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ORIGINAL ARTICLE

HCN4 Gene Polymorphisms Are Associated With Occurrence of Tachycardia-Induced Cardiomyopathy in Patients With Atrial Fibrillation

See Editorial by Tsai

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BACKGROUND: Tachycardia-induced cardiomyopathy (TIC) is a reversible cardiomyopathy induced by tachyarrhythmia, and the genetic background of the TIC is not well understood. The hyperpolarization-activated cyclic nucleotide-gated channel gene *HCN4* is highly expressed in the conduction system where it is involved in heart rate control. We speculated that the *HCN4* gene is associated with TIC.

METHODS: We enrolled 930 Japanese patients with atrial fibrillation (AF) for screening, 350 Japanese patients with AF for replication, and 1635 non-AF controls. In the screening AF set, we compared *HCN4* single-nucleotide polymorphism genotypes between AF subjects with TIC (TIC, n=73) and without TIC (non-TIC, n=857). Of 17 *HCN4* gene-tag single-nucleotide polymorphisms, rs7172796, rs2680344, rs7164883, rs11631816, and rs12905211 were significantly associated with TIC. Among them, only rs7164883 was independently associated with TIC after conditional analysis (TIC versus non-TIC: minor allele frequency, 26.0% versus 9.7%; $P=1.62\times10^{-9}$; odds ratio=3.2).

RESULTS: We confirmed this association of *HCN4* single-nucleotide polymorphism rs7164883 with TIC in the replication set (TIC=41 and non-TIC=309; minor allele frequency, 28% versus 9.9%; $P=1.94\times10^{-6}$; odds ratio=3.6). The minor allele frequency of rs7164883 was similar in patients with AF and non-AF controls (11% versus 10.9%; $P=0.908$).

CONCLUSIONS: The *HCN4* gene single-nucleotide polymorphism rs7164883 may be a new genetic marker for TIC in patients with AF.

Key Words: atrial fibrillation ■ cardiomyopathies ■ genetic markers ■ polymorphism, single nucleotide ■ tachycardia

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Tachycardia-induced cardiomyopathy (TIC) is a supraventricular or ventricular tachyarrhythmia that is reversible after control of the underlying arrhythmia. There are no established diagnostic criteria for TIC, but it should be suspected in patients with left ventricular (LV) dysfunction, tachycardia of >100 beats/min, and satisfying the following conditions: (1) no other identified cause of ischemic or nonischemic cardiomyopathy, (2) no enlargement of end-diastolic LV dimensions, and (3) recovery of LV function after control of tachycardia by rate control, cardioversion, or radiofrequency ablation.[1,2]

Many studies have examined alterations in cellular and neurohumoral regulation in TIC,[3,4] but the pathophysiology of TIC is still not completely elucidated, and little is known about patient factors that increase vulnerability to TIC.[5] In particular, no reliable genetic markers of TIC have been identified although one study reported that angiotensin-converting enzyme polymorphisms were associated with increased serum angiotensin-converting enzyme levels and a higher incidence of TIC.[6]

Atrial fibrillation (AF) is the most common cause of TIC in patients without a history of structural heart disease.[7] Poorly controlled ventricular rates may worsen ventricular function, but only a fraction of patients with AF develop TIC. Fourteen genetic loci have been linked to AF in European ancestry groups, and 12 new genetic variants associated with AF have just been reported.[8,9] Among these, only *HCN4* (encoding the cardiac hyperpolarization-activated cyclic nucleotide-gated I_f channel) and *KCNJ5* (encoding potassium voltage-gated channel subfamily J member 5) are cardiac ion channel genes.[8,9] The HCN4 is strongly expressed in the impulse conduction system, including the sinus node (SN) and atrioventricular node, and is known to have a critical function in autonomic control of heart rate.[10–12] Milanesi et al[13] reported progressive development of severe bradycardia and fatal atrioventricular block in cardiac-specific *HCN4* knockout mice. *HCN4* loss-of-function mutations were also reported to promote SN dysfunction.[14] However, an *HCN4* gain-of-function mutation was reported to increase cAMP sensitivity and associate with familial inappropriate sinus tachycardia.[15] Nakashima et al[16] found that *HCN4* overexpression in olfactory receptor neurons increased spontaneous firing rates by sensing basal cAMP levels and exacerbated firing induced by β-adrenergic stimulation. In addition, the I_f inhibitor ivabradine was shown to reduce ventricular rate during AF by inhibiting atrioventricular node conduction.[17]

Collectively, these studies indicate that the cardiac HCN4 channel is essential for normal heart impulse conduction and a critical mediator of heart rate control.[13–17] We thus speculated that *HCN4* is involved in TIC pathogenesis and investigated the association between *HCN4* single-nucleotide polymorphisms (SNPs) and TIC.

METHODS

The data, analytic methods, and study materials will be/have been made available to other researchers for purposes of reproducing the results or replicating the procedure. The Institutional Ethics Committee of the Graduate School of Biomedical Science at Hiroshima University approved all procedures that used human tissue. Written informed consent was obtained from all participants. Detailed methods are available in the Data Supplement.

