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ORIGINAL ARTICLE

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Bifid T waves on the ECG and genetic variation in *calcium channel voltage-dependent beta 2 subunit* gene (*CACNB2*) in acute Kawasaki disease

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Abstract

Background: We previously described the association of genetic variants in calcium channel genes and susceptibility to Kawasaki disease (KD), an acute, self-limited vasculitis, and the most common cause of acquired cardiac disease in children. Abnormal repolarization of cardiomyocytes and changes in T wave morphology have been reported in KD but have not been studied systematically.

Methods: We analyzed acute and convalescent ECG T wave morphology in two independent cohorts of KD subjects and studied the association between bifid T waves and genetic variants in previously reported genes with SNVs associated with cardiac repolarization.

Results: Bifid T waves in limb leads were identified in 24% and 27% of two independent cohorts of acute KD subjects. *Calcium channel voltage-dependent beta 2 subunit* gene (*CACNB2*) (rs1409207) showed association with bifid T waves in both cohorts (nominal P = .04 and P = .0003, respectively). This CACNB2 polymorphism also showed association with KD susceptibility in a previously published KD genome wide association study data (nominal P = .009).

Conclusion: This genotype/phenotype association study uncovered a variant in *CACNB2* that may be associated with both KD susceptibility and bifid T waves, a novel signature of altered myocardial repolarization. The present study combined with published reports suggests that genetic variants in calcium channels and intracellular calcium signaling play a prominent role in shaping susceptibility to KD.

KEYWORDS
bifid T wave, calcium channel, ECG, Kawasaki disease

1 | INTRODUCTION

Linking disease phenotypes with genetic variants is essential to understanding how changes in DNA sequence shape susceptibility to complex genetic diseases. Such analyses can also uncover critical pathways implicated in disease pathogenesis that may lead to identification of therapeutic targets or diagnostic biomarkers. Kawasaki disease (KD) is a pediatric vasculitis with complex genetics whose etiology is unknown. Coronary artery aneurysms occur in 25% of untreated children and 5%-7% of children treated with intravenous

immunoglobulin.[1] Varying degrees of myocardial inflammation also accompany the vasculitis during the acute illness.[2]

Host genetics play an important role in KD susceptibility and disease outcome. Variants in calcium signaling pathway genes, such as *solute carrier family 8, member 1 (SLC8A1)*, which influence KD susceptibility were discovered using pathway analysis followed by gene stability selection in the dataset from a KD genome wide association study (GWAS).[3] Because genetic variants in these calcium signaling pathway genes have been associated with abnormalities of myocardial repolarization[4-6] and QT intervals in other conditions, we performed a pilot study to analyze the electrocardiogram (ECG) in KD subjects carrying the validated risk alleles in *SLC8A1*.[4,7]

In the pilot study, we noted bifid T waves in the limb leads in a subset of subjects. In the present study, using cohorts with both genotypes and acute ECG tracings, we studied the association between bifid T waves and genetic variants in genes known to be associated with cardiac repolarization. The results were tested in an independent replication cohort with both genotypes and ECG tracings. The replicated SNV in CACNB2 associated with bifid T-waves was also nominally associated with KD susceptibility.

2 | METHODS

2.1 | Subjects

All KD subjects met AHA criteria for complete or incomplete KD according to current guidelines.[8] Detailed information regarding patient recruitment is described in the Supplementary Methods.

