Journal of Clinical & Experimental Cardiology

Fei and Chen, J Clin Exp Cardiolog 2013, S:12
DOI: 10.4172/2155-9880.S12-004

Research Article Open Access

Splice Site Mutation-Induced Alteration of Selective Regional Activity Correlates with the Role of a Gene in Cardiomyopathy

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Abstract

Splice site mutations of a number of genes are found to induce cardiomyopathy. The mutation alters the thermodynamic activity of nucleotide fragment around the splice sites, which causes exon skipping, leading to dysfunction of certain genes. In the present study, we analyzed the selective and regional characteristic of splice site mutations for genes contributed to the development of cardiomyopathy. 93 splice site mutations in total 16genes involved in cardiomyopathy were analyzed for the information content (R_i). Exon skipping (R_i value<0) appeared to correlate with hypertrophic cardiomyopathy and arrhythmogenic right ventricular dysplasia, the two cardiomyopathies with the highest mortality. We also found that most of the exon skipping was due to the donor splice site mutations instead of the acceptor site mutations at the splice junction. Our studies revealed that the regional preference of mutations at the donor sites is an important factor in determining whether or not the gene plays a role in the development of cardiomyopathies.

Keywords: Cardiomyopathy; Splice site; Exon skipping; Bioinformatics

Introduction

Cardiomyopathy represents a cluster of severe heart muscle diseases or syndromes leading to heart failure including hypertrophic cardiomyopathy (HCM), catecholaminergic polymorphic ventricular tachycardia (CPVT), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular dysplasia (ARVD), and restrictive cardiomyopathy (RCM) [1]. Patients with cardiomyopathies have a high risk of arrhythmia or sudden cardiac death [2]. Gene mutations have been identified in both inheritable cardiomyopathies [3] and acquired cardiomyopathies [4].

Gene mutations such as deletion, insertion, or fragment shifting normally do not alter RNA splice. However, nucleotide substitution at the junction of primary or natural splicing sites (NSS) can alter property of the splicing sites [5]. Moreover, most substitutions induce exon skipping or interruption of cryptic splicing sites (CSS) or alternative splicing sites (ALSS), resulting in genetic disorders [6-8]. Exon skipping is considered as the major consequence of abnormal splice. In addition, a number of CSSs are found near or around NSS with splice potentials. Activation or abnormal splice of these CSSs frequently induce genetic diseases [6]. Moreover, abnormal splice of intervening sequence (IVS) or intron has also been shown to alter functions of eukaryotic genes [9]. Therefore, it is very important to predict and analyze the impact of splicing site mutations on genetic disorder. In this report, an informative evaluation using Information-content Model has been conducted to evaluate effects of nucleotide substitutions on cardiomyopathies. Information theory-based model explains nucleotide variations in splice sites and has been frequently used to predict activities of normal or mutant splice sites in order to identify CSS [5]. Individual information reflects thermodynamic entropy and free energy of binding [10]. The information content (R_i, in bits) of a gene sequence describes the degree of the sequence contributing to the conservation of the entire gene [10]. Thus, the total effects of nucleotide alterations can evaluated based on cumulative R_i at every position in the splice-site [5]. By using the Information-content Model, we established the effect of regional activity alteration of splice sites in 16 genes on the development of cardiomyopathies, which predict the mechanism of a gene in inducing cardiomyopathy.

Methods

Selection and phenotypes of cardiomyopathies

Four main types of cardiomyopathies, i.e., HCM, CPVT, DCM and ARVD, were selected for this study. RCM was not included because RCM was not found to be a consequence of splice site mutation. Cardiac muscle myosin heavy chain 7-β (MYH7) mutation induces left ventricular non-compaction cardiomyopathy, which is classified as a HCM [11]. HCM phenotypes include increased left ventricular wall thickness, asymmetric septal hypertrophy and/or abnormal ECG [12,13]. CPVT is characterized by bidirectional ventricular tachycardia (VT) [14]. DCM is defined as decreased ejection fraction (<50%), left ventricular shortening fraction (<28%), and/or increased left ventricular internal diastolic diameter (>2.7 cm/m^2 body surface area) [15,16]. ARVD displays fibro-fatty penetration into right ventricular wall (RV) and RV wall thinning due to loss of cardiomyocyte [17].

Selection of candidate genes with mutation in cardiomyopathy

The human gene mutation database namely Biobase Database (http://www.biobase-international.com/product/human-gene-mutation-database) [18] was used to select genes which mutations are involved in cardiomyopathies. A total of 16 genes were found to be involved in cardiomyopathies due to splicing site mutations, and 93 splice site mutations were analyzed for their R_i values (Table 1).

Informatics analysis of splicing site mutations

DNA sequences with 150 nucleotides upstream and downstream of

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Received December 05, 2012; **Accepted** January 03, 2013; **Published** January 05, 2013

Citation: Fei J, Chen SY (2013) Splice Site Mutation-Induced Alteration of Selective Regional Activity Correlates with the Role of a Gene in Cardiomyopathy. J Clin Exp Cardiolog S12: 004. doi:10.4172/2155-9880.S12-004

normal or mutated splicing sites were obtained from Genbank. The R_i of each sequence was calculated using Delila system for Human Splice Junction Analysis (http://www.ccrnp.ncifcrf.gov/~toms/delilaserver.html) [5,10,19-21]. The user license was approved by National Institutes of Health.

The minimal information content ($R_{i\text{-}min}$) of a normal splice sites was calculated based on the boundary of nonfunctional site and normal transcript production. Comparison of multiple splice sites from a number of genes has established that an average value of 2.4 represents the $R_{i\text{-}min}$ for the normal splice sites [5].

R_i for both donor and acceptor sites in normal ($R_{i(n)}$) or mutated sequences ($R_{i(m)}$) was also calculated using Delila system. The correlation of R_i value with splice site mutation was determined by the following criteria: $R_{i(m)} \leq 0$ bit predicts that a junction mutation induces an exon skipping. $R_{i(m)}$<2.4 but >0 bits expects that a mutation completely inhibits or abolishes an normal splice site and contributes to a severe phenotype; $R_{i(m)}$>2.4 bits with a lower $R_{i(n)}$ of adjacent normal site forecasts that a mutation reduces the activity of an natural splice site resulting in a mild phenotype [5]; $R_{i(m)}>R_{i(n)}$ predicts that a mutation activates CSS. This newly-generated CSS would have its own R_i. $R_{i(m)}=R_{i(n)}$ suggests that a mutation does not affect normal splice sites [22].