RESULTS

Clinical Characteristics, Echocardiographic Parameters, and Electrophysiological Study Parameters in TIC and Non-TIC AF Patients of the Screening Set

We diagnosed 73 AF subjects with TIC (age, 61±9 years; 57 men). The ejection fraction improved in all TIC patients at 6 months after RFCA (34.5%±9.1% to 60.2%±6.2%; Figure 1). Recurrence of AF was detected in 5 patients, and they received rate control treatment.

Table 1 summarizes patient clinical characteristics, medications, and echocardiographic findings before RFCA in TIC (n=73) and non-TIC groups (n=857). The frequency of paroxysmal AF was lower in the TIC group than the non-TIC group (42.5% versus 64.6%; P=0.001). Left atrial diameter, left atrial volume index, and LV end-diastolic diameter were larger in the TIC group than the non-TIC group (41.8±8.0 versus 39.2±6.1 mm, P=0.001; 46.6±13.8 versus 41.5±12.7 mL/m^2, P=0.003; 51.0±6.0 versus 47.9±5.0, P=0.0001) while ejection fraction was lower (34.5%±9.1% versus 60.3%±6.0%, $P=3.52\times10^{-13}$). Before RFCA, the usage rate of class I antiarrhythmic drugs was lower in the TIC group than the non-TIC group (11.0% versus 28.1%; P=0.0343) while usage rates of amiodarone (class III) and β-blockers were higher (38.4% versus 10.3%, P=0.0001; 63.1% versus 25.4%, $P=2.8\times10^{-12}$). All antiarrhythmic drugs other than β-blockers were stopped at least 1 half-lives before the procedure in all subjects. The β-blockers were uninterrupted before the procedure only in the TIC group. Other clinical characteristics and echocardiographic findings were similar in both groups.

Association of *HCN4* Tag SNPs With TIC

To screen the entire *HCN4* gene, 17 tag SNPs were genotyped in patients with AF from TIC and non-TIC groups (Table I in the Data Supplement). The SNP rs7172796 upstream of *HCN4* as well as rs2680344, rs7164883, and rs11631816 in *HCN4* intron 1 and rs12905211 in intron 2 were significantly associated with TIC after Bonferroni correction. Among these,

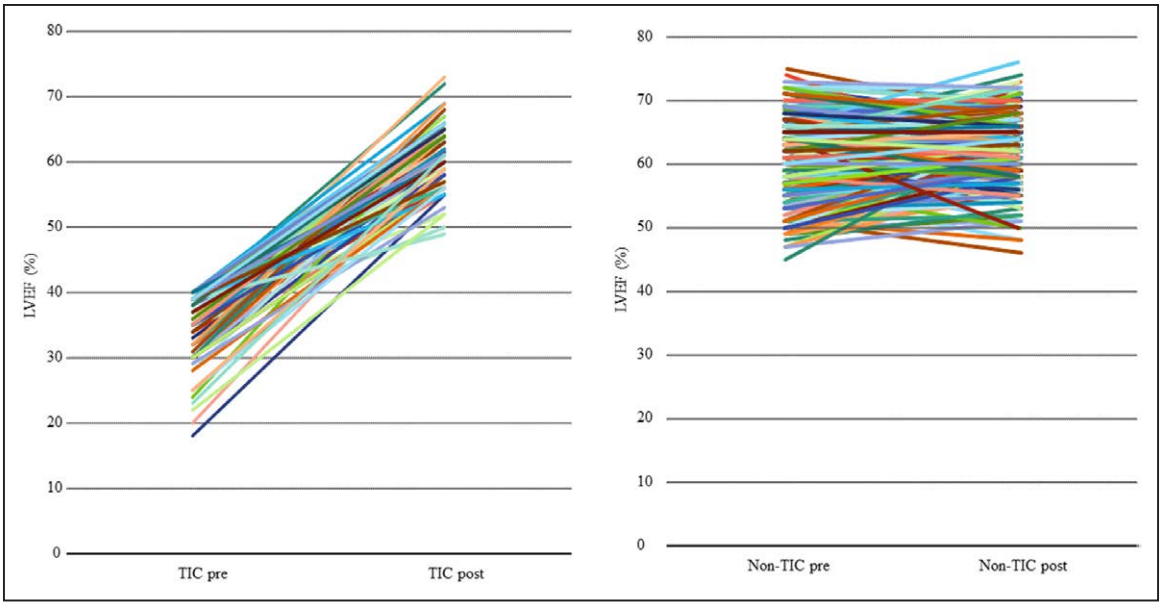

Figure 1. Changes of ejection fraction (EF) before and 6 mo after RFCA.
We diagnosed 73 atrial fibrillation (AF) subjects as tachycardia-induced cardiomyopathy (TIC; age, 61±9 y; male 57 patients). The EF improved in all TIC patients during 6 mo after AF RFCA (34.5%±9.1% to 60.2%±6.2%). LVEF indicates left ventricular ejection fraction; and RFCA, radiofrequency catheter ablation.

