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Sarcomeric Gene Variations and Phenotypic Plasticity of Cardiomyopathy

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ABSTRACT Hypertrophic Cardiomyopathy (HCM) is a primary cardiac disease characterized by increased thickness of the left ventricular walls in the absence of secondary causes and manifested by mutations in sarcomeric genes. Dilated Cardiomyopathy (DCM) is associated with dilation and impaired contraction of the ventricles and is associated with sarcomeric and cytoskeletal gene mutations. The present study is focused on screening for genetic variations in the sarcomeric genes viz., Myosin Heavy Chain gene (*MYH7*), Troponin I3 (*TNNI3*), Troponin T2 (*TNNT2*), Alpha-Actin (*ACTC*), Myosin Regulatory Light Chain (*MYL2*) and Myosin Essential Light Chain (*MYL3*) by SSCP and sequencing in 100 HCM, 97 DCM and 200 controls to elucidate the phenotypic plasticity associated with cardiomyopathy. Common variations were observed in exons 7, 12, 19 and 20 of MYH7; exon 9 and intron 16 of TNNT2; intron1, intron2, exon 5 and exon 7 of TNNI; exon 1 of MYL3 gene/s in both HCM and DCM cases. This can be explained on the basis of impaired energy compromise or dose effect of the mutant protein or environmental factors wherein a HCM could progress to a DCM phenotype affecting both the right and left ventricles, leading to heart failure. Genotype-phenotype correlations can be best explained by phenotypic plasticity where in mutations/ variations in the same gene and even same variations in a different environmental background may lead to HCM / DCM dispartite phenotypes.

INTRODUCTION

Cardiomyopathy is the disease of heart muscle caused by abnormalities in cardiac wall thickness, chamber size, contraction and relaxation. Based on the pathophysiology, cardiomyopathy is classified into four major groups viz., Hypertrophic, Dilated, Restrictive and Arrythmogenic Right Ventricular Dysplasia. Hypertrophic Cardiomyopathy (HCM) and Dilated Cardiomyopathy (DCM) are the most frequent forms of cardiomyopathy. Earlier reports state that a large number of pathogenic mutations in sarcomeric genes are associated with structural changes of the myocardium and pumping efficiency of the HCM and DCM heart (Tayler et al. 2006; Parvari and Levitas 2012; Maron and Maron 2013; Teekakirikul et al. 2013).

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HCM is characterized by hypertrophy of the left ventricle with the predominant involvement of the interventricular septum and diastolic dysfunction, and ventricular chamber volume decrease and disarray (Varnava et al. 2000). The diastolic dysfunction is responsible for heart failure and sudden cardiac death, with an incidence of 1 in 500 individuals (Maron and Maron 2013). Phenotypic heterogeneity exists in HCM with some individuals being asymptomatic and some symptomatic, exhibiting symptoms of progression like syncope or dyspnea with or without heart failure, and / or sudden cardiac death. The genetic basis of HCM is also characterized by greater inter / intra-allelic heterogeneity.

DCM is characterized by systolic dysfunction, which often leads to heart failure with a requirement of cardiac transplantation. DCM produces a prominent increase in chamber volumes as well as ventricular wall thickening, with the familial prevalence of 20% to 48% (Taylor et al. 2006). Most commonly DCM is inherited as an autosomal dominant disorder and exhibits genetic heterogeneity with the implication of both sarcomeric, cytoskeletal and calcium regulatory genes.

Over the past two decades approximately 700 mutations have been identified in the etiology of HCM and DCM phenotype (http://cardiogenomics.med.harvard.edu/).

Genotype-phenotype correlations can be best explained by phenotypic plasticity wherein mutations / variations in the same gene and even the same mutation / variation in a different background may cause disparatite phenotypes (Marian 2007). It is also hypothesized that phenotypic plasticity can be explained for an HCM heart progressing to a DCM phenotype resulting in heart failure. Phenotypic plasticity is the ability of an organism to alter its phenotype in response to changes in the environment and/or gene modifiers.The mutations in the sarcomeric genes results in varied types of cardiomyopathy, due to phenotypic plasticity. Hence the main objective of the present study is to screen for genetic variations in the sarcomeric genes viz., Myosin Heavy Chain gene (*MYH7*), Troponin I3 (*TNNI3*), Troponin T2 (*TNNT2*) Alpha-Actin (*ACTC*), Myosin Regulatory Light Chain (*MYL2*) and Myosin Essential Light Chain (*MYL3*) in the elucidation of phenotypic plasticity with respect to HCM and DCM.