Statistical analysis

R_i values were analyzed and showed a non-normal distribution. R_i was presented as mean (average) ± standard error of the mean (SEM). The significant difference between groups was analyzed using Mann Whitney test. A two-tailed value of $p<0.05$ indicates statistical significance [5].

Results

Gene mutation generated different impacts on splice site

To study the effect of gene mutation on the property of splice sites of genes involved in cardiomyopathy, we analyzed R_i of 93 splice site mutations found in 16 genes as aforementioned. We found that a great number of splice site mutations caused exon skipping (Table 2, Mutation allele #1, 10, 13, 14, 15, 18, 21, 22, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 41, 42, 43, 45, 47, 48, 49, 50, 51, 53, 56 and Table 3, # 6, 10, 11, 12, 16, 17, 21, 26, 27, 32). A less number of splice site mutations led to complete abolishment of natural splice sites (Table 2, Mutation allele #2, 5, 9, 11, 12, 20, 38, 39, 44, 46 and Table 3, #37). A number of mutations inhibited the activities of NSS and induced moderate cardiomyopathy phenotype (Table 2, Mutation allele #3, 4, 6, 7, 8, 16, 17, 19, 27, 37, 40, 54 and Table 3, # 2, 3, 4, 5, 7, 8, 13, 14, 20, 24, 25, 28, 30, 31, 33, 34, 35, 36, 37). In addition, several mutations activated CSS (Table 3, Mutation allele #2, 5, 9, 14, 15, 22, 25, 35). Only a few mutations had no effect on NSS (Table 2, Mutation allele #54 and Table 3, #1, 18, 19, 23, 29, 36).

Exon skipping was caused by donor site mutation

To determine what splice site mutation causes exon skipping, we quantified R_i value in both donor and acceptor sites of the genes with exon skipping, and found that R_i values at mutated donor sites were significantly decreased to -5.19 ± 0.79 as compared with their corresponding natural R_i value (7.17 ± 0.43 bits) ($P<0.0001$). In contrast, R_i values at mutated acceptor sites were reduced to -3.31 ± 1.09 as compared with their corresponding natural acceptor sites (4.77 ± 1.09) ($P<0.0001$). Since the difference of R_i value at the donor sites was much greater than the acceptor sites (14.3 ± 2.15 *vs.* 8.08 ± 0.22) ($P<0.05$), the donor site mutation, rather than the acceptor site mutation, was likely to be the major factor in inducing exon skipping.

Abolishment of normal splice sites was predicted by the similar reduction of R_i in both mutated donor and acceptor sites

A number of genes (Table 2, Mutation allele #2, 5, 9, 11, 12, 20, 38, 39, 44, 46 and Table 3, #37) involved in severe cardiomyopathies exhibited splice site mutations different from these found in Exon skipping. Analysis of these genes revealed that R_i values at the donor sites was dramatically reduced as compared to the natural splice sites (0.82 ± 0.21 *vs.* 8.31 ± 0.64) ($P<0.0001$). R_i values at the acceptor sites were also reduced as compared to the natural sequences (1.70 ± 0.75 *vs.* 10.0 ± 1.11) ($P<0.0001$). However, unlike Exon skipping, R_i value reductions at donor and acceptor sites were not significantly different (7.18 ± 1.32 *vs.* 8.30 ± 0.38, $P>0.05$). Based on the established requirement for Ri value (2.4) of a normal splice site, it was predicted that no normal splicing occurred in these genes.

Leaky splice was induced by either the donor or acceptor site mutations

Leaky splice was caused by an incomplete inhibition of natural

Gene ID	Description	Disorders/Phenotypes	Gene ID	Description	Disorders/Phenotypes
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	Dilated Cardiomyopathy	DSG2	Desmoglein 2	Arrhythmogenic right ventricular Cardiomyopathy
CASQ2	Calsequestrin 2 (cardiac muscle)	Catecholaminergic polymorphic ventricular tachycardia	DSP	Desmoplakin	Dilated Cardiomyopathy, Arrhythmogenic right ventricular Cardiomyopathy
CAV3	Caveolin 3	Hypertrophic Cardiomyopathy	LMNA	Laminin, alpha 1	Dilated Cardiomyopathy
COX15	cytochrome c oxidase assembly protein (yeast)	Hypertrophic Cardiomyopathy	MYBPC3	Myosin binding protein C, cardiac	Hypertrophic Cardiomyopathy
DES	Desmin	Dilated Cardiomyopathy	MYH7	Myosin, heavy chain 7, cardiac muscle, beta	Hypertrophic Cardiomyopathy
DMD	Dystrophin	Dilated Cardiomyopathy	MYL2	Myosin light chain 2, regulatory ventricular	Hypertrophic Cardiomyopathy
DNAJC19	DnaJ (Hsp40) homolog, subfamily C, member 19	Dilated Cardiomyopathy with ataxia syndrome	MYL3	Myosin light chain, alkali; ventricular and skeletal slow	Hypertrophic Cardiomyopathy
DSC2	Desmocollin 2	Arrhythmogenic right ventricular Cardiomyopathy	PKP2	Plakophilin 2	Arrhythmogenic right ventricular dysplasia/ cardiomyopathy
			TNNT2	Troponin T2, Cardiac	Hypertrophic Cardiomyopathy

Table 1: The genes with splice site mutations in cardiomyopathy.