HCN4 SNPs, rs7172796, rs2680344, rs7164883, and rs1163181 were closely linked. None of the haplotypes showed stronger associations than the single-marker association of rs7164883 (data not shown).

The genome-wide association study (GWAS) reported[8] that AF-related *HCN4* SNP rs7164883 was the most significantly associated with TIC. We performed conditional analysis accounting for rs7164883. Conditional analysis could not identify additional SNPs in this locus independently associated with TIC (Table II in the Data Supplement).

We downloaded Japanese genotype data (Hap Map JPT, n=104) of SNPs found in the 2-Mb region upstream and downstream of *HCN4* from 1000 genome data and created a map of chromosomal position of each SNP against its r^2 with rs7164883. The SNPs strongly linked with *HCN4* SNP rs7164883 did not extend to other genes on chromosome 15 (Figure 2).

The genotype distributions of HCN4 SNP (rs7164883) in TIC and non-TIC AF patients of both screening and replication sets are shown in Table 2. In the screening set, the minor allele (G) frequency (MAF) of rs7164883 was higher in the TIC group than the non-TIC group (AA/AG/GG: TIC 42/24/7 versus non-TIC 698/151/8; MAF: 26.0% versus 9.7%; allele frequency model: $P=1.6\times10^{-9}$, odds ratio [OR]=3.2, 95% confidence interval, 2.2–4.9; dominant model: $P=1.2\times10^{-6}$, OR=3.2, 95% confidence interval, 2.0–5.3; recessive model: $P=1.8\times10^{-8}$, OR=11.3, 95% confidence interval, 3.9–32.0). We also confirmed this significant association of *HCN4* SNP rs7164883 in the replication set (AA/AG/GG: TIC 20/19/2 versus non-TIC 249/59/81; MAF: 28.0% versus 9.9%; allele frequency model: $P=1.69\times10^{-6}$, OR=3.5; dominant model: $P=5.7\times10^{-6}$, OR=4.4; recessive model: $P=1.14\times10^{-5}$, OR=15.8).

We analyzed the relationships among TIC, total heart rate in 24-hour Holter ECG, and *HCN4* SNP (rs7164883) to

Table 1. Characteristics of AF Patients With or Without TIC

	Total		
	TIC	Non-TIC	*P* Value
No. of patients	73	857	
Clinical characteristics			
Age, y	61±9	61±11	0.9772
Male sex	57 (78.1%)	640 (74.6%)	0.5592
Duration of AF, mo	39±46	53±69	0.1600
AF type: PAF	31 (42.5%)	554 (64.6%)	0.0012
BMI, kg/m^2	23.9±5.5	24.1±3.3	0.7367
Hypertension	43 (58.9%)	445 (51.9%)	0.1876
Medications before RFCA			
Class I AADs	8 (11.0%)	241 (28.1%)	0.0343
Amiodarone	28 (38.4%)	88 (10.3%)	0.0001
Bepridil	10 (13.7%)	103 (12.2%)	0.6332
β-Blocker	46 (63.1%)	218 (25.4%)	2.8×10^{-12}
Echocardiographic findings			
LAD, mm	41.8±8.0	39.2±6.1	0.0010
LAVI, mL/m^2	46.6±13.8	41.5±12.7	0.0025
LVDd, mm	51.0±6.0	47.9±5.0	0.0001
EF	34.5±9.1	60.3±6.0	3.5×10^{-13}

AADs indicates antiarrhythmic drugs; AF, atrial fibrillation; BMI, body mass index; RFCA, radiofrequency catheter ablation; EF, ejection fraction; LAD, left atrial diameter; LAVI, left atrial volume index; LVDd, left ventricular end-diastolic dimension; PAF, paroxysmal AF; and TIC, tachycardia-induced cardiomyopathy.

