INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-2, April-June-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 5th Feb 2016 *Revised: 19th Feb-2016* *Accepted: 20th Feb 2016*

Research article

INFLUENCE OF *TGF-β1* -509C/T AND 869T/C POLYMORPHISMS ON CHANNEL CONDUCTIVITY IN LONG QT SYNDROME

Altaf Ali[1], Sameera F. Qureshi[1], Veronica M[1], Ananthapur Venkateshwari[2], Narsimhan Calambur[3], Hygriv Rao[4], Jayaprakash Shenthar[5], Kumarasamy Thangaraj[6] and Pratibha Nallari[12]*

[1]Department of Genetics, University College of Science, Osmania University, Hyderabad-500007, Telangana, India.

[2]Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad-500016, Telangana, India.

Care Hospital, Hyderabad, Telangana, India

[3]Krishna Institute of Medical Sciences, 1-8-31/1, Minister Road, Secunderabad-500 003, Telangana, India.

[4]Sri Jayadeva Institute of Cardiovascular Science and Research, Jayanagar 9th Block, Bannerghatta Road, Bangalore 560069, Karnataka, India.

[5]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, Telangana, India.

ABSTRACT: Long QT syndrome (**LQTS**) manifests itself with syncopal episodes ending in sudden cardiac death and abnormalities in the electrophysiology of the heart. The main cause of LQTS is the mutated ion channels encoded by faulty genes. Transforming growth factor-beta 1 is a cytokine involved in the development of cardiomyocytes. Incomplete penetrance and variable expressivity are phenomena commonly observed in **LQTS**; hence; the role of modifiers such as *TGF-β1* can be expected. A total of 219 unrelated control samples and 49 cases with 71 family members were enrolled for the study. Five ml of venous blood was collected from all the individuals and genomic DNA was isolated by standard protocol. Genotyping was performed for the two *TGF-β1* polymorphisms (-509C/T and 869T/C) by Allele specific PCR followed by calculation of relative risk estimates and *in silico* prediction of pre-mRNA secondary structures. Relative risk estimates showed a significant association between the "TC" genotype of the 869T/C polymorphism and **LQTS**. This was further corroborated by the "C" allele's pre-mRNA secondary structure of the 869T/C polymorphism. A significant association between the "TC" genotype and the "C" allele of the 869T/C polymorphism and **LQTS** was observed. The heterozygote disadvantage exhibited by "TC" could lead to expression of a haplo-insufficient protein product ending in improper folded ion channels or faulty anchoring of the ion channels.

Key words: Long QT Syndrome, TGF-β1, electrophysiology, 869T/C, torsade-de-pointes, sudden cardiac death.

*Corresponding author: Pratibha Nallari, [1]Department of Genetics, University College of Science, Osmania University, Hyderabad-500007, E-mail: prathinallari@yahoo.com Phone number: +91-8885486499

List of abbreviations

TGF-β1 – Transforming growth factor-beta 1
LQTS – Long QT Syndrome
FDRs – First degree relatives
ECG – Electrocardiograph
ARMS – Amplified refractory mutation system
CF – Common forward
RC – Reverse C
RT – Reverse T
CI – Confidence interval
SNP – Single nucleotide polymorphism
LD – Linkage disequilibrium

INTRODUCTION

The long QT syndrome (LQTS) is a rare hereditary disorder of the cardiac ion channels. The two cardinal manifestations of LQTS include syncopal episodes leading to cardiac arrest, sudden cardiac death and electrocardiographic (ECG) abnormalities such as prolongation of the QT interval and T wave abnormalities [Crotti et al., 2008]. LQTS is mainly manifested as a result of mutations in genes encoding the sodium and potassium ion channels causing prolongation of the cardiac action potential. LQTS exhibits incomplete penetrance and variable expressivity suggesting the involvement of modifiers in addition to the primary mutations in genes responsible for the phenotype manifestation. Identification of modifier gene/s in LQTS can help improve risk stratification among affected individuals and carriers of such mutations, providing information about the risk of life-threatening arrhythmias [http://www.geneticheartdisease.org/lqts.htm]. One such modifier gene is the Transforming Growth Factor β (*TGF-β*). The TGF-β family comprises a large number of structurally related polypeptide growth factors, each capable of regulating a multitude of cellular processes including cell proliferation, lineage determination, differentiation, motility, adhesion, and death [Massague, 1998]. The present study aims at identifying the possible influence of *TGF-β1* -509C/T and 869T/C polymorphisms in the modulation of ion flow across the channels peppering the cardiac sarcomere.

MATERIALS AND METHODS

The participants involved in the study were clinically evaluated based on a 12 lead ECG read by the consultant cardiologists at CARE Hospitals Nampally, Banjara Hills, Secunderabad, Sri Jayadeva Institute of Cardiovascular Science and Research, Bangalore and Institute of Maternal and Child Health, Calicut Medical College, Kerala.

A total of 219 unrelated individuals from Osmania General Hospital as controls and 49 LQTS probands with 71 available family members were enrolled in the study with prior informed consent. The study was designed in accordance with the ethical guidelines set under the 1975 Helsinki Declaration and approved by the Institutional Ethical Committee, Osmania University, Hyderabad.