Gene ID	Mutation allele	Cryptic (Alternative) Splice Site	Phenotype
	#, Coordinate (Ri(n) /Ri(m))	Coordinate (Ri(m))	
CASQ2	1, IVS 4, D:G→A, 1 (8.4/-4.1) [14]		CPVT
CAV3	2, IVS 1, D: T→C, 2 (8.1/0.6) [25]		HCM
DES	3, IVS 3, D:A→G, 3 (9.5/7.0) [26]		DCM
DMD	4, IVS 1, D:G→A, 5 (9.5/5.9) [27]		DCM
	5, IVS 1, D:G→T, 1 (9.5/1.6) [28]		DCM
	6, IVS 1, D:T→C, 6 (9.5/8.0) [29]		DCM
	7, IVS 5, D:G→T, 1 (11.1/3.3) [30]		DCM
DSC2	8, IVS 15, D:G→A, 5 (7.6 /4.1) [31]		ARVD
DSG2	9, IVS 5, D:T→C, 2 (7.5/0.0) [31]		ARVD
	10, IVS 6, D:G→A, 1 (11.0/-1.8) [32]		ARVD
DSP	11, IVS 2, D:G→A, 5 (5.4/1.9) [33]		ARVD
	12, IVS 15, D:G→C, 1 (10.0/0.2) [34]		DCM
LMNA	13, IVS 5, D:G→T, 1 (6.0/-1.8) [35]		DCM
	14, IVS 6, D:G→A, 1 (10.6/-2.2) [36]		DCM
	15, IVS 7, D:G→A, 1 (7.1/-5.7) [37]		DCM
MYBPC3	16, IVS 4, D:G→C, 5 (8.8/4.9) [38]		HCM
	17, IVS 6, D:G→A, 5 (9.3/5.8) [39]		HCM
	18, IVS 7, D:G→A, 1 (8.7/-4.1) [40]		HCM
	19, IVS 7, D:G→A, 5 (8.7/5.2) [41]		HCM
	20, IVS 7, D:G→T, 1 (8.7/0.9) [42]		HCM
	21, IVS 12, D:G→A, -1 (6.6/-9.3) [43]		HCM
	22, IVS 12, D:G→A, 1 (6.6/-26.1) [44]	IVS 12, D, 4 (3.5)	HCM
	23, IVS 12, D:G→C, 1 (6.6/-23.1) [45]	IVS 12, D, 2 (2.6)	HCM
	24, IVS 13, D:G→C, 1 (4.3/-5.5) [45]		HCM
	25, IVS 13, D:G→T, 1 (4.3/-3.5) [46]		HCM
	26, IVS 15, D:G→A, 1 (8.0/-4.8) [47]		HCM
	27, IVS 17, D:A→T, 4 (8.3/5.2) [13]		HCM
	28, IVS 17, D:G→A, 1 (8.3/-4.5) [48]		HCM
	29, IVS 17, D:G→C, 1 (8.3/-1.5) [49]		HCM
	30, IVS 21, D:G→A, 1 (4.0/-9.8) [50]		HCM
	31, IVS 21, D:T→G, 2 (4.0/-4.2) [51]		HCM
	32, IVS 21, D:T→G, 2 (4.0/-4.2) [51]		HCM
	33, IVS 23, D:G→A, 1 (7.3/-5.5) [41]		HCM
	34, IVS 23, D:G→T, 1 (7.3/-0.5) [40]		HCM
	35, IVS 27, D:G→A, 1 (4.9/-9.0) [52]		HCM
	36, IVS 29, D:G→A, 1 (5.9/-6.9) [53]		DCM
	37, IVS 29, D:G→A, 5 (5.9/2.4) [42]		HCM
	38, IVS 30, D:T→C, 2 (9.4/1.0) [46]		HCM
	39, IVS 30, D:T→G, 2 (9.4/0.2) [54]		HCM
	40, IVS 30, D:G→C, 5 (9.4/4.5) [55]		HCM
	41, IVS 31, D:G→A, 1 (3.6/-9.2) [56]		HCM
	42, IVS 31, D:G→T, 1 (3.6/-4.2) [57]		DCM
	43, IVS 32, D:G→A, 1 (9.9/-4.0) [40]		HCM
	44, IVS 32, D:T→A, 2 (9.9/1.0) [58]		HCM
MYH7	45, IVS 8, D:G→A, 1 (5.0/-7.8) [59]		HCM
	46, IVS 8, D:G→C, 3 (5.0/0.6) [59]		HCM
PKP2	47, IVS 1, D:G→A, 1 (10.5/-2.3) [31]		ARVD
	48, IVS 4, D:G→A, 1 (10.3/-2.5) [31]		ARVD
	49, IVS 5, D:G→A, 1 (4.8/-9.0) [60]		ARVD
	50, IVS 5, D:G→C, 1 (4.8/-5.0) [31]		ARVD
	51, IVS 7, D:G→A, 1 (9.4/-4.4) [31]		ARVD
	52, IVS 10, D:G→C, 1 (9.4/-1.4) [61]		ARVD
	53, IVS 12, D:G→T, 1 (11.0/3.2) [61]		ARVD
	54, IVS 12, D:C→T, -6 (11.0/11.0) [61]		ARVD
	55, IVS 12, D:G→A, 1 (11.0/-1.8) [62]		ARVD
TNNT2	57, IVS 16, D:G→A, 1 [63]		HCM

The coordinate is the numerical location of the base in Genbank sequence. It is illustrated as the relative position in the numbered IVS (Intervening sequence) donor (D) splice site. Positive integers indicate 3' (downstream) location; negative integers indicate 5' (upstream) location. $R_{i(n)}$--R_i value of natural splice sites; $R_{i(m)}$--R_i value of mutant splice sites

Table 2: Information contents of the splice-mutations at donor sites.