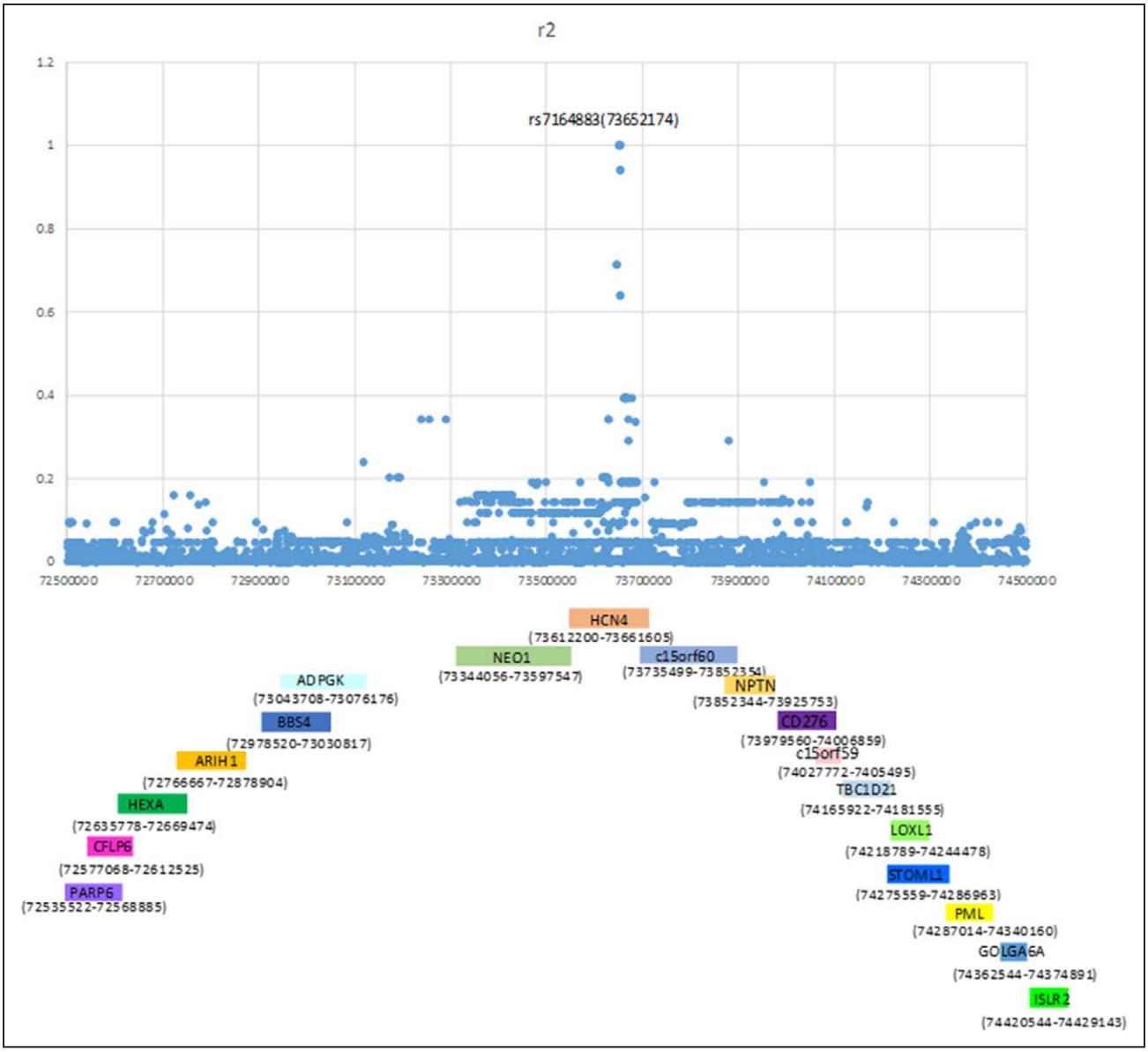

Figure 2. Link of *HCN4* SNP rs7164883 and single-nucleotide polymorphisms (SNPs) in upstream and downstream of *HCN4*.
We downloaded Japanese genotype data of SNPs existed in 2 Mb upstream and downstream of *HCN4* in Chr15 from 1000 genome data and created map by gene positions and r^2. The linkage SNPs with the *HCN4* SNP rs7164883 did not spread to the other genes in chromosome 1.

rule out spurious associations and found that total heart rate and *HCN4* SNP rs7164883 were independently associated with TIC (total heart rate: OR=1.0, P=5.05×10^{-4}; rs7164883: OR=3.31, P=2.50×10^{-8}; Table 3).

Multivariate analysis for TIC using significant factors by univariate analysis, age, and sex was performed and revealed that nonparoxysmal AF and *HCN4* SNP rs7164883 minor allele were independently associated with TIC after adjusting for these confounders (Table 4; OR, 2.15, P=2.86×10^{-3} and OR, 3.05, P=2.16×10^{-7}, respectively).

Finally, we compared these results on rs7164883 in patients with AF (n=930) to non-AF controls (n=1635) and found that the MAF of rs7164883 was similar (MAF, 11% versus 10.9%; P=0.908). Nagelkerke pseudo R^2 of Rs7164883 was calculated in the logistic regression model. This SNP explained 7.9% of the variance for TIC.