MATERIAL AND METHODS

Blood samples were collected from 197 patients of cardiomyopathy (100 HCM and 97 DCM) of South Indian origin. The patients were confirmed based on the physical and clinical diagnosis by electrocardiogram and echocardiogram. In addition to these, 200 voluntary healthy donors, with no history of any cardiac disorder were included as control group. Informed consent and institutional ethical committee clearance was also obtained.

Genomic DNA was isolated from the peripheral blood collected from the patients and healthy controls (Lahiri et al. 1991; Ali et al. 2008). This was followed by amplification of DNA using specific primers. Polymerase chain reaction (PCR) was carried out in 0.2 ml tubes containing 100 ng of genomic DNA, 50 pmol each of forward and reverse primers, 0.5–1 U of Taq DNA polymerase enzyme, 200 μM of dNTPs, 1× PCR buffer and water to make up the final volume to 25 μl. Initial denaturation was carried out at 95°C for 3 min, followed by a denaturation step at 95°C for 30sec. The annealing temperature varied from 54°C to 65°C based on the exon for 30 s and extension at 72°C for 1 min. A final extension step of 72°C was for 2 min. The amplified DNA samples were subjected to SSCP analysis on native polyacryalamide gels. Samples revealing any kind of band pattern variations / mobility shifts were commercially sequenced on a 3730 × l DNA analyzer.

RESULTS

MYH7 Gene

Screening of *MYH7* revealed 8 exonic variations and 3 intronic variations, with 7 being novel, of which three are missense mutations (G377R, A682G, R787H). Most of the SNPs identified (exon 7, 12, 19 and 20) were heterozygous in HCM and homozygous in DCM patients revealing the dose effect of the protein (Table 1). The variation in dose effect may influence the gross anatomical changes in the ventricles of a HCM heart further culminating to heart failure as comonly seen in a DCM heart.

Troponin I3 Gene

Screening of TNNI3 revealed a total of 10 variations. Four common variations observed in both types of cardiomyopathy with two being intronic and two exonic (exon 5: R68R, exon 7: E179E). The mutation was found to occur in functionally significant domain that lies within the troponin T binding domain. However the variations observed in HCM patients, were of magnitude indicating the possible implication of these variations in disease pathogenesis of HCM and follow up of these cases is warranted to elucidate the phenotypic plasticity, as an HCM heart may progress to a DCM associated with heart failure.

Troponin T2 Gene

Screening of TNNT2 gene in cardiomyopathy patients and control group has revealed 1 exonic and 3 intronic variations. Of which the two variations were found to be common (Table 2), with little LV hypertrophy and high incidences of sudden cardiac death, are features of cardiac troponin T mutations.

Cardiac Alpha Actin Gene

Cardiac actin (ACTC) is the first gene to be simultaneously related to two distinct forms of