Gene ID	Mutation allele	Cryptic (Alternative) splice site	Phenotype
	#, Coordinate (Ri(n) /Ri(m))	Coordinate (Ri)	
ABCC9	1, IVS 13, A:A→C,76 (4.9/4.9) [64]		DCM
COX15	2, IVS 3, A: C→G, -3 (9.9/4.0), -5 (4.3/4.8) [65]	IVS 3, A, -4 (4.8)	HCM
DES	3, IVS 2, A:G→A, -1 (12.0/4.4) [66]	IVS 2, A, 1 (5.9)	DCM
DNAJC19	4, IVS 3, A:G→C, 3 (11.6/4.3) [67]		DCM
DSC2	5, IVS 5, A:A→G, -2 (14.2/6.0), -3(6.9 /7.8) [68]	IVS 5, A, -3 (3.8)	ARVD
DSG2	6, IVS 12, A:A→G, -2 (6.2/-2.0) [69]		ARVD
DSP	7, IVS 3, A:G→A, -1 (12.4/4.9) [70]	IVS 3, A, 1 (4.3)	ARVD
LMNA	8, IVS 1, A:G→T, -1 (12.4/3.6), 3 (5.5/5.4) [71]		DCM
	9, IVS 3, A:A→G, -10 (11.2/11.6) [72]		DCM
MYBPC3	10, IVS 1, A:A→G, -2 (5.8/-2.4) [73]		HCM
	11, IVS 5, A:A→C, -2 (6.4/-1.1) [74]		HCM
	12, IVS 5, A:G→A, -1 (6.4/-1.2) [45]		HCM
	13, IVS 8, A:C→A, -20 (4.3/3.7) [75]		HCM
	14, IVS 9, A:G→A, -36 (8.3/14.1), -35 (5.9/2.5) [76]		HCM
	15, IVS 9, A:G→C, -1 (2.5/5.4) [76]		HCM
	16, IVS 11, A:A→G, -2 (-1.3/-9.5) [40]		HCM
	17, IVS 11, A:G→A, -9 (-1.3/-9.5) [77]		HCM
	18, IVS 13, A:A→G, -2 (8.3 /8.3), -1 (D5.9/D5.9) [78]		HCM
	19, IVS 13, A:G→A, -19 (8.3/8.3), -1 (D5.9/D5.9) [46]		HCM
	20, IVS 14, A:G→A, -13 (8.3/7.0) [74]		HCM
	21, IVS 15, A:G→A, -1 (5.5/-2.1) [79]		HCM
	22, IVS 16, A:G→A, -6 (1.6/1.8) [75]	IVS 16, A, -5 (4.3)	HCM
	23, IVS 20, A:A→G, -2 (9.5/9.5) [80]		HCM
	24, IVS 22, A:C→T, -1 (9.1/7.8) [45]		HCM
	25, IVS 23, A:A→G, -26 (D5.1/D6.3), -1 (A5.1/A4.5) [41]		HCM
	26, IVS 23, A:A→G, -2 (5.1/-3.1) [73]		HCM
	27, IVS 24, A:A→G, -2 (3.7/-4.5) [44]		HCM
	28, IVS 26, A:C→G, -3 (12.6/3.0) [81]	IVS 26, A, -1 (6.7)	HCM
MYH7	29, IVS 32, A:C→T, -26 (10.4/10.4) [82]		HCM
MYL3	30, IVS 4, A:A→G, -2 (8.6/2.8), 1(11.1/8.9) [83]	IVS 4, A, -1 (0.5)	HCM
MYL2	31, IVS 5, A:A→G, -2 (14.1/5.9) [78]		HCM
	32, IVS 6, A:G→C, -1 (7.2/-0.1) [84]		HCM
PKP2	33, IVS 4, A:A→G, -2 (11.6/3.4), 1 (3.1/2.7) [85]		ARVD
	34, IVS 7, A:G→C, -1 (12.7/5.4) [31]	IVS 7, A, 5 (4.2)	ARVD
	35, IVS 10, A:A→T, -2 (11.9/4.5), -4 (3.0/3.5)[86]	IVS 10, A, 5 (3.6)	ARVD
	36, IVS 10, A:G→C, -1 (11.9/4.6), -4 (3.0/ 3.0) [61]	IVS 10, A, 3 (3.4)	ARVD
	37, IVS 12, A:G→C, -1 (7.6/0.3), -3 (3.7/3.3) [87]		ARVD

The coordinate is illustrated as the relative position in the numbered IVS (Intervening sequence) acceptor (A) splice site. Positive integers indicate 3' (downstream) location; negative integers indicate 5' (upstream) location. $R_{i(n)}$--R_i value of natural splice-sites; $R_{i(m)}$--R_i value of mutant splice-sites

Table 3: Information content of splice mutations at acceptor sites.

splice due to splice site mutations. These mutations had a reduced R_i values that were greater than $R_{i\text{-}min}$ (2.4 bits) and were observed to induce mild cardiomyopathy phenotypes. Compared to NSS, these mutations significantly decreased the R_i in mutated donor sites (5.04 ± 0.51 *vs.* 9.05 ± 0.40) ($p<0.0001$, n=12). The mutations may also significantly reduced R_i value of mutated acceptor sites (5.43 ± 0.52 *vs.* 10.73 ± 0.76) ($p<0.0001$). There were no significant difference in R_i values between donor and acceptor sites (4.01 ± 0.59 *vs.* 4.88 ± 0.88), similar to splice elimination. Therefore, leaky splice was not caused by regional mutations.

The nearby CSS detection and classification

CSS activated by splice site mutations in or around splice sites occurred as a result of new formation of secondary cryptic splice sites with an abnormal R_i value. CSS may be categorized as following:

1. CSS with a high R_i value: Mutations induced an increased CSS R_i value accompanied by a decreased or unaltered R_i value of NSS. In cardiomyopathy related genes, two CSSs were found at donor site mutations (Table 2, # 22, 23) and four at acceptor site mutations (Table 3, #2, 3, 22, 28). This type of CSS was shown in Figure 1.
2. CSS with a low R_i value: The newly-created CSS was characterized by a R_i value lower than that of NSS after mutations (Table 3, # 5, 7, 30, 34, 35, 36). Desmoplakin (DSP) gene exhibited this type of CSS, as shown in Figure 2.
3. CSS with residual splice: This type of CSS occurred when splice site mutations had no effect on R_i values (Table 3, # 2, 8, 9, 35, 37). This phenomenon is likely to result in the competition between NSS and CSS.