Five ml of peripheral blood was collected from all the participants and DNA isolated following standard protocol [**Lahiri and Nurnberger, 1991**]. The two SNPs were genotyped by amplified refractory mutation system PCR (**ARMS-PCR**). Two sets of reaction were carried out for each sample with a common primer (**CF**) and one of the reverse primer (**RC/RT**) specific for the different alleles of the polymorphic locus. The primer sequences and amplicon lengths for the two SNPs in the study are given in **Table 1** (**Supplementary 1**).

Statistical analysis was carried out to determine the allelic and genotypic frequencies for the polymorphic loci. Relative risk estimates (Odd's ratio) at 95% confidence intervals (**95% CI**) and $p \leq 0.05$ were estimated to test for association between various genotypic combinations using SNPSTAT software [**http://bioinfo.iconcologia.net /snpstats/custom.php]**. Differences in the control group and LQTS group were tested by Chi square test for association [**http://www.socscistatistics.com/tests/chisquare2/default2.aspx**]. To identify the differences between the allelic make-ups of different populations, hap-map analysis was carried out. LD plots were generated by Haploview ver4.2 program [**http://www.broad.mit.edu/mpg/haploview**].

In silico analysis was carried out to identify the stability of the pre-mRNA secondary structures [http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi], and the changes in the various transcription factors binding to the variants [http://www.gene-regulation.com/pub/programs/alibaba2/index.html] of the individual SNPs of the *TGF-β1* gene.

RESULTS

Table 1 represents the epidemiological data of the samples in the present study wherein it is observed that nine patients out of the 49 were aLQTS cases (18.4%), and five out of these nine cases were syncope positive (55.5%), and 26 (65%) out of the 40 cLQTS (81.6%) cases were syncope positive. The 21 male cases (42.9%) had a mean age of 19.23 years (SD=16.5) whereas the 28 females cases (57.1%) had a mean age of 22.95 years (SD=18.4) clearly pointing towards an early onset in the male cases when compared to the female cases. Twelve (24.5%) out of the 49 LQTS cases revealed consanguinity with majority being female cases highlighting the presence of recessive alleles of LQTS genes in the general population and the homozygosity of these being responsible for the recessive inheritance of LQTS (Table 2 – Supplementary 1). The cLQTS cases showed a pooled ECG value of 490±34ms when compared to the aLQTS cases with a value of 494±58ms. However, a larger sample size is required to substantiate the findings.

The genotype and allele frequency distributions of the two SNPs are represented in Table 2. The "CC" (28.6%) genotype of *TGF-β1* -509C/T was higher in LQTS when compared to the controls (28.3%) and FDRs (25.4%), while the FDRs showed higher percentage of "CT" (64.8%) when compared to the other two genotypes. However, no significant deviation from the Hardy-Weinberg equilibrium was observed, strengthening the fixation of alleles in the gene pool.

The "TC" (77.6%) genotype of *TGF-β1* 869T/C was higher in the LQTS when compared to the other two groups exhibiting heterozygote disadvantage and the Hardy-Weinberg equilibrium was found to be disturbed because of the specific association in LQTS ($\chi 2$= 8.97; p= 0.01) (Table 2).

Relative risk estimates were calculated to establish the risk of different alleles and genotype combinations in LQTS group and FDRs compared to the controls (Tables 3a, 3b and 3c).

Calculation of relative risk estimates for the *TGF-β1* -509C/T polymorphism did not reveal any variation in the distribution of the genotypes (Table 3a, 3b and 3c).

The *TGF-β1* 869T/C polymorphism showed interesting result wherein the "TC" genotype revealed a fourfold increased risk towards LQTS under the co-dominant and dominant model (OR= 3.69; CI= 1.48 – 9.15; p= 0.0067 and OR= 3.44; CI= 1.40 – 8.45; p= 0.0026 respectively) and a threefold risk under the over-dominant mode of inheritance (OR= 2.75; CI= 1.33 – 5.65; p= 0.0037).

No significant observations were seen when the FDRs were compared against the LQTS and controls, reflecting the rarity and small sample size of LQTS.

Table 2 (Supplementary 1) gives the mutation analysis data produced from the ongoing study. The table shows the distribution of variations in correspondence to the risk alleles of the *HSP-70* in the LQTS cases and FDRs, which strengthens the association of *TGF-β1* and its role as a modifier in the onset and progression of LQTS.

Hapmap Analysis

Hapmap analysis was carried out to identify the allelic distribution of the two *TGF-β1* polymorphisms in the South Indian cohort comparing it to the various populations available in the SNP database (NCBI). The hapmap of *TGF-β1* -509C/T (Fig. 1) revealed the "C" allele to be dominant in the various populations analyzed. The frequency of "T" allele of *TGF-β1* +869T/C was higher in all the populations analyzed, but interestingly, it was observed that the dominant "T" allele was fixed in the European, Asian and the Sub-Saharan African populations (Fig. 2) highlighting the unique genetic makeup of these populations.

