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Research Article

A Common 5bp (CTTCT) Deletion Polymorphism in *TNNT2* is Significantly Associated with Hypertrophic Cardiomyopathy

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Abstract

Troponin T is a component of the Troponin complex, which plays a major role in the regulation of myocardial contraction in response to changes in the intracellular Ca2+ion concentration. Deletion of 5bp (CTTCT) in the polypyrimidine tract of intron 3 of the cardiac Troponin T gene (*TNNT2*) results in cTnT2 and cTnT4 isoforms by skipping of exon 4, which was reported to be associated with hypertrophic cardiomyopathy (HCM). In the present study, we screened a common 5bp deletion polymorphism in *TNNT2* gene using direct sequencing in 178 cardiomyopathy patients with significant LV hypertrophy from North India against 197 ethnically matched and clinically well-characterized healthy controls. Our study revealed a high frequency of del/del genotype in patients with HCM vs controls (p=0.000007). The deletion and insertion allele frequencies in patients and controls were (75% & 25%) and (56% & 44%), respectively. Further, analysis of 2056 caste and tribe groups belonging to highly diverse endogamous people inhabited in different states of India have revealed higher deletion allele frequency in both South and North Indian states compare to control group whereas the North-eastern states showed a slightly higher frequency of insertion allele. In conclusion, the deletion allele frequency in the HCM patient group is found to be significantly higher than the control group (p=<.0001). Therefore, the deletion allele frequency is not only associated with LV hypertrophy in Japanese, French, and South Indian HCM patients but also in North Indian HCM patients.

Keywords: HCM; Hypertrophic Cardiomyopathy; 5bp polymorphism; TNNT2; allele frequency; genotype frequency

Introduction

Studies have reported four isoforms of human cardiac Troponin T (cTnT) in myocardial muscle [1]. The cTnT3 (missing exon 5) is a major mRNA isoform of a normal adult heart and cTnT4 (missing exons 4 and 5) is a minor isoform and the cTnT1 (all exons present) and cTnT2 (missing exon 4) mRNAs isoforms are found to be expressed in a barely detectable quantity [2 3]. The four isoforms are reported to be a result of alternative splicing of a single gene Troponin T (*TNNT2*), which is located on chromosome 1q32 [4-7]**.** Farza et al have described the sequence and the organization of the *TNNT2* gene and a possible mechanism by which some of the cTnT mRNA are generated [8].

In humans, pre-mRNA splicing involves the removal of introns and the ligation of the exons forming mature mRNA. Accurate removal of introns required several cis-acting sequences and trans-acting factors. The polypyrimidine tract is one of the important cis-acting sequence elements, which dictate the removal of the intron from pre-mRNA splicing. Deletion of 5bp (CTTCT) at the polypyrimidine tract in intron 3 of the *TNNT2* gene was affecting the branch site selection and splicing [9 10]. The del/del

polymorphism significantly affects the mRNA expression pattern by skipping exon 4 during splicing; it has been proved by *in vitro* mRNA (mini gene construct) expression study [11]. The missing exon 4 in cardiac Troponin T corresponding to isoforms cTnT2 and cTnT4, and the isoform cTnT4 have been reported in failing hearts [4 5]. The two isoforms cTnT2 and cTnT4 are found to be functionally distinct based on the structure, Ca2+ sensitivity, and inhibition of force development [1].

India is known for its genetic diversity, a high level of endogamy created by social boundaries within and between castes and tribes, along with the influence of evolutionary forces such as long-term isolation, fragmentation, and genetic drift has kept the Indian populations diverse and distant from each other [12 13]. Previously, we reported 5bp (del/del) polymorphism and its association in South Indian HCM cases [14]. In the present study, we screened the frequency of 5bp (del/del) polymorphism in North Indian Hypertrophic Cardiomyopathies (HCM).