DISCUSSION

Various structural and hemodynamic changes have been reported in TIC, including elevated LV filling pressure, impaired LV systolic and diastolic functions, LV cavity dilation, and reduced cardiac output.[18] Depletion of myocardial energy stores, reduced myocardial blood flow, increased oxidative stress, decreased β-adrenoceptor responsiveness, and abnormal calcium handling have been reported to be involved in TIC pathogenesis.[19] AF is the most common cause of TIC while rapid heart rate, loss of atrial contraction, and rhythm

Table 2. The Genotype Distribution of *HCN4* SNP (rs7164883) in Patients With TIC and Non-TIC in Screening and Replication

		Genotype Distribution			HW Test	MAF	Allelic Model (A vs G)		Dominant Model (AG+GG vs AA)		Recessive Model (GG vs AG+AA)	
		AA	AG	GG	*P* Value		*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)
Screening	TIC	42	24	7	0.21	0.260	1.62×10^{-9}	3.26 (2.18–4.88)	1.15×10^{-6}	3.24 (1.98–5.31)	1.75×10^{-8}	2.09 (3.96–32)
	Non-TIC	698	151	8	0.95	0.097						
Replication	TIC	20	19	2	0.34	0.280	1.94×10^{-6}	3.56 (2.06–6.17)	5.71×10^{-6}	4.36 (2.22–8.56)	1.14×10^{-5}	15.8 (1.4–178.2)
	Non-TIC	249	59	1	0.20	0.099						

The results were tested by the χ^2 test and the Cochran-Armitage trend test. Fisher exact test was used instead of the χ^2 test when any number in the proportion was <5. The A allele was considered as the reference allele in the allelic model. CI indicates confidence interval; HW, Hardy-Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; and TIC, tachycardia-induced cardiomyopathy.

irregularity may contribute to progression. However, not all patients with AF tachycardia develop TIC.[3,4,6] The mechanisms underlying development of cardiomyopathy in patients with AF have not been clearly elucidated, and little is known about patient factors that increase vulnerability to TIC. In addition, genetic markers for TIC have not been sufficiently studied.

The *HCN*-gated channels are responsible for the I_f pacemaker current activated by hyperpolarization and are thought to influence SN function in cardiac impulse generation.[19] This channel family comprises 4 members (*HCN1-4*), each composed of 6 transmembrane domains (S1–S6) and a linking site for cAMP in the C-terminal region of the peptide. Of these channel isoforms, *HCN4* is known to have the strongest influence on heart rate.[20] Molecular studies of the conduction system have found that *HCN4* is expressed not only in the SN but also the atrioventricular node.[21,22] Multiple studies have reported that loss-of-function *HCN4* mutations induce SN dysfunction[23,24] and that *HCN4* overexpression induces sinus tachycardia, suggesting that *HCN4* plays a crucial role in heart rate control.[13–16] Heart rate reduction or prevention of AF after cardiac surgery with combined administration of the I_f inhibitor ivabradine and the β-blocker metoprolol was reported to be more effective than treatment with metoprolol alone.[25] In another report, ivabradine significantly decreased ventricular rate during AF compared with placebo in nonparoxysmal AF patients.[26]

We conducted typing of 17 *HCN4* tag SNPs in patients with AF (TIC=73 and non-TIC=857). The some closely linked *HCN4* SNPs on upstream, intron 1, or intron 2 were significantly associated with TIC. Among them, only the *HCN4* SNP rs7164883, G>A was independently associated with TIC by conditional analysis. We then confirmed this significant association in a replication set.

However, the *HCN4* SNP did not reach significance association between Japanese non-AF controls and Japanese AF patients also in our study. A GWAS in European ancestry populations reported that the *HCN4* SNP rs7164883 was associated with AF, but in the Japanese Bio Bank, study showed a negative replication for SNPs in *HCN4*.[8] More recently, another GWAS replication in the Japanese population reported that the *HCN4* SNP rs7164883 was not statistically significantly associated with AF in the Japanese population, indicating population heterogeneity in genetics of AF.[27] In our study, the minor (G) allele frequency of rs7164883 was 10.9% in non-AF controls and 11.0% in total AF patient cohort, which was consistent with the frequencies reported in the Hap Map Project (11.6% in Japanese and 8.1% in East Asian populations). The *HCN4* SNP rs7164883 may be a genetic marker of TIC rather than AF in Japanese.

A 2013 GWAS reported that the minor allele of rs4489968 in *HCN4* increased heart rate.[28] We genotyped 21 heart rate-associated SNPs, but none were associated with TIC after Bonferroni correction (data not shown). In our study, total heart rate/d and *HCN4* SNP rs7164883 were independently associated with TIC.

The mechanisms by which the intron SNPs of *HCN4* regulate *HCN4* function or expression are not clear. The presence of a myocyte enhancer factor 2C–binding site has been reported in intron 1 of Rat *HCN4*.[29,30] The myocyte enhancer factor 2C–binding site (Ch15:73658006-73658022) also exists in intron 1 of human *HCN4* gene, but it is not perfectly matched with the rs7164883 site (Ch15: 73652174). According to the JASPER[2018] (http://jaspar.genereg.net/) CORE, upstream transcription factor (USF-1, Ch15:73652295-73652584) 1 and USF-2 (Ch15:73652441-73652640) exist in intron 1 of *HCN4* and the rs7164883 site is included in the USF site. USF is a family of transcription factors characterized by a highly conserved basic-helix-loop-helix leucine zipper (bHLH-zip) DNA-binding domain. The USF-1 and USF-2 were reported as transcriptional repressor and negative regulator of prolif-

Table 3. Relationship Between TIC and Total HR/HCN4 SNP (rs7164883)

Parameters	Odds Ratio	95% CI	*P* Value
Total heart rate, beats/d	1.02	1.01–1.03	1.95×10^{-4}
HCN4 SNP (rs7164883)	3.27	2.19–4.87	5.92×10^{-9}

CI indicates confidence interval; HR, heart rate; SNP, single-nucleotide polymorphism; and TIC, tachycardia-induced cardiomyopathy.