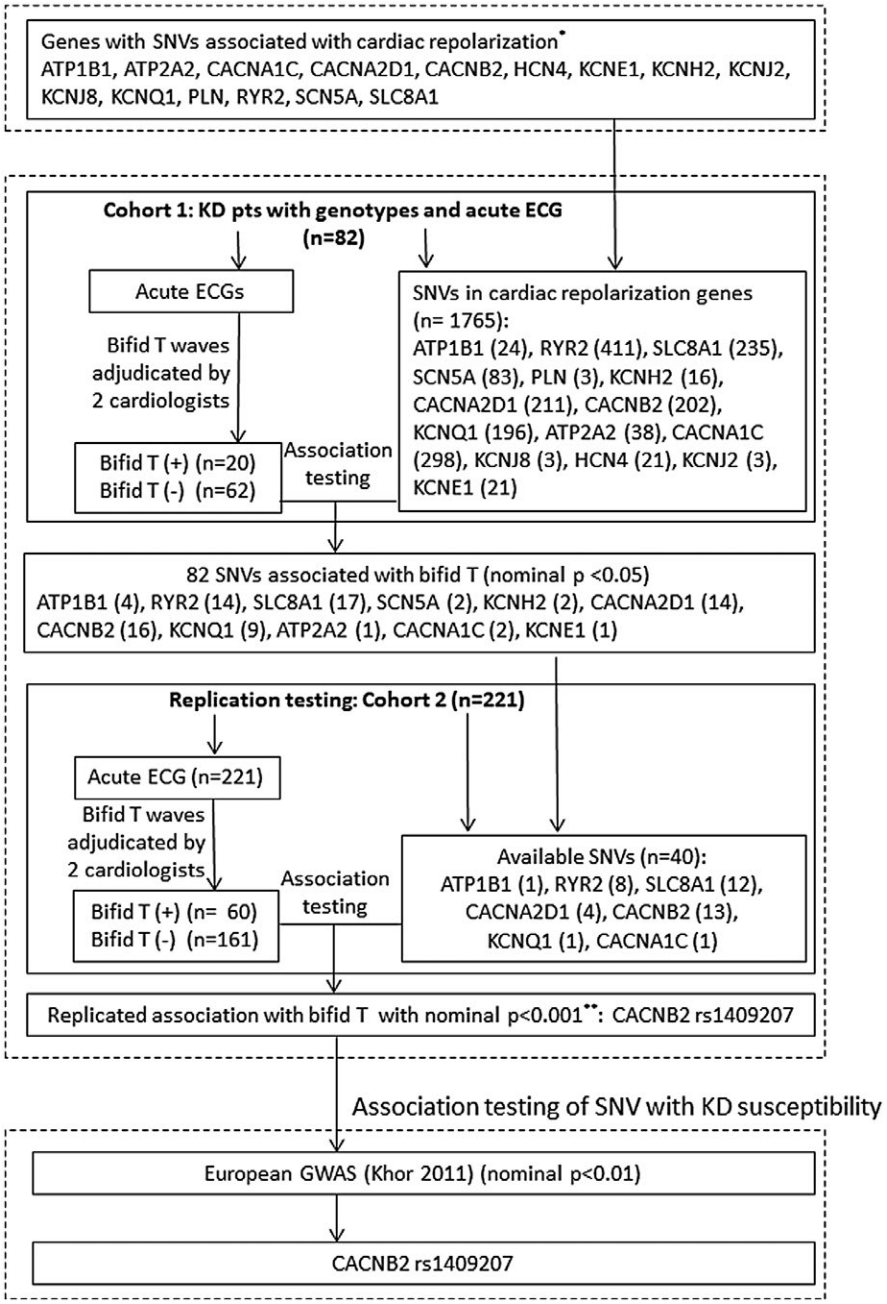

FIGURE 1 Study work flow*. *ATP1B1, ATPase Na+/K+ transporting subunit beta 1; ATP2A2, ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2; CACNA1C, calcium channel voltage-dependent L-type alpha 1C subunit; CACNA2D3, calcium channel voltage-dependent alpha 2/delta subunit 3; CACNB2, calcium channel voltage-dependent beta 2 subunit; HCN4, hyperpolarization activated cyclic nucleotide gated potassium channel 4; KCNE1, potassium voltage-gated channel subfamily E regulatory subunit 1; KCNH2, potassium voltage-gated channel subfamily H member 2; KCNJ2, potassium voltage-gated channel subfamily J member 2; KCNJ8, potassium voltage-gated channel subfamily J member 8; KCNQ1, potassium voltage-gated channel subfamily Q member 1; PLN, phospholamban; RYR2, ryanodine receptor 2; SCN5A, sodium voltage-gated channel alpha subunit 5; SLC8A1, solute carrier family 8 member A1*; GWAS, genome-wide association studies; SNV, single nucleotide variant. *Refs. 3-5; **Bonferroni multiple comparison cutoff .05/40 = .00125, numbers of SNVs in each gene shown in ()

The two independent, multi-ethnic cohorts, Cohort 1 (n = 82) and Cohort 2 (n = 221), included KD subjects with both genotypes and adequate quality ECGs obtained during the acute phase of their illness (Figure 1 and Table 1). The Institutional Review Board of the University of California San Diego approved this study, and parent consent and assent as appropriate were obtained from parents and participants.

2.2 | ECG analysis

ECGs (12 leads) were obtained during the acute phase (illness day 3-17) from subjects in Cohorts 1 and 2. ECGs from the late convalescent phase (interval from the onset: 1-3 years) were also evaluated in a subset of Cohort 2 subjects (n = 31) with a bifid T wave noted on the acute phase ECG. Measurements were made using a hand caliper for five ECG variables on each tracing: PR interval, QRS duration, corrected QT interval using Bazett's method (QTc), QT dispersion (QTd), and peak-to-end interval of the T wave (Tp-e). T wave morphology was also evaluated. We defined a bifid T wave as an indented T wave resulting in two visible peaks in either Lead I or II. Two pediatric cardiologists (JCP and JO) independently read ECGs and evaluated T wave morphology in Cohort 1. For Cohort 2, the presence or absence of a bifid T wave was independently assessed by the two pediatric cardiologists (JCP and JO) for both the initial ECG and late convalescent ECGs when bifid T was noted on the acute study. Discordant interpretations for both cohorts were adjudicated by a third pediatric cardiologist (MW). All ECG readers were blinded to the genotypes of the subjects.