Haplotype analysis and linkage disequilibrium

Linkage disequilibrium (LD) plot of the two SNPs was constructed with the help of Haploview 4.2 and D' values are given in the Fig. 3. As seen from Fig. 3 the two SNPs are being inherited as 'n block' and co-segregate together as a single unit. To study the association between possible haplotype combinations of the *TGF-β1* gene variants and LQTS, haplotype analysis was performed. The estimated *TGF-β1* haplotype frequencies are given in Table 4. Haplotypes with a frequency of $p<0.01$ were excluded from the analysis, and odds ratio at 95% confidence interval (CI) for each haplotype compared with the reference haplotype group are given at $*p \leq 0.05$. The most common haplotype observed was used as the reference haplotype for comparison. No significant haplotype combination conducive for disease onset was observed. This could be due to the rarity of the disease and small sample size.

***In silico* analysis**

Secondary pre-mRNA structures were built as per the background algorithm at the Vienna webserver [http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi].

***TGF-β1* -509C/T (rs1800469)**

Secondary structure analysis of the pre-mRNA revealed distinct changes with the entropy being lesser in the wild type allele (-91.50 kcal/mol). The alternate 'T' allele (-91.20 kcal/mol) may encode for decreased pre-mRNA molecules, and mature protein, thus, leading to reduced developmental efficiency of the cardiomyocytes.

The *TGF-β1-509 C/T* promoter polymorphism leads to deletion of T3R alpha belonging to the thyroid hormone receptor sub-family which plays a role in cellular development and homeostasis by serving as a biological signal to control cell growth and differentiation.

[http://www.gene-regulation.com/pub/programs/alibaba2/index.html].

***TGF-β1* 869T/C (rs1800470)**

As seen from the above figures, distinct structural changes corroborated by free energy change could be observed. The lower free energy of the 'C' allele leads to its higher stability, hence allowing it to express at increased rate creating a conducive environment for disease progression.

Table 1: Distribution of the epidemiological variables in congenital LQTS and acquired LQTS

Variable	**Controls n (%)**	**LQTS n (%)**
aLQTS	-	9 (18.3)
cLQTS	-	40 (81.7)
Gender		
1. Males	100 (45.67)	21 (42.9)
2. Females	119 (54.33)	28 (57.1)
Sex ratio	0.84:1	0.75:1
Syncope	-	aLQTS – 5 cLQTS – 26
Consanguinity	-	**12 (24.5%)**
Deafness	-	4 (8.69%)
Variable	**Controls (N= 219) (Mean±SD)**	**LQTS (N= 49) (Mean±SD)**
Age in years		
1. Males	31.1±8.26	19.23±16.5
2. Females	27.05±10.08	22.95±18.4
Pooled	29.08 ±9.41	21.3±17.4

Type of LQTS	**Males n (%)**	**Females n (%)**	**Pooled n (%)**
cLQTS	17 (42.5)	23 (27.5)	40 (81.6)
aLQTS	4 (44.4)	5 (55.6)	9 (18.4)
Consanguinity (N= 49)			
Type of LQTS	**Males n (%)**	**Females n (%)**	**Pooled n (%)**
cLQTS	4 (40)	6 (60)	10 (21)
aLQTS	-	2 (100)	2 (4.3)
Family History of sudden death (N= 49)			
Type of LQTS	**Males n (%)**	**Females n (%)**	**Pooled n (%)**
cLQTS	6 (50)	6 (50)	12 (26)
aLQTS	1 (50)	1 (50)	2 (4.3)
Type of LQTS	Males (Mean±SD) (msec)	Females (Mean±SD) (msec)	Pooled (Mean±SD) (msec)
cLQTS	500±36	478±30	490±34
aLQTS	517±9.2	479±76.7	494±58

The addition of binding site for C/EBPalpha and Sp1 (Table 5) indicate there is an enhancing of the transcription of the mutant pre-mRNA leading to higher expression of mutant polypeptides of the 869 T/C SNP, which might contribute to disease progression. The loss of MyoD; a muscle differentiation factor could lead to developmental defects of the myocardiocytes creating a conducive environment for mis-lodging/faulty anchoring of the channel proteins, thus, contributing to disease phenotype.

Table 2: Genotype and Allele frequency distribution of the *TGF-β1* gene polymorphisms

SNP ID	SNP Locus	Genotype	Controls (C) (N=219) n (%)	LQTS (P) (N=49) n (%)	FDR (N=71) n (%)	$\chi 2^*$ (p value)		
						C vs P	FDR vs P	C vs FDR
rs1800469 *TGF-β1*	**-509**	CC CT TT	62 (28.3) 114 (52.05) **43 (19.65)**	**14 (28.6)** 27 (55.1) 8 (16.3)	18 (25.4) **46 (64.8)** 7 (9.8)	0.30 (0.89)	1.53 (0.47)	4.72 (0.09)
rs1800470 *TGF-β1*	**+869**	TT TC CC	**71 (32.4)** 122 (55.7) **26 (11.9)**	6 (12.2) **38 (77.6)** 5 (10.2)	15 (21.1) 49 (69) 7 (9.9)	8.97* (0.01)	1.6 (0.45)	4.11 (0.13)
		Alleles	**Controls (N=438)**	**LQTS (N=98)**	**FDR (N=142)**	**C vs P**	**FDR vs P**	**C vs FDR**
rs1800469 *TGF-β1*	**-509**	C T	238 200	55 43	82 60	0.1 (0.75)	0.06 (0.8)	0.5 (0.48)
rs1800470 *TGF-β1*	**+869**	T C	264 174	50 48	79 63	2.83 (0.09)	0.5 (0.48)	0.96 (0.33)

* $\chi 2_T = 5.991$ at 2 degrees of freedom.