Materials and Methods

Patients, controls, and population samples

A total of 178 unrelated cardiomyopathy patients with significant LV hypertrophy from North India were included in this study. All the patients fulfilled the selection criteria, based on electrocardiographic and echocardiographic measurements, without hypertension or the patient currently not on any anti-hypertensive medications were recruited from the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, (Table 1), along with one ninety-seven healthy normal individuals from the same ethnic background, without hypertension and cardiomyopathy as controls based on the electrocardiographic and echocardiographic measurements.

DNA isolation

DNA was isolated from the samples using the following protocol: Erythrocytes were lysed with 15.0 ml of erythrocyte lysis buffer for 5 minutes (10 mM Tris pH 8.0, 320 mM sucrose, 5 mM MgCl2, 1% Triton X-100; Sigma Chemical Company, St Louis, MO, USA). After complete lysis, the leukocytes were pelleted by centrifugation. The leukocyte pellet was dissolved in 8.0 ml of leukocyte lysis buffer (400 mM Tris, 60 mM EDTA, 150 mM NaCl, and 1% SDS; Sigma) and mixed thoroughly. To this, 2.0 ml of 5M sodium perchlorate (E. Merck, Darmstadt, Germany) was added and mixed thoroughly for 2–3 minutes. The DNA of both the patients and the controls was Precipitated by extracting once with phenol: chloroform, and again with chloroform. The precipitated DNA was then washed with 70% ethanol and dissolved in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA).

PCR assay for 5bp (del/del; ins/del; ins/ins) Genotyping

A pair of primers to amplify the exons 3 and 4 of *TNNT2*gene, covering the exons and exon boundaries of *TNNT2* were designed and synthesized using an ABI 392 Oligo synthesizer (Perkin-Elmer. Foster City. CA). The nucleotide sequence of both FP (forward primer) *TNNT2*: 3F = (5'atgagaacggcaggccaggctagtg 3') and the RP (reverse primer) *TNNT2:* 4R = (5'gtttgcctcaagacccgagcaacc 3'). PCR of each sample was set in a 0.2ml thin-wall tube using 5.0 ng of DNA, 10 pM of each primer, 200 mM of dNTPs, 1X PCR buffer containing 1.5 mM MgCl2 and 2U of AmpliTaq Gold (Perkin Elmer). The cycling conditions used for amplification are as follows: initial denaturation at 94^0C for 1 min followed by 30 cycles of denaturation for 45 sec at 94^0C, annealing for 40 sec at 58^0C, and extension for 37 sec at 70^0C (final extension for 7 min).

Sequencing of the PCR amplicon

The PCR amplicon was directly sequenced using 50.0 ng (2.0 ml) of PCR product and 2 pM (0.5ml) of primer, 2.0 ml of BigDye Terminator (v3.1) ready reaction mix (Perkin Elmer), and 0.5ml of double distilled water (DDW) to make up the volume to 5.0ml. The PCR cycle sequencing was carried out in a GeneAmp 9600 Thermal cycler (Perkin Elmer) employing the following conditions: 96^0C for 10 seconds, 55^0C for 5 seconds, and 60^0C for 4 minutes for 30 cycles. Extended products have been purified and dissolved in 10 ml of 50% Hi-Di formamide to analyze in an ABI 3730 Genetic Analyzer (Perkin Elmer, Foster City, CA, USA).

Statistical analysis

Chi-square $\chi 2$ test and odds ratio were carried out and obtained *p-value* to analyze (http://faculty.vassar.edu/lowry/VassarStats.html) the significance of the 5bp *(D/D, I/D, I/I)* polymorphism in HCM patients and controls of Indian origin.