Table 4. Multivariate Analysis of TIC and Non-TIC Groups

	TIC	Non-TIC	Odds Ratio	95% CI	*P* Value
No. of patients	73	857			
Age	61±9	61±11	1.00	0.97–1.03	0.92
Male sex	57 (78.1%)	640 (74.6%)	1.36	0.72–2.56	0.34
AF type: non-PAF	42 (57.5%)	303 (35.4%)	2.15	1.30–3.56	2.86×10^{-3}
HCN4 SNP (rs7164883) additive model (AA/AG/GG)	42/24/7	698/151/8	3.05	2.00–4.65	2.16×10^{-7}

AF indicates atrial fibrillation; CI, confidence interval; PAF, paroxysmal AF; and TIC, tachycardia-induced cardiomyopathy.

eration.[31,32] The binding affinity of transcription factors may be modulated by rs7164883. Further functional analyses are desirable to reveal that allelic change of the SNP modulate binding affinity between the SNP and some transcription factors.

The *HCN4* (I_f channel) mediates protein kinase A–dependent phosphorylation of sarcoplasmic reticular, mitochondrial, and ion channel proteins, which in turn regulates Ca^{2+} cycling and drives generation of spontaneous rhythmic action potentials.[33] Recently, Mueller et al[34] reported that TIC is characterized by changes in cardiomyocyte and mitochondrial morphology. Milano et al,[35] who first found that mutations in *HCN4* are associated with structural abnormalities of the myocardium, linked *HCN4* mutations to LV noncompanction cardiomyopathy.[36]

We queried expression quantitative trait locus data acquired from 264 human left atrial appendage samples available in the Genotype-Tissue Expression website (http://gtexportal.org; V7 release) for *cis*-expression quantitative trait locus effects of *HCN4* rs7164883. We analyzed genes located within 1 Mb upstream and downstream of *HCN4* rs7164883 but found no genes, including *HCN4*, for which expression was significantly associated with rs7164883. However, these expression quantitative trait locus expression data were not from the target tissue of interest, and we could not find any publicly available expression quantitative trait locus-database using optimal tissue, therefore we further have to evaluate its possible association with other gene.

We think that *HCN4* SNPs are potential genetic risk markers for TIC in patients with AF. Stratification of TIC risk is important because it can facilitate early therapeutic intervention (eg, stricter heart rate control or early rhythm control) to prevent heart failure in patients with an *HCN4* minor allele. Differential diagnosis of TIC and dilated cardiomyopathy with AF tachycardia is also difficult but critical for selection of patient treatment strategy. Greater attention should be paid to recurrence of heart failure in patients with the *HCN4* minor allele. Ivabradine may be an effect treatment for TIC.

In conclusion, the *HCN4* SNP rs7164883 independently increases risk of TIC. Furthermore, *HCN4* SNP may be a reliable genetic marker for TIC risk. Persistent AF patients with diabetes mellitus and the *HCN4* SNP rs7164883 minor allele may be at particularly high risk and so should be considered for early therapeutic intervention.

Study Limitations

Diagnosis of TIC is difficult because LV ejection fraction is dependent on heart rate during AF. Thus, patients susceptible to TIC may have been selected as control patients if persistent AF and ventricular rate were well controlled. In addition, enrollment of patients with AF treated by RFCA may introduce selection bias as this population does not represent the entire AF population. Specifically, the proportion of AF patients with TIC may be higher than in the general AF population because of selection bias. We did not perform GWAS analysis and so could not elucidate precise relationship with nearby genes. Also, mechanisms by which rs7164883 regulates HCN4 function or expression are not clear. TIC accounted for ≈10% of the total subjects, so the total number of cases was small. We, therefore, must validate the association between TIC and *HCN4* SNP rs7164883 in a larger sample of cases and controls. Nonetheless, *HCN4* SNPs are promising genetic markers for TIC and may be useful for selecting the most appropriate therapeutic intervention.

ARTICLE INFORMATION

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Disclosures

None.