Table 3a: Genotype distribution of the *TGF-β1* gene polymorphisms in LQTS compared to controls

SNP (rs number)	Model	Genotype	Controls	LQTS	Unadjusted OR (95%CI)	p
rs1800469 *TGF-β1* **-509C/T**	**Codominant**	C/C C/T T/T	62 114 43	14 27 8	1.00 1.05 (0.51 – 2.15) 0.82 (0.32 – 2.13)	0.86
	Dominant	C/C C/T-T/T	62 157	14 35	1.00 0.99 (0.5 – 1.96)	0.97
	Recessive	C/C-C/T T/T	176 43	41 8	1.00 0.8 (0.35 – 1.83)	0.59
	Over dominant	C/C-T/T C/T	105 114	22 27	1.00 1.13 (0.61 – 2.11)	0.7
rs1800470 *TGF-β1* **869T/C**	**Codominant**	T/T T/C C/C	71 122 26	6 38 5	1.00 **3.69 (1.48 – 9.15)*** 2.28 (0.64 – 8.10)	**0.0067***
	Dominant	T/T T/C-C/C	71 148	6 43	1.00 **3.44 (1.40 – 8.45)***	**0.0026***
	Recessive	T/T-T/C C/C	193 26	44 5	1.00 0.84 (0.31 – 2.32)	0.74
	Over dominant	T/T-C/C T/C	97 122	11 38	1.00 **2.75 (1.33 – 5.65)***	**0.0037***
SNP (rs number)	**Allele**		Controls	LQTS	**Unadjusted OR(95%CI)**	**p**
rs1800469 *TGF-β1* **-509 C/T**	**C** **T**		238 200	55 43	1.00 0.93 (0.59 – 1.48)	0.84
rs1800470 *TGF-β1* **869 T/C**	**T** **C**		264 174	50 48	1.00 1.46 (0.92 – 2.31)	0.12

***: relative risk calculated at p≤0.05**

Table 3b: Genotype distribution of the *TGF-β1* gene polymorphisms in FDRs compared to LQTS group

SNP (rs number)	Model	Genotype	FDRs	Cases	Unadjusted OR(95% CI)	p
rs1800469 *TGF-β1* -509 C/T	Codominant	C/C C/T T/T	18 46 7	14 27 8	1.00 0.75 (0.32 – 1.76) 1.47 (0.43 – 5.04)	0.47
	Dominant	C/C C/T-T/T	18 53	14 35	1.00 0.85 (0.37 – 1.93)	0.7
	Recessive	C/C-C/T T/T	64 7	41 8	1.00 1.78 (0.60 – 5.29)	0.3
	Over dominant	C/C-T/T C/T	25 46	22 27	1.00 0.67 (0.32 – 1.4)	0.29
rs1800470 *TGF-β1* 869 T/C	Codominant	T/T T/C C/C	15 49 7	6 38 5	1.00 1.94 (0.69 – 5.47) 1.79 (0.40 -7.91)	0.44
	Dominant	T/T T/C-C/C	15 56	6 43	1.00 1.92 (0.69 – 5.36)	0.2
	Recessive	T/T-T/C C/C	64 7	44 5	1.00 1.04 (0.31 – 3.48)	0.95
	Over dominant	T/T-C/C T/C	22 49	11 38	1.00 1.55 (0.67 – 3.59)	0.3
SNP (rs number)	**Allele**		**FDRs**	**Cases**	**Unadjusted OR(95% CI)**	**p**
rs1800469 *TGF-β1* -509 C/T	**C** **T**		82 60	55 43	1.00 1.07 (0.61 – 1.86)	0.91
rs1800470 *TGF-β1* 869 T/C	**T** **C**		79 63	50 48	1.00 1.20 (0.70 – 2.09)	0.57

Table 3c: Genotype distribution of the *TGF-β1* gene polymorphisms in FDRs compared to controls