Results

In humans, myocardial TnT expression has been found to influence the disease pathogenesis and development. Studies have reported that the human myocardium has expressed in four TnT isoforms (Figure.1). In the present study, we screened a five bp (del/del) polymorphism in the *TNNT2* gene (Figure.2) in 178 unrelated hypertrophy cardiomyopathy patients from North India (Table 1), against 197 ethnically matched and clinically well-characterized controls. We analysed the genotype and the allele frequencies in HCM patients from North India against controls (Tables 2 and 3). We found significantly higher *del/del* genotype (64%) and deletion allele (75%) frequencies in HCM patients (Tables 2 and 3). The frequencies of *del/del*; *del/ins*; *ins/ins* genotypes in the HCM patients were 64%, 22%, and 14 %, respectively (Table 2), whereas, in the control group, they were 41%, 30%, and 29%, respectively (Table 2). The *del/del genotype* frequency in the hypertrophy patients showed significantly higher P value (P=0.000007) than that of the control group (Table 2). The frequency of deletion and insertion alleles in patients were 75% and 25%, respectively (Table 3), whereas in the control group, they were 56% and 44%, respectively (Table 3). The deletion allele frequency in hypertrophic patient group was significantly higher than that of the control group (p=<.0001) (Table 3).

Baseline characteristics of the Hypertrophic cardiomyopathy patients studied	Cases (n=178)
Age, Yrs	44 ± 10
Sex, Males, %	74
NYHA class III, IV (%)	33
Dyspnea, %	71
Angina Pectoris, %	59
Syncope, %	37
Hypertrophic Cardiomyopathy (n) HCM	178
LVEDD, mm	39 ± 6.8
LVESD, mm	24.3 ± 3.7
Septum, mm	24.2 ± 4.2
Abnormal ECG, %	69

Table 1: Clinical features of Hypertrophic cardiomyopathy patients studied

S.NO	Genotype	HCM		Controls		Odds Ratio	Chi-square		Fisher's exact probability test	
		N=178	%	N=197	%		Yates	Pearson	p value (one-tailed)	p value (two-tailed)
1	Deletion/Deletion	114	64	81	41	0.392	18.79	19.69	0.000007	0.000012
2	Insertion/Deletion	39	22	59	30	1.5238	2.73	3.13	0.048990	0.079128
3	Insertion/Insertion	25	14	57	29	2.4917	11.28	12.13	0.000344	0.000672

Table 2: Genotype Frequency of a common 5bp Polymorphism (D/D, I/D, I/I) in TNNT2- Observed in Hypertrophic Cardiomyopathy Patients from North India

S.NO	Allele frequency	HCM		Controls		Odds Ratio	Chi-square		Fisher's exact probability test	
		N=356	%	N=394	%		Yates	Pearson	P value (one-tailed)	P value (two-tailed)
1	Deletion alleles	267	75	221	56	0.4258	28.59	29.42	<.0001	<.0001
2	Insertion alleles	89	25	173	44					

Table 3. Allele Frequency of a common 5bp Polymorphism (Deletion/Insertion) in TNNT2- Observed in Hypertrophic Cardiomyopathy Patients from North India

Further, analysis of 2056 caste and tribe groups belonging to highly diverse endogamous populations inhabited in different states of India has revealed their 5bp del/del polymorphism frequencies (Table 4). Interestingly, the frequency of *del/del* genotype was high in Beastha Chaurasia, Gamit, Jain Meena, Subba, and Great Andamanese as compared to the controls.