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HCN4 Gene Polymorphisms Are Associated With Occurrence of Tachycardia-Induced Cardiomyopathy in Patients With Atrial Fibrillation

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SUPPLEMENTAL MATERIAL

Supplementary Table 1. Relationship of HCN4 SNPs in TIC with AF

SNP[a]	Position	Region	Allele	TIC (N=73)			non-TIC (N=857)			OR	(95%CI)	P value[b]	HWE[c]
			1/2	11	12	22	11	12	22				
rs7172796	73661437	upstream	T vs G	60	12	1	811	43	3	3.604	(1.939 - 6.698)	1.59E-05	0.274
rs2680344	73653235	intron 1	A vs G	40	26	7	660	186	10	2.759	(1.865 - 4.082)	1.46E-07	0.838
rs7164883	73651924	intron 1	A vs G	42	24	7	698	151	8	3.259	(2.179 - 4.875)	1.62E-09	0.216
rs477377	73649343	intron 1	C vs T	71	2	0	815	40	2	1.897	(0.058 - 3.226)	0.58	0.907
rs11631816	73646344	intron 1	G vs A	49	18	6	717	134	6	2.778	(1.796 - 4.295)	1.87E-06	0.160
rs512943	73632619	intron 2	C vs T	64	10	1	709	136	12	1.184	(0.642 - 2.183)	0.59	0.105
rs3784807	73631580	intron 2	G vs A	30	32	11	255	409	193	1.474	(1.039 - 2.090)	0.03	0.415
rs497859	73629491	intron 2	A vs G	63	9	1	713	131	13	1.238	(0.655 - 2.338)	0.51	0.325
rs2623997	73628464	intron 2	T vs C	10	36	27	108	369	380	1.201	(0.848 - 1.701)	0.30	0.273
rs8030574	73627964	intron 2	A vs C	26	32	15	381	381	95	0.677	(0.48 - 0.954)	0.03	0.738
rs12905211	73627918	intron 2	C vs T	29	27	15	470	322	60	1.915	(1.346 - 2.724)	2.51E-04	0.207
rs2623998	73627836	intron 2	T vs C	10	38	27	110	373	383	1.211	(0.859 - 1.707)	0.27	0.286
rs548525	73627621	intron 2	C vs G	0	34	39	20	221	616	1.690	(1.126 - 2.536)	0.01	0.404
rs546564	73627520	intron 2	A vs C	53	20	0	631	205	21	1.061	(0.649 - 1.733)	0.81	0.624
rs3784801	73623532	intron 3	A vs G	4	34	35	80	373	404	1.118	(0.77 - 1.622)	0.56	0.487
rs529004	73614584	exon 8	T vs C	1	13	59	7	148	698	1.933	(0.464 - 8.055)	0.58	0.864
rs3743496	73614158	exon 8	G vs T	14	45	14	184	412	261	1.197	(0.854 - 1.679)	0.30	0.751

TIC: tachycardia induced cardiomyopathy, SNPs: single nucleotide polymorphisms, AF: atrial fibrillation, OR: odds ratio

The odds ratios are calculated for reference alleles (allele1).

a: Tagging-SNPs other thanrs7164883 were selected based on the selection criteria of r 2 > 0.8 and minor allele frequency > 0.01in the Hap Map-JPT population.

b: chi-square test P value in allele frequency model (uncorrected)

c: Hardy-Weinberg equilibrium tests in control subjects

Supplementary Table 2. Conditional analysis accounting for rs7164883

SNP	Position	Region	Allele 1/2	OR	Single marker P value and OR (95%CI)	Association P value and OR (95%CI) of Conditioning SNP (rs7164883)	Conditional P value and OR (95%CI) of Tested SNPs
rs7164883	73651924	intron 1	A vs G	3.259	$1.62x10^{-9}$ (2.18-4.88)		
rs7172796	73661437	upstream	T vs G	3.604	$1.59x10^{-5}$ (1.94-6.70)	$3.55x10^{-7}$, 2.96 (1.95-4.49)	0.952, 1.02 (0.47-2.22)
rs2680344	73653235	intron 1	A vs G	2.759	$1.46x10^{-7}$ (1.87-4.08)	$5.79x10^{-6}$, 2.84 (1.81-4.45)	0.629, 1.13 (0.69-1.82)
rs11631816	73646344	intron 1	G vs A	2.778	$1.87x10^{-6}$ (1.80-4.30)	$4.82x10^{-6}$, 2.81 (1.81-4.38)	0.704, 1.18 (0.70-1.98)
rs12905211	73627918	intron 2	C vs T	1.915	$2.51x10^{-4}$ (1.35-2.72)	$1.73x10^{-7}$, 3.03 (2.00-4.58)	0.610, 0.91 (0.62-1.32)