SNP (rs number)	Model	Genotype	Controls	FDRs	Unadjusted OR(95% CI)	p
rs1800469 *TGF-β1* **-509** C/T	Codominant	C/C C/T T/T	62 114 43	18 46 7	1.00 1.39 (0.74 – 2.6) 0.56 (0.22 – 1.46)	0.08
	Dominant	C/C C/T-T/T	62 157	18 53	1.00 1.16 (0.63 – 2.14)	0.63
	Recessive	C/C-C/T T/T	176 43	64 7	1.00 0.45 (0.19 – 1.05)	0.047
	Over dominant	C/C-T/T C/T	105 114	25 46	1.00 1.69 (0.97 – 2.95)	0.059
rs1800470 *TGF-β1* **869 T**/C	Codominant	T/T T/C C/C	71 122 26	15 49 7	1.00 1.90 (0.99 – 3.63) 1.27 (0.47 – 3.48)	0.12
	Dominant	T/T T/C-C/C	71 148	15 56	1.00 1.79 (0.95 – 3.38)	0.064
	Recessive	T/T-T/C C/C	193 26	64 7	1.00 0.81 (0.34 – 1.96)	0.64
	Over dominant	T/T-C/C T/C	97 122	22 49	1.00 1.77 (1.00 – 3.13)*	0.045*
SNP (rs number)	**Allele**		**Controls**	**FDRs**	**Unadjusted OR(95% CI)**	**p**
rs1800469 *TGF-β1* **-509** C/T	**C** **T**		238 200	82 60	1.00 0.87 (0.58 – 1.3)	0.54
rs1800470 *TGF-β1* **869 T**/C	**T** **C**		264 174	79 63	1.00 1.21 (0.81 – 1.81)	0.38

Table 4: *TGF-β1* haplotypes among Controls and LQTS and their association with LQTS

Haplotype	Frequency		Odds Ratio (95% CI)	p
	Controls	LQTS		
C-T	0.3387	0.3173	1.00 (Ref)	---
T-T	0.264	0.193	0.92 (0.26 – 3.30)	0.9
C-C	0.2046	0.244	1.69 (0.44 – 6.49)	0.44
T-C	0.1926	0.2458	1.49 (0.75 – 2.96)	0.25
	Global haplotype association p-value:			0.28

Table 5: Loss/addition of Transcription factors in 869T/C polymorphism of *TGF-β1*

Type	Transcription factor	Location	Function
Addition	C/EBPalpha	115 – 124	Binds to the promoter and modulates the expression of genes.
Addition	Sp1	119 – 128	Involved in many cellular processes, including cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, and chromatin remodelling.
Loss	MyoD	125 – 134	Plays a major role in regulating muscle differentiation.

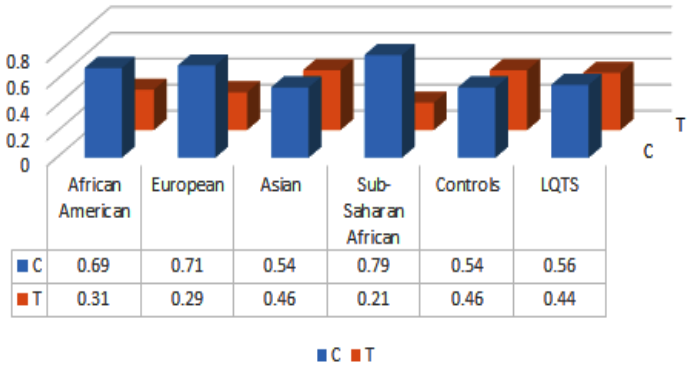

Fig. 1: Hapmap analysis of *TGF-β1* -509C/T polymorphism in different populations.

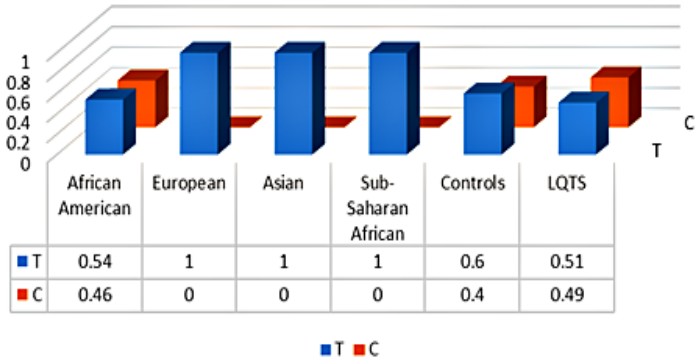

Fig. 2: Hapmap analysis of *TGF-β1* 869T/C polymorphism in different populations.

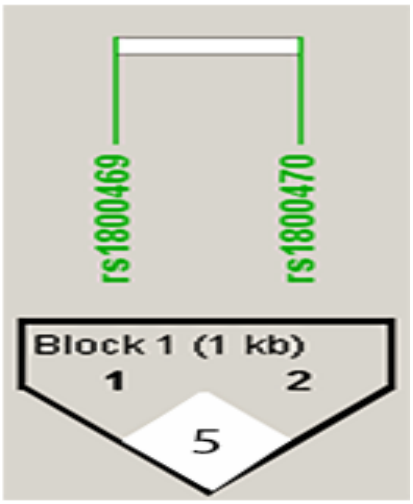

Fig. 3: Linkage disequilibrium plot of the two SNPs of the *TGF-β1* in LQTS and controls. (LD plots were generated using Haploview 4.2 software. D' values were indicated in the respective squares).

