currently being used to stratify arrhythmic risk in patients with inherited syndromes of SCD, may also be associated with occurrence of electrical storm in patients with remote myocardial infarction. The clinical relevance of this study is the possibility that identification of such genetic variants may provide us with the opportunity to better risk stratify high-risk MI subsets prone to recurrent VT. Studies with ethnically different and larger cohorts of ischemic cardiomyopathy patients are required to establish a consistent genetic patterns, as multiple genes among the panel seem to influence the function and a cumulative effect of various mutations play a role in the manifestation of the conditions.

Limitation of the study: This is a small cohort of patients but given the very critical nature of the clinical situation and attempt to maintain homogeneity of the cohort, the inclusion criteria were restricted. This also explains for the absence of a control population which would have made the observations more robust.

5. Conclusion

In the present study, genetic analysis in patients exhibiting ES with remote MI was carried out which revealed variants in ion channel genes, indicating that the underlying pathophysiology of SCD due to ES of unstable VT may be similar to that of ion channelopathies. Hence, screening of these genes could be helpful in risk stratification of patients who are genetically predisposed to electrical storm. However, studies on larger cohorts in various ethnic groups are required for more concrete results.

Conflict of interest

None.

Acknowledgment

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