Splice site mutation regional effect on cardiomyopathy phenotype

ARVD, DCM and HCM were selected to analyze if the regional

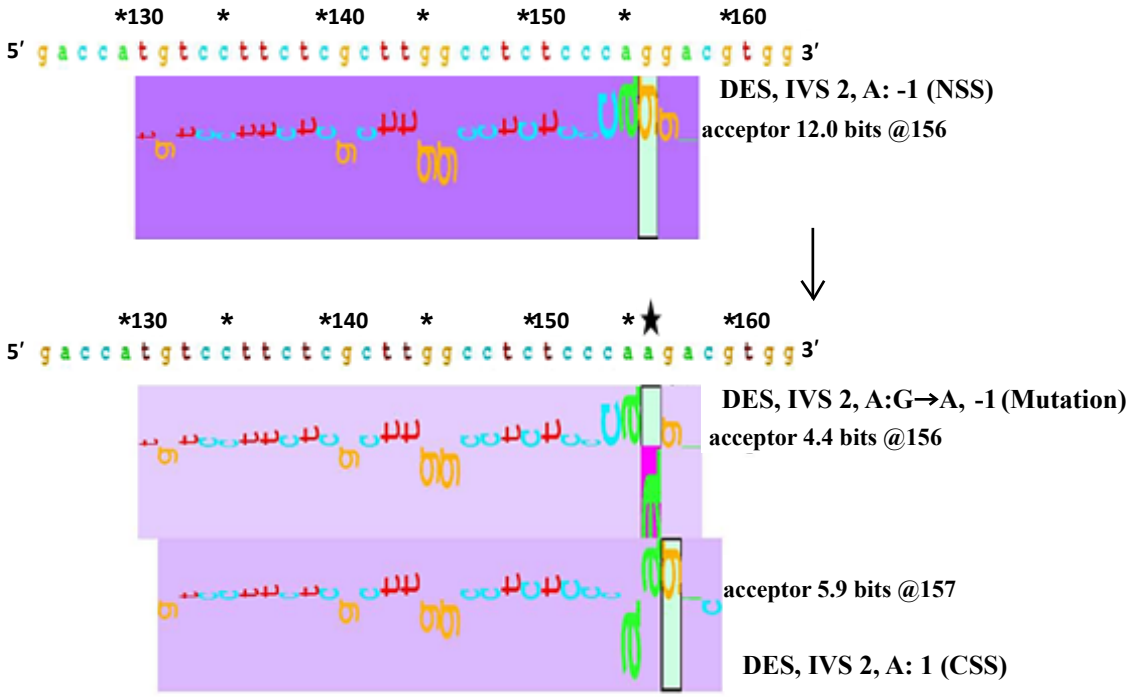

Figure 1: Splice site mutation-induced CSS with a high R_i value. G→A mutation (black star) of DES gene at IVS acceptor site resulted in a decline of NSS Ri value (from 12.0 to 4.4 bits). Anewly-formed cryptic splice site (CSS) was shown at one nucleotide downstream of NSS with a R_i value of 5.9 bits.

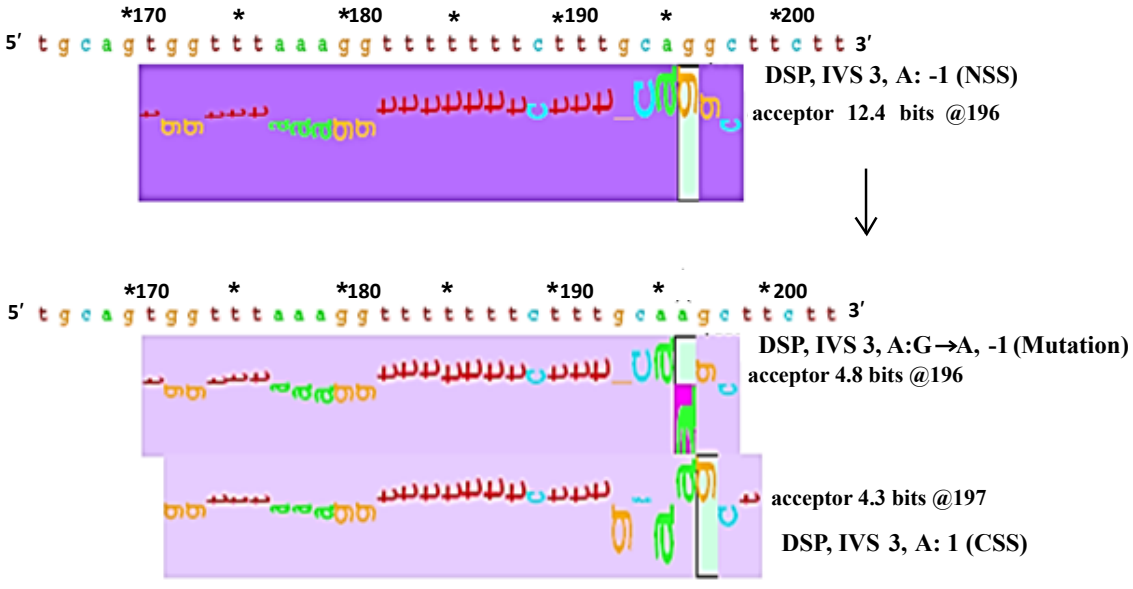

Figure 2: Splice site mutation-induced CSS with a low R_i value. G→A mutation (black star) of DSP gene at IVS acceptor site resulted in a decline of NSS R_i value (from 12.4 to 4.8). Anewly-formed cryptic splice site (CSS) was shown at one nucleotide downstream of NSS with a R_i value of 4.3 bits.

mutation of a gene was a determining factor for its involvement in the development of a specific type of cardiomyopathy. As shown in table 4, ARVD was associated with exon skipping due to donor site mutation while ARVD was associated with leaky splice due to acceptor site mutation of certain genes. Both exon skipping and leaky splice showed equal contributions to DCM due to donor site mutation of different

R_i value (cutoff) bits	Phenotype*					
	ARVD		DCM		HCM	
	Donor	Acceptor	Donor	Acceptor	Donor	Acceptor
< or = 0	8/13	1/8	5/11	0/5	18/31	9/24
<2.4	2/13	1/8	2/11	0/5	6/31	0/24
> or = 2.4	2/13	7/8	4/11	3/5	6/31	9/24
> or =ASS	1/13	3/8	0/11	2/5	0/31	6/24

*No. of mutations in each category

Table 4: R_i value for each cardiomyopathy phenotype.

genes. The activation of CSS or leaky splice was involved in DCM as a result of acceptor site mutation. Exon skipping was the major cause of donor site mutation-induced HCM. Interestingly, acceptor site mutation-induced HCM may be due to exon-skipping, leaky splice, CSS, or splice elimination.

Discussion

It is evident that gene mutations result in cardiomyopathies. Splice site mutations play a critical role in the onset of cardiomyopathies. Nucleotide substitutions significantly induce exon skipping, splice abolishment and leaky splice. Although mutations contribute to the significant change of R_i value in both donor and acceptor sites, the significant effect is only evident on exon skipping between donor and acceptor sites, suggesting that donor sites are more susceptible to mutation than acceptor sites in the development of cardiomyopathy.