Table 1:Total variations observed in the present study

S.No.	*Location*	*Position*	*SNP reference*		*Nucleotide change*	*Codon change*	*Controls*	*HCM*	*DCM*
MYH7									
1	Exon 4	g.6350	Novel	C/T	Y110Y	Nil	2	Nil	
2	Exon 7	g.7647	Novel	A/G	A199A	Nil	1	1	
3	Exon 12	g.9600	Reported	C/T	G354G	1	6	5	
4	Exon 12	g.9633	Reported	G/A	K365K	Nil	1	3	
5	Exon 12	g.9667	Novel	G/C	G377R	Nil	Nil	1	
6	Exon 12	g.9567	Novel	A/C	S343S	Nil	Nil	1	
7	Exon 19	g.13353	Novel	C/G	A682G	Nil	Nil	5	
8	Exon 19	IVS19-64	Reported	A/G	-	Nil	1	2	
9	Intron 19	IVS19-86	Reported	A/-	-	Nil	Nil	1	
10	Exon 20	IVS19-56	Novel	A/G	-	Nil	1	5	
11	Exon 21	g.14088	Novel	G/A	R787H	Nil	Nil	1	
TNNT2									
1	Exon9	g.8815	rs3729547	C/T	I106I	HP	HP	HP	
2	Intron10	g.10544	rs3730237	G/A	-	p	p	p	
3	Intron14	g.14373	rs2275863	C/T	-	p	p	p	
4	Intron16	g.14896	Novel	C/T	-	Nil	1	4	
TNNI3									
1	Intron 1	g.1389	rs11667847	T/C	-	Nil	4	2	
2	Intron 1	g.1403	rs11671293	A/G	-	Nil	1	2	
3	Intron 2	g.1698	rs3729836	T/A	-	Nil	4	8	
4	Intron 3	g.1810	rs3729837	G/A	-	Nil	3	Nil	
5	Intron 3	g.1897	rs3729838	G/A	-	Nil	22	Nil	
6	Exon5	g.2560	rs3729711	G/T	R68R	Nil	3	3	
7	Exon5	g.2601	Mogensen et al 2003		C/G	P82S	Nil	1	Nil
8	Exon5	g.2577	Reported	G/C	R74P	Nil	1	Nil	
9	Exon5	g.2627	Reported	G/A	A91T	Nil	1	Nil	
10	Intron 5	g.2653	Novel	G/A	-	Nil	1	Nil	
11	Intron 6	g.4003	Novel	C/T	-	Nil	1	Nil	
12	Exon7	g.4797	rs3729841	G/A	E179E	Nil	17	3	
MYL2									
1	Exon3	g.4929	rs2301610	T/C	I44I	p	p	p	
2	Intron4	g.6504-13,14	Repeats	(CA) 9-10	-	p	p	p	
3	Intron5	g.7455	rs3833910	G del	-	p	p	p	
4	Intron5	g.7486	rs2233260	C/T	-	P	p	p	
5	Intron5	g.7477	rs3216817	C ins	-	P	p	p	
6	Intron6	g.7609	Novel	C/T	-	Nil	2	Nil	
7	Intron6	g.7612	Novel	C/A	-	Nil	3	Nil	
MYL3									
1	Exon 1	g.1120	rs2233264	C/T	P23P	Nil	2	1	
2	Intron 1	g.1224	rs936175	T/G	-	P	p	P	
3	Intron 6	g.6249	Novel	C/T	-	Nil	Nil	4	

P: Polymorphic, HP: Highly Polymorphic

cardiomyopathy (Mogensen et al. 1999). Screening of ACTC gene revealed no variations, in both types of cases, attributing to the high consensus sequence the gene.

MYL2 Gene

Screening of MYL2 revealed four SNPs, of which three are intronic and one exonic. However no common variations were observed in both the phenotypes.

MYL3 Gene

Screening of MYL3 has revealed a total of 3 variations, of which one SNP (rs2233264) was found to be common in both HCM and DCM respectively.

DISCUSSION

In the present study, MYH7 gene mutations were found to be more prevalent in DCM while TNNI3 gene mutations in HCM. Surprisingly some of the variations in Myosin Heavy Chain gene (*MYH7*) and Myosin Essential Light Chain (*MYL3*) genes were found to be common, emphasizing on the phenotypic plasticity of the dispartite phenotypes (Table 2), with the implication of genetic modifiers and environmental factors. Alternatively with impaired energy compromise and gene dosage effects can also lead to phenotypic plasticity of HCM and DCM hearts.

Table 2: Common variations of sarcomeric genes in HCM and DCM

Gene	*Location*	*Position*	*SNP reference*	*Nucleotide change*	*Codon change*	*Controls*	*HCM*	*DCM*
MYH7								
	Exon 7	g.7647	Novel	A/G	A199A	Nil	1	1
	Exon 12	g.9600	Novel	C/T	G354G	Nil	1	3
	Exon 12	g. 9633	Reported	G/A	K365K	1	6	5
	Exon 19	IVS 19-64	Reported	A/G	-	Nil	1	2
	Exon 20	IVS19-56	Novel	A/G	-	Nil	1	5
TNNT2								
	Exon 9	g.8815	rs3729547	C/T	I106I	18	5	6
	Intron 16	g.14896	Novel	C/T	-	Nil	1	4
TNNI3								
	Intron 1	g.1389	rs11667847	T/C	-	Nil	4	2
	Intron 1	g.1403	rs11671293	A/G	-	Nil	1	2
	Intron 2	g.1698	rs3729836	T/A	-	Nil	4	8
	Exon 7	g.4797	rs3729841	G/A	E179E	Nil	17	3
MYL3								
	Exon 1	g.1120	rs2233264	C/T	P23P	Nil	2	1