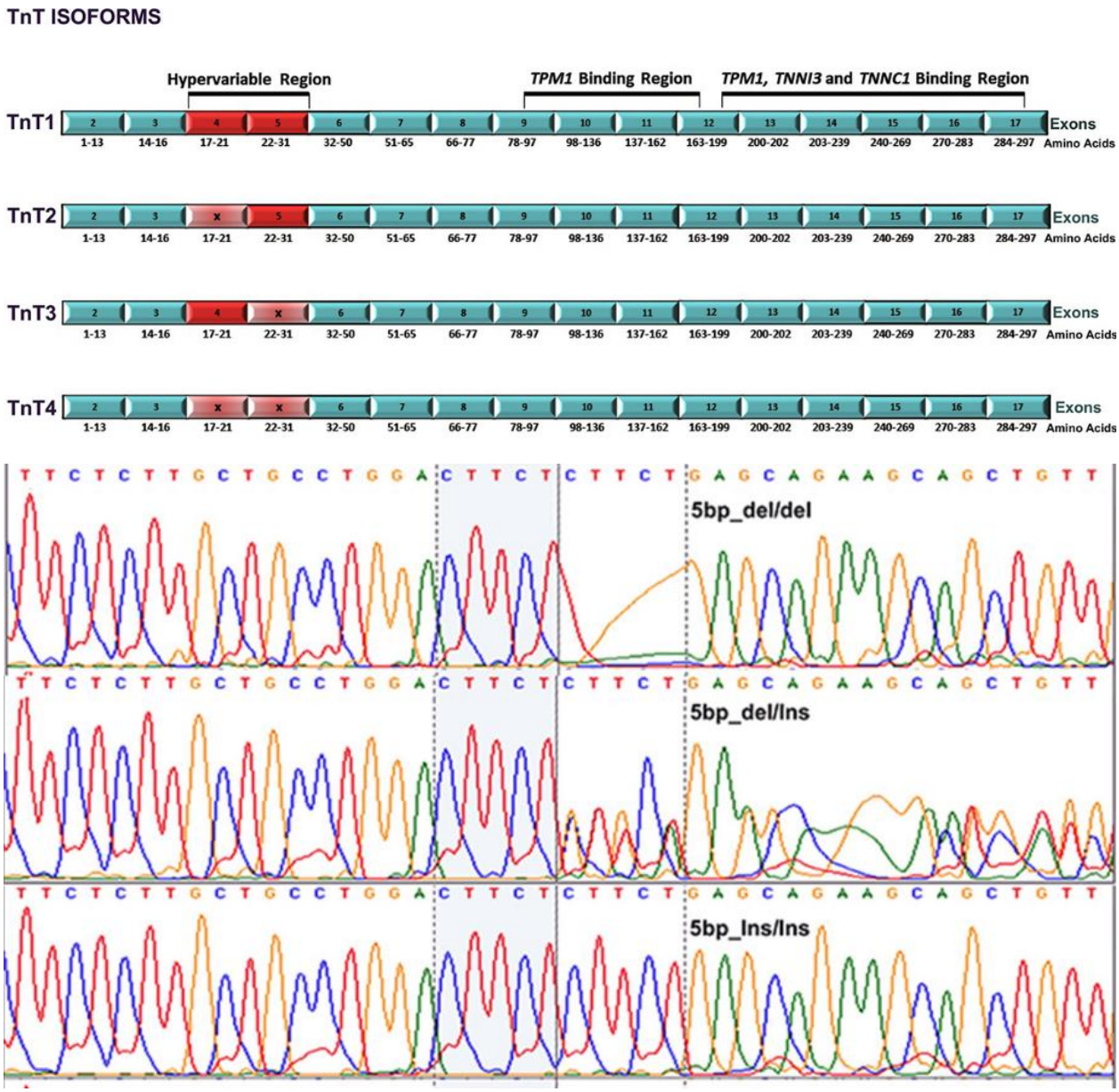
However, the insertion allele frequency found to be high in Nishi, Ao-Naga, Chakesang Naga, Mizo, Kandha, Sah, mixed people from West Bengal, Siddis from Gujarat and Karnataka, Medar from Karnataka and Onges of the Andaman Island (Table 4). Our study revealed a very high *del/del* genotype and deletion allele frequency in HCM (Table 2 and 3), compared to most of the diverse people from different states of India (Table 4).