The relationship analyses indicate that mutation-induced R_i value alteration contributes to the selective splice site abnormalities. These abnormalities selectively occur at either donor or acceptor sites depending on the types of cardiomyopathies. Such regional abnormalities appear to determine particular genes that may be involved in certain cardiomyopathies. For example, MYBPC3 mutations induce hypertrophic cardiomyopathy. Most of the splice site mutations are found in donor sites. Similar phenomena are observed in Laminin α1 gene and Plakophilin 2, in which donor site mutations cause DCM and ARVD, respectively. Moreover, donor site mutation-induced exon skipping with large decline of R_i value ($R_i < 0$) is closely associated with the onset of ARVD and HCM. Comparing to the other types of cardiomyopathies, ARVD and HCM are recognized as the most threatening disorders due to sudden death. Therefore, donor site mutation may be useful for predicting the potential role of a particular gene in inducing severe cardiomyopathies. The smaller R_i alterations induced by the relatively mild splice site mutations randomly occur in most of less severe cardiomyopathies. Exon skipping, splice abolishment, and leaky splice due to acceptor site mutations are found in all types of cardiomyopathies, suggesting that mutation in acceptor sites has less selective effects.

CSS obtains its own R_i value although it is lower compared to NSS. Most of CSS occur in acceptor sites because acceptor sites appear to be used for defining and recognizing exons in vertebrate species [23]. It has been reported that certain nucleotide compositions such as alternative GC-AG introns exhibit weak donor sites but enhanced spliceosome intron-recognizing ability in acceptor sites in human [24]. Most of the newly-created CSS have relative low R_i value compared to the mutant sites, suggesting that CSS activation may not be the major cause for cardiomyopathy. The function of CSS in cardiomyopathies requires further investigations. Individual information analysis simulates thermodynamic entropy of nucleotides, and thus reflects the importance of the regional splice site mutation in the selection of genes that may be involved in the onset of cardiomyopathies. This finding may be useful for developing a novel method to screen candidate genes potentially implicated in these diseases.

Acknowledgement

This work was supported by grants from National Institutes of Health (HL093429 and HL107526 to Dr. Chen).

References

1. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, et al. (1995) Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation 91: 1512-1519.
2. Kasper DL, Hauser S, Longo D, Jameson JL, Fauci AS (2005) Harrison's Principles of Internal Medicine 16e.
3. Gomes AV, Liang J, Potter JD (2005) Mutations in human cardiac troponin I that are associated with restrictive cardiomyopathy affect basal ATPase activity and the calcium sensitivity of force development. J Biol Chem 280: 30909-30915.
4. Tang Z, Diamond MA, Chen JM, Holly TA, Bonow RO, et al. (2007) Polymorphisms in adenosine receptor genes are associated with infarct size in patients with ischemic cardiomyopathy. Clin Pharmacol Ther 82: 435-440.
5. Rogan PK, Faux BM, Schneider TD (1998) Information analysis of human splice site mutations. Hum Mutat 12: 153-171.
6. Wang GS, Cooper TA (2007) Splicing in disease: disruption of the splicing code and the decoding machinery. Nat Rev Genet 8: 749-761.
7. Hastings ML, Resta N, Traum D, Stella A, Guanti G, et al. (2005) An LKB1 AT-AC intron mutation causes Peutz-Jeghers syndrome via splicing at noncanonical cryptic splice sites. Nat Struct Mol Biol 12: 54-59.
8. López-Bigas N, Audit B, Ouzounis C, Parra G, Guigó R (2005) Are splicing mutations the most frequent cause of hereditary disease? FEBS Lett 579: 1900-1903.
9. Cech TR (1990) Self-splicing of group I introns. Annu Rev Biochem 59: 543-568.
10. Schneider TD (1997) Information content of individual genetic sequences. J Theor Biol 189: 427-441.
11. Arad M, Penas-Lado M, Monserrat L, Maron BJ, Sherrid M, et al. (2005) Gene mutations in apical hypertrophic cardiomyopathy. Circulation 112: 2805-2811.
12. Doolan A, Tebo M, Ingles J, Nguyen L, Tsoutsman T, et al. (2005) Cardiac troponin I mutations in Australian families with hypertrophic cardiomyopathy: clinical, genetic and functional consequences. J Mol Cell Cardiol 38: 387-393.
13. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, et al. (2005) Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet 42: e59.
14. Postma AV, Denjoy I, Hoorntje TM, Lupoglazoff JM, Da Costa A, et al. (2002) Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circ Res 91: e21-26.
15. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, et al. (1996) Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. Circulation 93: 841-842.
16. Li D, Czernuszewicz GZ, Gonzalez O, Tapscott T, Karibe A, et al. (2001) Novel cardiac troponin T mutation as a cause of familial dilated cardiomyopathy. Circulation 104: 2188-2193.
17. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist

C, et al. (1994) Diagnosis of arrhythmogenic right ventricular dysplasia/ cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. Br Heart J 71: 215-218.

18. Krawczak M, Cooper DN (1997) The human gene mutation database. Trends Genet 13: 121-122.

19. Rogan PK, Schneider TD (1995) Using information content and base frequencies to distinguish mutations from genetic polymorphisms in splice junction recognition sites. Hum Mutat 6: 74-76.

20. Schneider TD, Stormo GD, Haemer JS, Gold L (1982) A design for computer nucleic-acid-sequence storage, retrieval, and manipulation. Nucleic Acids Res 10: 3013-3024.

21. Schneider TD, Stormo GD, Yarus MA, Gold L (1984) Delila system tools. Nucleic Acids Res 12: 129-140.

22. Schneider TD (1997) Sequence walkers: a graphical method to display how binding proteins interact with DNA or RNA sequences. Nucleic Acids Res 25: 4408-4415.

23. Berget SM (1995) Exon recognition in vertebrate splicing. J Biol Chem 270: 2411-2414.

24. Thanaraj TA, Clark F (2001) Human GC-AG alternative intron isoforms with weak donor sites show enhanced consensus at acceptor exon positions. Nucleic Acids Res 29: 2581-2593.

25. Müller JS, Piko H, Schoser BG, Schlotter-Weigel B, Reilich P, et al. (2006) Novel splice site mutation in the caveolin-3 gene leading to autosomal recessive limb girdle muscular dystrophy. Neuromuscul Disord 16: 432-436.

26. Park KY, Dalakas MC, Goebel HH, Ferrans VJ, Semino-Mora C, et al. (2000) Desmin splice variants causing cardiac and skeletal myopathy. J Med Genet 37: 851-857.

27. Kimura S, Ikezawa M, Ozasa S, Ito K, Ueno H, et al. (2007) Novel mutation in splicing donor of dystrophin gene first exon in a patient with dilated cardiomyopathy but no clinical signs of skeletal myopathy. J Child Neurol 22: 901-906.