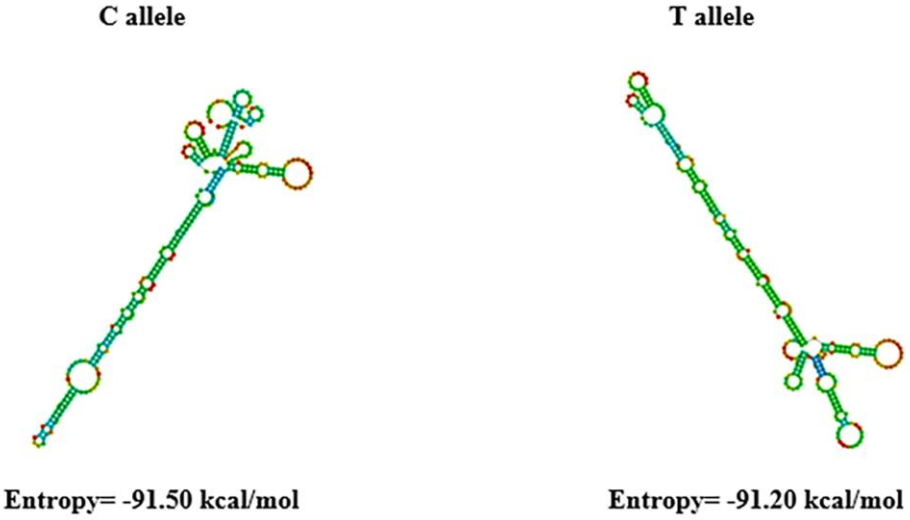

Fig. 4: Secondary pre-mRNA structures for the two alleles of -509C/T polymorphism of the *TGF-β1*.

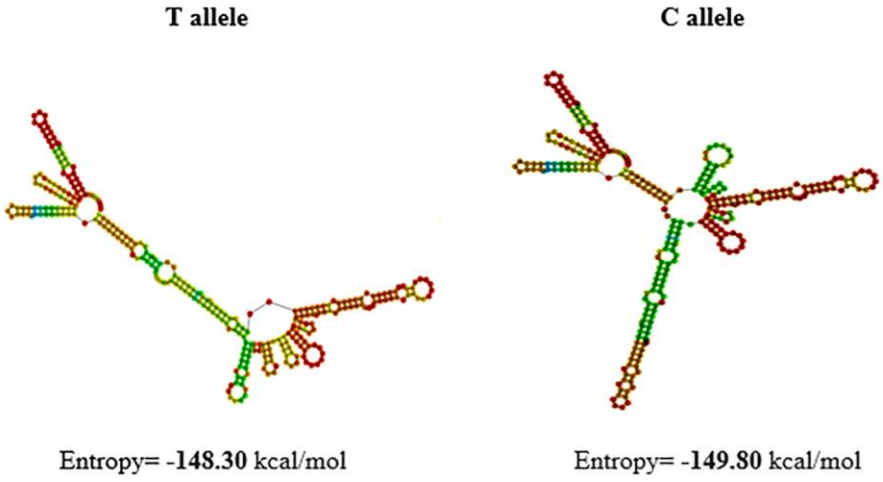

Fig. 5: Secondary pre-mRNA structures for the two alleles of 869T/C polymorphism of the *TGF-β1*.

Supplementary 1

Table 1: Primer for the -509C/T and 869T/C *TGF-β1* polymorphisms

SNP	Primers	Amplimer (bp)	PCR
-509C/T	CF antisense: 5'-CTACGGCGTGGAGTGCTGAG-3' (RC) Sense: 5'-AGGGGCAACAGGACACCTGGG-3' (RT) Sense: 5'-AAGGGGCAACAGGACACCTGGA-3'	349	ARMS PCR
869T/C	(sense): 5'-TCCGTGGGATACTGAGACAC-3' primer C (antisense): 5'-CAGCGGTAGCAGCAGCG-3' primer T (antisense): 5'- GCAGCGGTAGCAGCAGCA-3'	241	ARMS PCR

Table 2: Ion channel gene defects in correlation to *TGF-b1* risk alleles

SNP	Risk Allele	Cases	KCNQ1						KCNE1			SCN5A	
			IVS13+36A/G			S546S			S38G			C3417A (S1074A)	
			AA	AG	GG	AA	AG	GG	AA	AG	GG	CC	CA
rs1800469 *TGF-β1*	T	35	12	11	12	12	11	12	5	16	14	22	13
rs1800470 *TGF-β1*	C	43	15	11	17	14	12	17	6	17	20	27	16
SNP	**Risk Allele**	**FDRs**	**KCNQ1**						**KCNE1**			**SCN5A**	
			IVS13+36A/G			S546S			S38G			C3417A (S1074A)	
			AA	AG	GG	AA	AG	GG	AA	AG	GG	CC	CA
rs1800469 *TGF-β1*	T	53	9	19	25	9	19	25	4	28	21	17	36
rs1800470 *TGF-β1*	C	56	10	18	28	10	18	28	3	33	20	17	39

***The table represents a part of the mutation analysis data from the ongoing study.**

DISCUSSION

TGF-β plays an important role in the signaling cascade of hierarchy of genes involved in lung fibrosis [Willis and Borok, 2007], renal, and liver injury, Alzheimer [Ramos-Mondragón et al., 2008], cancer [Roberts and Wakefield, 2003], and cardiac disorders [Bujak and Frangogiannis, 2007, Burstein and Nattel, 2008].