Methods

Participants

We enrolled 953 Japanese AF patients who underwent catheter ablation at Hiroshima University Hospital between November 2009 and August 2016 as candidates. We excluded those with severe valvular disease (n = 1), congenital heart disease (n = 2), hypertrophic cardiomyopathy (n = 10), dilated cardiomyopathy (n = 1), angina pectoris (n = 6), and old myocardial infarction (n = 3). Ultimately, we included 930 AF patients (734 men and 196 women, 61 ± 12 years) in the screening cohort. We also enrolled 350 Japanese AF patients (240 men and 110 women, 66 ± 10 years) who underwent catheter ablation at Hiroshima University Hospital or Japanese Red Cross Nagoya Daini Hospital between September 2016 and June 2017 for replication. All patients diagnosed with TIC (TIC group) had acute cardiac decompensation with left ventricular ejection fraction (LVEF) of <40% requiring hospitalization and AF tachycardia >140 beats/min, but no underlying structural heart disease, including congenital heart disease, valvular heart disease, cardiomyopathy, or ischemic heart disease. Further, they also exhibited improved clinical condition and LVEF within 6 months of heart rate control or sinus conversion after radiofrequency catheter ablation (RFCA).[3,4, 37] Some patients in the TIC group were treated during prior hospitalization because of decompensated heart failure. They had received medication and/or underwent electrical cardioversion during prior hospitalization and were

thereafter admitted to our hospital for AF ablation. Atrial fibrillation patents without TIC (non-TIC group) underwent RFCA during the same period. We also enrolled 1,635 non-AF controls (794 males and 841 females, mean age 54 ± 18 years) to compare the minor allele frequency of *HCN4* SNPs between AF patients and non-AF controls. The non-AF controls are all Japanese and they are volunteers collected at the Hiroshima University. Those with cardiac diseases, hyperthyroidism, severe liver and kidney dysfunction and AF were excluded by interview.

The Institutional Ethics Committee of the Graduate School of Biomedical Science at Hiroshima University approved all procedures that used human tissue. Written informed consent was obtained from all participants.

Echocardiography

Transthoracic echocardiography was performed within 24 h before RFCA using a commercially available ultrasonography system (iE33; Philips Medical Systems, Best, The Netherlands) to exclude structural heart disease. In TIC patients, transthoracic echocardiography was performed while in hospital for decompensation, was repeated on admission for the ablation procedure, and repeated again 6 months after ablation. Echocardiography measurements were taken following the recommendations of the American Society of Echocardiography.[38]

Holter monitoring

All AF subjects in the screening cohort set underwent 24-hour Holter monitoring within one week before RFCA although they had already received rate control therapy. Holter recordings were performed using a standard unit (RAC-2512 or RAC-2503, NIHON KOHDEN, Tokyo, Japan) and automatically analyzed by a PC-based Holter system (DSC-5500, NIHON KOHDEN, Tokyo, Japan).

Radiofrequency Ablation (RFCA) of AF

The AF subjects were treated with antiarrhythmic drugs (AADs) before the RFCA. The AADs were stopped at least one half-lives before the procedure. As an exception, theβ-blocker administration remained uninterrupted throughout the periprocedural period only in patients with TIC to avoid recurrence of heart failure.

Extensive encircling pulmonary vein isolation (EEPVI) and bidirectional cavotricuspid isthmus blocks were performed as previously reported.[39] If AF persisted after completion of EEPVI, cardioversion was performed to restore sinus rhythm. Successful PV isolation was defined as the loss of all PV potentials (entrance block) and failure to capture the left atrium (LA) when pacing from the PV (exit block) using circular multipolar mapping catheters.

Tag SNP selection

Tagging SNPs were selected for the *HCN4* (Chr 15) region spanning approximately 49.4 kb, from approximately 5 kb upstream of the transcription start site to 5 kb downstream of the 3'-untranslated region from Phase III Hapmap Japanese data with a minor allele frequency >0.05 for the Japanese population using SNPinfo web server (http://www.niefs.nih.gov/snpinfo).

HCN4 genotyping

Peripheral blood was obtained and genomic DNA was extracted from leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the standard protocol. We genotyped 17 *HCN4* SNPs in all participants using the Taq Man or Invader assay as previously described.[40]

We examined whether *HCN4* haplotypes show more significant associations with TIC than single-marker analysis. We removed SNPs in strong linkage disequilibrium with an r-squared value>0.8. We constructed haploblocks of *HCN4* haplotypes from tagging- *HCN4* SNPs based on the confidence interval using the Haploview 3.2 software.

Statistical analysis

Normally distributed continuous variables were reported as mean ± standard deviation (SD). Between-group differences were compared using the Welch's t test. Multivariable analysis was performed using a logistic regression analysis. To test the additive genetic effect for the minor

allele, the common alleles were coded as 0 (reference), heterozygotes were coded as 1, and minor allele homozygotes were coded as 2. Deviation from the Hardy–Weinberg equilibrium was tested in cases and controls with the chi-square test. Fisher's exact test was used instead of the chi-square test when any number in the proportion was less than five. *P*-values <0.05 was considered significant. The Bonferroni-corrected P value threshold for discovery was P < 0.0029 (0.05/17). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the reference allele or genotype. To test the genetic relationships between cases and controls, we used the chi-square test and the Cochran–Armitage trend test. We performed conditional analysis to determine which SNPs were independently associated with TIC after adjusting for the most significantly associated SNP. Statistical analyses were conducted using R3.3.1 and the JMP statistical package (version 12, SAS Institute, Cary, NC).

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