With respect to *MYH7* gene, the heterozygous genotypes are vulnerable to hypertrophy while the homozygous to DCM phenotype revealing the gross anatomical variations in the ventricles of a DCM heart due to dose effect of the mutant protein. HCM and DCM result from alterations in one or more of myosin's fundamental mechanical properties, wherein HCM-causing mutations lead to enhanced and DCM causing mutations to lowered function (Debold et al. 2007). This is further supported by a previous study in which heterozygous mice expressing Arg403Gln á-MHC developed left ventricular hypertrophy as commonly seen in HCM, while homozygous mice developed progressive DCM, leading to neonatal death (Fatkin et al. 1999; Geisterfer-Lowrance et al. 1996). One such mutation is R787H mutation, which has been previously reported both in hypertrophic and dilated cardiomyopathy (Richard et al. 2003; Rai et al. 2009). Hence HCM and DCM may be considered partially as allelic disorders whereby same genes have been implicated in the pathogenesis of two distinct phenotypes.

The role of epidemiological factors in the pathogenetic process gains more prominence in view of the fact that a single mutation may sometimes give rise to two very divergent phenotypes in members of the same family. For example, mutations in the TNNI3 gene can lead to either HCM or RCM in the same family, emphasizing on the role of gene modifiers and environmental factor implication in phenotypic plasticity (Fatkin and Graham 2002; Mogensen et al. 2004). The situation may also be explained by dose effect of the identified genotype. In the case of sarcomeric genes, it appears that a gain of function usually results in increased energy demand, inefficient adenosine triphosphate utilization, and hypertrophy, whereas a loss of function results in decreased contractility (Mestroni 2009). The end phenotype is determined not only by the causal mutation but also by the modifier genes, each exerting a modest effect. But nevertheless epigenetic modifications / factors also seem to influence phenotypic plasticity (Marian 2007). Phenotypic plasticity is best illustrated and explained in cardiomyopathy, wherein mutations in the MYH7 and TNNT2, could cause either HCM or DCM, the opposite ends of the spectrum of phenotypic responses of the heart to injury, stress and mutations (Kamisago et al. 2000). MYL3, encoding the essential light chain (ELC) of myosin, gene variations are rare and have been associated with sudden death. The functional role of ELC variations in disease manifestation has not been defined, in normal heart it appears to involve in force development and fine tuning of muscle contraction (Hernandez et al. 2007; Andersen et al. 2012).

Phenotypic plasticity of the mutant sarcomeric proteins could partly be explained by the effect of mutant proteins on different structural and regulatory components of force generation and relaxation complex. Mutations in TNNT2 can cause either a HCM or DCM phenotype, wherein variations in HCM are known to enhance Ca2+ sensitivity of myofilament force generation and ATPase activity, while the same impose opposite effects in DCM phenotype. Hence the clinical variability in allelic disorders may lies in the differential function of the mutant proteins.

For example *ANKRD1* mutations in HCM increase binding of CARP to titin and myopalladin, conversely, *ANKRD1* mutations in DCM cause a loss of CARP binding to talin 1, potentially leading to loss of stretch sensing, disruption of the link between titin complex and cytoskeletal network (Arimura et al. 2009; Moulik et al. 2009). These mutation specific changes in mechanical properties may initiate a distinct signaling cascade that ultimately leads to the dispartite phenotypic responses (Debold et al. 2007). The researchers' results have reinforced the fact that the progression of hypertrophy to dilation could eventually lead to heart failure and sudden cardiac death based on the gene variations in an appropriate environmental background. Genetic heterogeneity also represents an important limitation in the understanding of the complex disorders and hence establishment of genotype-phenotype correlations is warranted in these dispartite phenotypes.

CONCLUSION

It is emphasized that variations in the same gene may cause both HCM and DCM, in spite of the two being divergent phenotypes. The dose effect of the protein and progression from HCM to DCM associated heart failure could be explained on the basis of phenotypic plasticity. Hence follow up of the cohort is warranted for appropriate pharmaceutical intervention.

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