Reports by numerous studies state that in a number of cardiac diseases, the expression of type 1 isoform is increased [Bujak and Frangogiannis, 2007, Burstein and Nattel, 2008]. Thomson et al. (1988) elucidated the specific role played by *TGF-β* in cardiac pathophysiology, wherein the infarcted heart ventricular myocytes over expressed TGF-β1 protein and its mRNA; developing increased vulnerability to atrial fibrillation and decreased epicardial conduction velocity [Verheule et al., 2004]. Several authors have suggested the role of TGF-β signaling in heart development [Ng et al., 2004, Bujak and Frangogiannis, 2007, Li et al., 2005, Liao, 2005 and Okada et al., 2005].

Alternatively, *TGF-β1* plays an important role in disrupting the integrity of the alveolar epithelial barrier contributing to impaired fluid and ion transport dynamics in acute lung injury [Noseworthy and Newton-Cheh 2008], a similar mechanism can account to LQTS. TGF-β1 induced dose- and time-dependent increase in current across the sodium channel, reflects upregulation of trans-epithelial active ion transport. Studies in the presence and absence of inhibitors of apical Na^+ channels (amiloride) and basolateral sodium pumps (ouabain) indicated that the observed increase in Isc in the presence of TGF-β1 is accounted by an increase in transepithelial Na^+ transport [Massague, 1998].

Remodeling of the myocardium is an important event that occurs in later stages of cardiac diseases. No literature exists that sheds light on the relationship between TGF-β isoforms and the electrophysiology/the electrical modulation of ion flow in the myocardium. TGF-β signaling is essential for epithelial–mesenchymal transformation (EMT), which determines formation of cardiac valves and the septa [Ramos-Mondragón et al., 2008, Nakajima et al., 2000]. Hence, similar transformation may be promoted by TGF-β cytokines in ion channel development.

TGF-β1 differentially affects cardiac ion channel function by targeting signaling pathways that modify transcription and gene expression of ion channel proteins. TGF-β is a locally generated cardiac cytokine that contributes to myofibroblast activation and proliferation, as well as production of extracellular matrix components [Eghbali et al., 1991, Gingery et al., 2008, Kaur et al., 2013, and Pittet et al., 2001].

The observation that tachyarrhythmias modify electrophysiological properties in cardiac diseases can culminate in "electrical or electrophysiological remodeling" as seen in LQTS, which could be correlated to the expression profiles of the *TGF-β1*. However, the precise alterations of *TGF-β1* involved in this process, with respect to ion channel function and expression still needs to be substantiated [Pandozi and Santini, 2001].

In the present study, female preponderance was observed with their mean age higher than the male patients. This shows that in males the advent of the disease condition is much earlier and severe (Table 2) when compared to the females which could be accounted to selective disadvantage of male fetuses, hence preponderance of females over males. Mutation analysis also returned a result wherein majority of the individuals were harboring the recessive alleles for the genes analyzed further strengthening the role of consanguinity in the etiology of LQTS (Table 2 – Supplementary 1). This clearly indicates the recessive inheritance pattern observed in the study cohort of LQTS making it clear that the disease manifests due to the presence of common recessives and homozygosity is brought about by consanguinity.

CONCLUSION

Since the role played by *TGF-β1* in ion transport of the alveolar membrane is already established, a hypothesis can be formed wherein a similar mechanism could be stated to exist in the cardiac myocardium being associated with arrhythmias and syncope. According to our study, the "TC" genotype of the *TGF-β1* 869T/C polymorphism displays heterozygote disadvantage, creating haplo-sufficiency wherein the affected females being carriers show variable levels of expressivity and transmit their lethal alleles to their fetuses, which if in homozygous recessive state are lost by natural selection such that the lethal genotype is not expressed. Even if expressed the heterozygous condition provides a conducive environment for improper functioning of the final protein product encoded by the mutant 'C' allele overshadowing that of the 'T' allele, thus, leading to improper folding or translocation of the ion channels to the myocardial membrane. Additional studies are warranted to further strengthen the above hypothesis and corroborate the effect of the mutant 869C allele on the disease condition.

ACKNOWLEDGEMENT

Financial assistance from Department of Biotechnology, New Delhi and Maulana Azad National Fellowship, University Grants Commission, New Delhi is sincerely acknowledged.

Declaration: "On behalf of all the authors, I, Prof. Pratibha Nallari, the corresponding author, would like to declare and take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation". I would also like to state that no conflict of interests exist among the authors.

REFERENCES

Bujak. M and Frangogiannis. N.G (2007). The role of TGF-β signaling in myocardial infarction and cardiac remodeling. Cardio. Res: Vol.74, 184 – 195.

Burstein. B and Nattel. S (2008). Atrial Fibrosis: Mechanisms and Clinical Relevance in Atrial Fibrillation. J. Amer. Col. Cardio: Vol.51, 8, 802 – 809.