Discussion

The human myocardium has the potential to express multiple TnT isoforms. The major TnT isoforms expressed in the failing human heart are the products of alternative splicing of mRNA [15] (Figure.1), rather than post-translational modifications of a single protein or the result of proteolysis [4]. The basis of TnT isoform expression in chicken and rat [13] hearts and rabbit,[16] rat, [17] and avian [18] skeletal muscles are the result of alternative splicing

						I/D		D/D		I/I		Del	Ins
					2056	No	%	No	%	No	%	%	%
1	Andhra Pradesh	Nayee Brahmin	Dravidian	46	307	23	50.2	16	34.6	7	15.2	59.78	40.21
		Gond		91		41	45.1	49	53.84	1	1.09	76.36	23.61
		Yerukula		65		34	52.3	29	44.61	2	3.07	48.65	29.22
		Thoti		61		23	37.7	29	47.54	9	14.8	66.39	33.6
		Mondi		44		20	45.5	16	36.36	8	18.2	59.09	40.9
							46.1		43.4		10.5		
2	Karnataka	Gram Vokkal	Dravidian	44	145	21	47.7	19	43.18	4	9.09	67.04	32.95
		Medar		50		27	54	10	20	13	26	47	53
		Korova		31		14	45.2	12	38.7	5	16.1	61.28	38.7
		Siddi	Indo-European	20		7	35	3	15	10	50	32.5	67.5
							45.5		29.22		25.3		
3	Tamil Nadu	Pillai	Dravidian	102	225	54	52.9	26	25.49	22	21.4	51.96	47.82
		Paravar		40		22	55	9	22.5	9	22.5	50	50
		Arunthathiyar		83		54	65.1	29	34.93	0	0	67.46	32.53
							57.7		27.64		14.6		
4	Madhya Pradesh	Sahariya	Austro-Asiatic	86	249	39	45.3	35	40.69	12	14	63.36	36.62
		Bharia	Dravidian	42		20	47.6	12	28.57	10	23.8	52.37	47.61
		Bhil	Indo-European	40		20	50	14	35	6	15	60	40
		Chaurasia		81		35	43.2	38	46.91	8	9.87	68.51	31.47
							46.5		37.79		15.7		
5	Uttar Pradesh	Agaria	Indo-European	44	44	24	54.5	14	31.81	6	13.6	59.08	40.9
6	Maharashtra	Mahadeo Koli	Indo-European	82	227	37	45.1	32	39.02	13	15.9	61.58	38.41
		Maratha Desai		62		38	61.2	16	25.8	8	12.9	56.4	43.5
		Warli		83		43	51.8	31	37.34	9	10.8	63.24	36.74
							53.2		33.49		13.3		
7	Gujarat	Gamit	Proto-Australoid	88	88	37	42	43	48.86	8	9.09	69.88	30.11
		Siddi	Indo-European	63	143	34	54	8	12.69	21	33.3	39.67	60.31
		Patel		80		33	41.3	32	40	15	18.8	60.63	39.38
							45.8		33.85		20.4		
8	Rajasthan	Jain	Indo-European	86	153	36	41.6	43	50	10	9.9	70.93	22.63
		Meena	Indo-European	67		29	42	32	47.76	8	8.95	67.16	28.35
							41.8		48.8		9.4		
9	Chhattisgarh	Sindhi	Indo-European	52	52	25	48.1	20	38.46	7	13.5	62.49	37.49
10	West Bengal	Subba	Tibeto-Burman	14	60	6	42.9	8	57.14	0	0	78.57	21.43
		Mixed	Indo-European	46		11	44	6	24	8	32	46	54
							43.4		40.57		16		
11	Haryana	Ho Munda	Austro-Asiatic	9	9	4	44.4	4	44.44	1	11.1	66.66	33.32
12	Nagaland	Ao Naga	Tibeto-Burman	34	71	13	38	7	21	14	41	40	60
		Chakesang Naga		37		17	46	4	11	16	43	34	67
							42		16		42		
13	Mizoram	Mizo	Tibeto-Burman	26	26	9	35	8	31	9	35	48	52
14	Jharkhand	Gorait	Austro-Asiatic	63	63	31	49	27	43	5	8	67	33
15	Uttaranchal	Sah	Indo-European	5	5	2	40	1	20	2	40	40	60
16	Jammu & Kashmir	Kashmiri Pandits	Indo-European	15	15	7	47	5	33	3	20	57	43
17	Orissa	Kandha	Indo-European	85	85	41	48	20	24	24	28	48	52
18	Andaman islands	Andamanese	Andamanese	16	39	5	31	10	63	1	6	78	22
		Onge	Andamanese	23		12	52	3	13	8	35	39	61
							42		38		21		
19	Nicobar islands	Nicobarese	Austro-Asiatic	23	23	15	65	5	22	3	13	54	46
20	Assam	Nyshi	Tibeto-Burman	27	27	11	41	7	26	9	33	46	54