28. Milasin J, Muntoni F, Severini GM, Bartoloni L, Vatta M, et al. (1996) A point mutation in the 5' splice site of the dystrophin gene first intron responsible for X-linked dilated cardiomyopathy. Hum Mol Genet 5: 73-79.

29. Feng J, Yan JY, Buzin CH, Sommer SS, Towbin JA (2002) Comprehensive mutation scanning of the dystrophin gene in patients with nonsyndromic X-linked dilated cardiomyopathy. J Am Coll Cardiol 40: 1120-1124.

30. Feng J, Yan J, Buzin CH, Towbin JA, Sommer SS (2002) Mutations in the dystrophin gene are associated with sporadic dilated cardiomyopathy. Mol Genet Metab 77: 119-126.

31. Fressart V, Duthoit G, Donal E, Probst V, Deharo JC, et al. (2010) Desmosomal gene analysis in arrhythmogenic right ventricular dysplasia/cardiomyopathy: spectrum of mutations and clinical impact in practice. Europace 12: 861-868.

32. Syrris P, Ward D, Asimaki A, Evans A, Sen-Chowdhry S, et al. (2007) Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: a genotype-phenotype characterization of familial disease. Eur Heart J 28: 581-588.

33. Basso C, Thiene G, Nava A (2006) Letter regarding article by Norman et al, "novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy". Circulation 113: e68.

34. Elliott P, O'Mahony C, Syrris P, Evans A, Rivera SC, et al. (2010) Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy. Circ Cardiovasc Genet 3: 314-322.

35. Arbustini E, Grasso M, Ansaldi S, Malattia C, Pilotto A, et al. (2005) Identification of sixty-two novel and twelve known FBN1 mutations in eighty-one unrelated probands with Marfan syndrome and other fibrillinopathies. Hum Mutat 26: 494.

36. Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, et al. (2008) Long-term outcome and risk stratification in dilated cardiolaminopathies. J Am Coll Cardiol 52: 1250-1260.

37. van Tintelen JP, Hofstra RM, Katerberg H, Rossenbacker T, Wiesfeld AC, et al. (2007) High yield of LMNA mutations in patients with dilated cardiomyopathy and/or conduction disease referred to cardiogenetics outpatient clinics. Am Heart J 154: 1130-1139.

38. Lin J, Zheng DD, Tao Q, Yang JH, Jiang WP, et al. (2010) Two novel mutations of the MYBPC3 gene identified in Chinese families with hypertrophic cardiomyopathy. Can J Cardiol 26: 518-522.

39. Ortiz MF, Rodriguez-Garcia MI, Hermida-Prieto M, Fernandez X, Veira E, et al. (2009) A homozygous MYBPC3 gene mutation associated with a severe phenotype and a high risk of sudden death in a family with hypertrophic cardiomyopathy. Rev Esp Cardiol 62: 572-575.

40. Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, et al. (1998) Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. N Engl J Med 338: 1248-1257.

41. Carrier L, Bonne G, Bährend E, Yu B, Richard P, et al. (1997) Organization and sequence of human cardiac myosin binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. Circ Res 80: 427-434.

42. Caramins M, Halliday G, McCusker E, Trent RJ (2003) Genetically confirmed clinical Huntington's disease with no observable cell loss. J Neurol Neurosurg Psychiatry 74: 968-970.

43. Roncarati R, Latronico MV, Musumeci B, Aurino S, Torella A, et al. (2011) Unexpectedly low mutation rates in beta-myosin heavy chain and cardiac myosin binding protein genes in Italian patients with hypertrophic cardiomyopathy. J Cell Physiol 226: 2894-2900.

44. Girolami F, Olivotto I, Passerini I, Zachara E, Nistri S, et al. (2006) A molecular screening strategy based on beta-myosin heavy chain, cardiac myosin binding protein C and troponin T genes in Italian patients with hypertrophic cardiomyopathy. J Cardiovasc Med (Hagerstown) 7: 601-607.

45. Millat G, Bouvagnet P, Chevalier P, Dauphin C, Jouk PS, et al. (2010) Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy. Eur J Med Genet 53: 261-267.

46. Waldmüller S, Müller M, Rackebrandt K, Binner P, Poths S, et al. (2008) Array-based resequencing assay for mutations causing hypertrophic cardiomyopathy. Clin Chem 54: 682-687.

47. Ho CY (2009) [Clinical spectrum of preclinical hypertrophic cardiomyopathy: characterizing carriers of sarcomere gene mutation]. Zhonghua Xin Xue Guan Bing Za Zhi 37: 289-293.

48. Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, et al. (2006) Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. J Med Genet 43: 829-832.

49. Brito D, Madeira H (2005) Malignant mutations in hypertrophic cardiomyopathy: fact or fancy? Rev Port Cardiol 24: 1137-1146.

50. Konno T, Shimizu M, Ino H, Fujino N, Uchiyama K, et al. (2006) A novel mutation in the cardiac myosin-binding protein C gene is responsible for hypertrophic cardiomyopathy with severe ventricular hypertrophy and sudden death. Clin Sci (Lond) 110: 125-131.

51. Golubenko MV, Puzyrev KV, Puzyrev VP, Pavlukova EN, Makeeva OA, et al. (2004) Gene symbol: MYBPC3. Disease: Hypertrophic cardiomyopathy. Hum Genet 114: 406.

52. Kimura A (1997) Symposium on gene abnormalities in medical diseases. 3. Molecular genetics of hypertrophic cardiomyopathy in Japan. Intern Med 36: 152-154.

53. Hershberger RE, Norton N, Morales A, Li D, Siegfried JD, et al. (2010) Coding sequence rare variants identified in MYBPC3, MYH6, TPM1, TNNC1, and TNNI3 from 312 patients with familial or idiopathic dilated cardiomyopathy. Circ Cardiovasc Genet 3: 155-161.

54. Xin B, Puffenberger E, Tumbush J, Bockoven JR, Wang H (2007) Homozygosity for a novel splice site mutation in the cardiac myosin-binding protein C gene causes severe neonatal hypertrophic cardiomyopathy. Am J Med Genet A 143A: 2662-2667.

55. Watkins H, Anan R, Coviello DA, Spirito P, Seidman JG, et al. (1995) A de novo mutation in alpha-tropomyosin that causes hypertrophic cardiomyopathy. Circulation 91: 2302-2305.