Crotti. L, Celano. G, Dagradi. F, and Schwartz. P.J (2008). Congenital long QT syndrome. Orph. J. of Rare Dis: Vol.3, 18, 1 – 16.

Eghbali. M, Tomek. R, Sukhatme. V.P, Woods. C, and Bhambi. B (1991). Differential effects of transforming growth factor-beta 1 and phorbol myristate acetate on cardiac fibroblasts. Regulation of fibrillar collagen mRNAs and expression of early transcription factors. Circ. Res: Vol.69, 483–490.

Gingery. A, Bradley. E.W, Pederson. L, Ruan. M, Horwood. N.J, and Oursler. M.J (2008). TGF beta coordinately activates TAK1/MEK/AKT/NFkB and SMAD pathways to promote osteoclast survival. Exp. Cell Res: Vol.314, 2725–2738.

http://bioinfo.iconcologia.net/snpstats/custom.php
http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
http://www.broad.mit.edu/mpg/haploview
http://www.gene-regulation.com/pub/programs/alibaba2/index.html
http://www.geneticheartdisease.org/lqts.htm.
http://www.socscistatistics.com/tests/chisquare2/default2.aspx

Kaur. K, Zarzoso. M, Ponce-Balbuena. D, Guerrero-Serna. G, Hou. L, Musa. H, and Jalife. J (2013). TGF-□1, Released by Myofibroblasts, Differentially Regulates Transcription and Function of Sodium and Potassium Channels in Adult Rat Ventricular Myocytes. PLOS ONE: Vol.8, 2, e55391.

Lahiri. D.K and Nurnberger. J.I Jr. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res: Vol.19, 5444.

Li. T.S, Hayashi. M, Ito. H, Furutani. A, Murata. T, Matsuzaki. M, and Hamano. K (2005). Regeneration of Infarcted Myocardium by Intramyocardial Implantation of Ex Vivo Transforming Growth Factor-β–Preprogrammed Bone Marrow Stem Cells. Circ: Vol.111, 2438-2445.

Liao. R (2005). Yin and Yang of Myocardial Transforming Growth Factor-β1 Timing Is Everything. Circ: Vol.111, 2416-2417.

Massague. J (1998). Tgf-B Signal Transduction. Annu. Rev. Biochem: Vol.67, 753 – 91.

Nakajima. Y, Yamagishi. T, Hokari. S, and Nakamura. H (2000). Mechanisms Involved in Valvuloseptal Endocardial Cushion Formation in Early Cardiogenesis: Roles of Transforming Growth Factor (TGF)-β and Bone Morphogenetic Protein (BMP). Anato. Rec: Vol.258, 119–127.

Ng. C.M, Cheng. A, Myers. L.A, Martinez-Murillo. F, Jie. C, Bedja. D, Gabrielson. K.L, Hausladen. J.M, Mecham. R.P, Judge. D.P and Dietz. H.C (2004). TGF-β–dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J. of Clin. Inves: Vol.114, 1586 – 1592.

Noseworthy. P.A and Newton-Cheh. C (2008). Genetic Determinants of Sudden Cardiac Death. Circ: Vol.118, 1854-1863.

Okada. H, Takemura. G, Kosai. K, Li. Y, Takahashi. T, Esaki. M, Yuge. K, Miyata. S, Maruyama. R, Mikami. A, Minatoguchi. S, Fujiwara. T and Fujiwara. H (2005). Postinfarction Gene Therapy Against Transforming Growth Factor-β Signal Modulates Infarct Tissue Dynamics and Attenuates Left Ventricular Remodeling and Heart Failure. Circ: Vol.111, 2430-2437.

Pandozi. C and Santini. M (2001). Update on atrial remodelling owing to rate. Does atrial fibrillation always 'beget' atrial fibrillation? Euro. Heart J: Vol.22, 541–553.

Pittet. J.F, Griffiths. M.J, Geiser. T, Kaminski. N, Dalton. S.L, Huang. X, Brown. L.A, Gotwals. P.J, Koteliansky. V.E, Matthay. M.A and Sheppard. D (2001). TGF-β is a critical mediator of acute lung injury. J Clin Invest: Vol.107, 1537–1544.

Ramos-Mondragón. R, Galindo. C.A and Avila. G (2008). Role of TGF-βon cardiac structural and electrical remodeling. Vasc. Health and Risk Manag: Vol.4, 6, 1289–1300.

Roberts. A.B and Wakefield. L.M (2003). The two faces of transforming growth factorβ in carcinogenesis. PNAS: Vol.100, 15, 8621– 8623.

Verheule. S, Sato. T, Everett. T IV, Engle. S.K, Otten. D, Lohe. M.R.D, Nakajima. H.O, Nakajima. H, Field. L.J and Olgin. J.E (2004). Increased Vulnerability to Atrial Fibrillation in Transgenic Mice with Selective Atrial Fibrosis Caused by Overexpression of TGF-β1. Circ Res: Vol.94, 1458-1465.

Willis. B.C and Borok. Z (2007). TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am. J. Physiol. Lung Cell. Mol. Physiol: Vol.293, L525–L534.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com