Table 4. Genotype and Allele frequency of a common 5bp (D/D, I/D, I/I) Polymorphism Detected in Caste and Tribe groups belonging to different states of India



(Figure.2). The C/T polymorphism in intronic polypyrimidine tract was found to influence splicing [19]. The polypyrimidine tract is one of the important cis-acting sequence elements directing intron removal in pre-mRNA splicing, however, the deletion of the polypyrimidine tract has been found to abolish correct lariat formation, spliceosome assembly, and thus the alternate splicing results in skipping of exon [7,8].

The 5bp (CTTCT) polymorphism in intron 3 of *TNNT2* was reported to be in the polypyrimidine tract sequence which results in the skipping of exon 4, located downstream of this polymorphism [6,9]. Missing exon 4 in cardiac *TNNT2* corresponds to isoforms cTnT2 and cTnT4, and found to be elevated in heart failure and the fetal heart [3-5]. The 5bp deletion polymorphism reported in the French population was found to be influencing the splicing accuracy and efficiency, although the clinical significance of this polymorphism has not been elucidated [6]. The minigene construct (*in vitro mRNA expression*) has shown that the 5bp deletion had affected the mRNA expression pattern by skipping of exon four cardiac *TNNT2* [9].

We have therefore screened the 5bp *(*D/D, D/I, I/I) polymorphism in the intron 3 of the *TNNT2* gene in North Indian HCM patients and found that the deletion (*del/del*) genotype and the deletion allele frequencies were significantly higher in the hypertrophic cases, compared to the healthy controls. Hence, the *del/del* genotype (73%) might be associated with prominent concentric LV hypertrophy in such cases. The difference in deletion allele frequencies between the patients (HCM) and healthy controls has shown a statistically significant p-value (p=0.000172).

In Japanese patients, the frequency of insertion and deletion alleles in the HCM was 33.1 and 66.9%, respectively [9]. Our study revealed that the *del/del* genotype frequency in North Indians are 75% higher than that of the Japanese patients, suggesting a *strong* association. This indicates that the 5bp *del/del* genotype and the deletion alleles of Troponin T2 are associated with a predisposition to prominent left ventricular hypertrophy not only in French, Japanese, and South Indian patients but also in North Indian HCM patients.

Most Indians descend from a mixture of two genetically divergent populations: Ancestral North Indians (ANI) and Ancestral South Indians (ASI)[10]. Our study of 2056 Caste and Tribe groups belonging to highly diverse endogamous populations inhabited in different states of India

showed that the frequency of 5bp (del/del) polymorphism was significantly higher not only in South Indians but also in North Indians (p=0.000535). Therefore, our study helps to understand the frequency of 5bp (del/del) polymorphism in the admixture of the Indian population. In conclusion, the *TNNT2* del/del genotype and the deletion allele frequency are not only associated with LV hypertrophy in Japanese, French, and South Indian hypertrophic cardiomyopathy but also in HCM patients from North India.

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Conflict of Interest

The authors have declared that there is no conflict of interest exist.