56. Rottbauer W, Gautel M, Zehelein J, Labeit S, Franz WM, et al. (1997) Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization Of cardiac transcript and protein. J Clin Invest 100: 475-482.

57. Zeller R, Ivandic BT, Ehlermann P, Mücke O, Zugck C, et al. (2006) Large-scale mutation screening in patients with dilated or hypertrophic cardiomyopathy: a pilot study using DGGE. J Mol Med (Berl) 84: 682-691.

58. Frisso G, Limongelli G, Pacileo G, Del Giudice A, Forgione L, et al. (2009) A child cohort study from southern Italy enlarges the genetic spectrum of hypertrophic cardiomyopathy. Clin Genet 76: 91-101.

59. Klaassen S, Probst S, Oechslin E, Gerull B, Krings G, et al. (2008) Mutations in sarcomere protein genes in left ventricular noncompaction. Circulation 117: 2893-2901.

60. Fidler LM, Wilson GJ, Liu F, Cui X, Scherer SW, et al. (2009) Abnormal connexin43 in arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 mutations. J Cell Mol Med 13: 4219-4228.

61. den Haan AD, Tan BY, Zikusoka MN, Lladó LI, Jain R, et al. (2009) Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ Cardiovasc Genet 2: 428-435.

62. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, et al. (2004) Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. Nat Genet 36: 1162-1164.

63. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, et al. (1994) Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell 77: 701-712.

64. Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'Cochlain F, et al. (2004) ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. Nat Genet 36: 382-387.

65. Antonicka H, Mattman A, Carlson CG, Glerum DM, Hoffbuhr KC, et al. (2003) Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. Am J Hum Genet 72: 101-114.

66. Taylor MR, Slavov D, Ku L, Di Lenarda A, Sinagra G, et al. (2007) Prevalence of desmin mutations in dilated cardiomyopathy. Circulation 115: 1244-1251.

67. Davey KM, Parboosingh JS, McLeod DR, Chan A, Casey R, et al. (2006) Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. J Med Genet 43: 385-393.

68. Heuser A, Plovie ER, Ellinor PT, Grossmann KS, Shin JT, et al. (2006) Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet 79: 1081-1088.

69. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, et al. (2006) Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. Circulation 113: 1171-1179.

70. Bauce B, Basso C, Rampazzo A, Beffagna G, Daliento L, et al. (2005) Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. Eur Heart J 26: 1666-1675.

71. Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, et al. (2008) Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. Am Heart J 156: 161-169.

72. Otomo J, Kure S, Shiba T, Karibe A, Shinozaki T, et al. (2005) Electrophysiological and histopathological characteristics of progressive atrioventricular block accompanied by familial dilated cardiomyopathy caused by a novel mutation of lamin A/C gene. J Cardiovasc Electrophysiol 16: 137-145.

73. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, et al. (2004) Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol 44: 1903-1910.

74. Jääskeläinen P, Kuusisto J, Miettinen R, Kärkkäinen P, Kärkkäinen S, et al. (2002) Mutations in the cardiac myosin-binding protein C gene are the predominant cause of familial hypertrophic cardiomyopathy in eastern Finland. J Mol Med (Berl) 80: 412-422.

75. Andersen PS, Havndrup O, Bundgaard H, Larsen LA, Vuust J, et al. (2004) Genetic and phenotypic characterization of mutations in myosin-binding protein C (MYBPC3) in 81 families with familial hypertrophic cardiomyopathy: total or partial haploinsufficiency. Eur J Hum Genet 12: 673-677.

76. Frank-Hansen R, Page SP, Syrris P, McKenna WJ, Christiansen M, et al. (2008) Micro-exons of the cardiac myosin binding protein C gene: flanking introns contain a disproportionately large number of hypertrophic cardiomyopathy mutations. Eur J Hum Genet 16: 1062-1069.

77. Rodriguez-Garcia MI, Monserrat L, Ortiz M, Fernandez X, Cazon L, et al. (2010) Novel human pathological mutations. Gene symbol: MYBPC3. Disease: cardiomyopathy, hypertrophic. Hum Genet 127: 484.

78. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, et al. (2003) Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation 107: 2227-2232.

79. Song L, Zou Y, Wang J, Wang Z, Zhen Y, et al. (2005) Mutations profile in Chinese patients with hypertrophic cardiomyopathy. Clin Chim Acta 351: 209-216.

80. Erdmann J, Raible J, Maki-Abadi J, Hummel M, Hammann J, et al. (2001) Spectrum of clinical phenotypes and gene variants in cardiac myosin-binding protein C mutation carriers with hypertrophic cardiomyopathy. J Am Coll Cardiol 38: 322-330.

81. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, et al. (2002) Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. Circulation 105: 446-451.

82. Hougs L, Havndrup O, Bundgaard H, Køber L, Vuust J, et al. (2005) One third of Danish hypertrophic cardiomyopathy patients with MYH7 mutations have mutations [corrected] in MYH7 rod region. Eur J Hum Genet 13: 161-165.

83. Andersen PS, Havndrup O, Hougs L, Sørensen KM, Jensen M, et al. (2009) Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. Hum Mutat 30: 363-370.

84. Andersen PS, Havndrup O, Bundgaard H, Moolman-Smook JC, Larsen LA, et al. (2001) Myosin light chain mutations in familial hypertrophic cardiomyopathy: phenotypic presentation and frequency in Danish and South African populations. J Med Genet 38: E43.

85. La Gerche A, Robberecht C, Kuiperi C, Nuyens D, Willems R, et al. (2010) Lower than expected desmosomal gene mutation prevalence in endurance athletes with complex ventricular arrhythmias of right ventricular origin. Heart 96: 1268-1274.

86. Christensen AH, Benn M, Tybjaerg-Hansen A, Haunso S, Svendsen JH (2010) Missense variants in plakophilin-2 in arrhythmogenic right ventricular cardiomyopathy patients--disease-causing or innocent bystanders? Cardiology 115: 148-154.

87. Xu T, Yang Z, Vatta M, Rampazzo A, Beffagna G, et al. (2010) Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol 55: 587-597.

Citation: Fei J, Chen SY (2013) Splice Site Mutation-Induced Alteration of Selective Regional Activity Correlates with the Role of a Gene in Cardiomyopathy. J Clin Exp Cardiolog S12: 004. doi:10.4172/2155-9880.S12-004