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References

1. Gomes AV, Guzman G, Zhao J, Potter JD. (2002). Cardiac troponin T isoforms affect the Ca2+ sensitivity and inhibition of force development. Insights into the role of troponin T isoforms in the heart. J Biol Chem;277(38):35341-35349.
2. Mesnard-Rouiller L, Mercadier JJ, Butler-Browne G, et al. (1997). Troponin T mRNA and protein isoforms in the human left ventricle: pattern of expression in failing and control hearts. J Mol Cell Cardiol;29(11):3043-3055.
3. Anderson PA, Malouf NN, Oakeley AE, Pagani ED, Allen PD. (1991). Troponin T isoform expression in humans. A comparison among normal and failing adult heart, fetal heart, and adult and fetal skeletal muscle. Circ Res;69(5):1226-1233.
4. Townsend PJ, Farza H, MacGeoch C, et al. (1994). Human cardiac troponin T: identification of fetal isoforms and assignment of the TNNT2 locus to chromosome 1q. Genomics;21(2):311-316.
5. Anderson PA, Greig A, Mark TM, et al. (1995). Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart. Circ Res;76(4):681-686.
6. Mesnard L, Logeart D, Taviaux S, Diriong S, Mercadier JJ, Samson F. (1995). Human cardiac troponin T: cloning and expression of new isoforms in the normal and failing heart. Circ Res;76(4):687-692.
7. Breitbart RE, Nguyen HT, Medford RM, Destree AT, Mahdavi V, Nadal-Ginard B. (1995). Intricate combinatorial patterns of exon splicing generate multiple regulated troponin T isoforms from a single gene. Cell 1985;41(1):67-82.
8. Farza H, Townsend PJ, Carrier L, et al. (1998). Genomic organisation, alternative splicing and polymorphisms of the human cardiac troponin T gene. J Mol Cell Cardiol;30(6):1247-1253.
9. Coolidge CJ, Seely RJ, Patton JG. (1997). Functional analysis of the polypyrimidine tract in pre-mRNA splicing. Nucleic Acids Res;25(4):888-896.
10. Norton PA. (1994). Polypyrimidine tract sequences direct selection of alternative branch sites and influence protein binding. Nucleic Acids Res;22(19):3854-3860.
11. Komamura K, Iwai N, Kokame K, et al. (2004). The role of a common TNNT2 polymorphism in cardiac hypertrophy. J Hum Genet;49(3):129-133.
12. Reich D, Thangaraj K, Patterson N, Price AL, Singh L. (2009). Reconstructing Indian population history. Nature;461(7263):489-494.
13. Burke W, Coughlin SS, Lee NC, Weed DL, Khoury MJ. (2001). Application of population screening principles to genetic screening for adult-onset conditions. Genet Test;5(3):201-11.
14. Rani DS, Nallari P, Dhandapany PS, et al. (2012). Cardiac Troponin T (TNNT2) mutations are less prevalent in Indian hypertrophic cardiomyopathy patients. DNA Cell Biol;31(4):616-624.
15. Cooper TA, Ordahl CP. (1985). A single cardiac troponin T gene generates embryonic and adult isoforms via developmentally regulated alternate splicing. J Biol Chem;260(20):11140-11118.
16. Briggs MM, Schachat F. (1989). N-terminal amino acid sequences of three functionally different troponin T isoforms from rabbit fast skeletal muscle. J Mol Biol;206(1):245-249.
17. Jin JP, Lin JJ. (1989). Isolation and characterization of cDNA clones encoding embryonic and adult isoforms of rat cardiac troponin T. J Biol Chem;264(24):14471-1447.
18. Hastings KE, Bucher EA, Emerson CP, Jr. (1985). Generation of troponin T isoforms by alternative RNA splicing in avian skeletal muscle. Conserved and divergent features in birds and mammals. J Biol Chem;260(25):13699-13703.
19. Roscigno RF, Weiner M, Garcia-Blanco MA. (1993). A mutational analysis of the polypyrimidine tract of introns. Effects of sequence differences in pyrimidine tracts on splicing. J Biol Chem;268(15):11222-11229.

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