2.3 | Echocardiography and genotyping

Detailed information is described in the Supplementary Methods.

2.4 | Statistical analysis

Mann-Whitney *U* test was used for two-group comparisons of continuous variables. Chi-square test and Fisher's exact test were used for categorical variables and allele frequency analysis. Cohen's Kappa was used to measure interrater agreement for bifid T wave detection. *P* values < .05 were considered statistically significant.

3 | RESULTS

3.1 | T wave variations in acute KD

Cohort 1 consisted of 82 KD subjects for whom genotypes and acute ECGs were available (Table 1). A single pediatric electrophysiologist (JCP) evaluated the PR, QRS, QTc, QTd, and Tp-e intervals and T wave morphology in the acute ECG and no interval abnormalities were noted (Table 1). Several variant T wave morphologies were observed. The unique finding of bifid T waves in the limb leads emerged from this analysis (Figure 2). Only subjects with true bifid T waves (n = 21 (26%)) were used in the genetic studies. Other T-wave morphologies (flat peak in 10 (12%), inverted in 3 (4%), early repolarization in 5 (6%), and late peak in 5 (6%)) were noted but were not included for the subsequent analysis. Although bifid T waves have been reported in healthy children in leads V2 and V3 with normal QTc intervals, the finding of bifid T waves in the limb leads appeared to be a novel finding.[9] The acute ECGs from Cohort 1 were then independently assessed for bifid T wave morphology by a second pediatric cardiologist (JO) blinded to the assessment of the first cardiologist. Three (4%) ECGs had discordant readings between the two independent readers. The Cohen's Kappa values for ECG interrater agreements with their 95% confidence intervals were .9 [.78, 1]. A third pediatric cardiologist (MW) adjudicated one of the three ECGs as having a bifid T wave. Thus, a total of 20/82 (24%) ECGs from acute KD subjects had a bifid T wave (Figure 1). All 20 patients also had bifid T waves in the precordial leads V5 and 6, as would be expected because these leads are electrocardiographically similar to the limb leads I and II.

3.2 | SNVs associated with bifid T waves

Subtle variations in the time course of repolarization of cardiomyocytes contribute to the morphology of the T wave. Genetic variants associated with abnormalities of myocardial repolarization have been reported.[4-6] We studied the association between bifid T waves and genetic variants in genes with SNVs reported to influence cardiac repolarization in Cohort 1 (1765 SNVs in 15 genes: *ATP1B1*, *ATP2A2*, *CACNA1C*, *CACNA2D3*, *CACNB2*, *HCN4*, *KCNE1*, *KCNH2*, *KCNJ2*, *KCNJ8*, *KCNQ1*, *PLN*, *RYR2*, *SCN5A*, *SLC8A1*) (Figure 1). Overall, 82 SNVs in 11 genes were associated with bifid T waves (nominal $P <$ .05) (Figure 1 and Table S1).

3.3 | Validation of SNVs associated with KD susceptibility and bifid T waves

Cohort 2 consisted of 221 KD subjects for whom both genotypes and acute ECGs were available. ECGs were evaluated by a single pediatric cardiologist (JO) for intervals and bifid T waves. No interval abnormalities were identified and 54/221 (24%) ECGs had a bifid T wave. Independent interpretation by the pediatric electrophysiologist (JCP) yielded discordant findings in 14/221 (6%) ECGs. The Cohen's Kappa value for ECG interrater agreement with the 95% confidence interval was .83 [.75, .92]. Of these 14 discrepant interpretations, 11/14 (79%) ECGs were adjudicated as having bifid T waves by a third independent reader (MW). Thus, 60/221 (27%) acute ECGs were judged as having a bifid T wave.

Genotyping for Cohort 2 was performed on a different platform from Cohort 1. Therefore, only 40 of the 82 associated SNVs from Cohort 1 were available for validation testing in Cohort 2 (Figure 1 and Table S1). Only one SNV in *CACNB2* was replicated following Bonferroni correction for multiple comparisons (nominal $P <$ .001) (G allele of rs1409207, P = .0003, OR 2.4, 95% CI 1.5-3.8) (Table 2). This variant is located in a large (250 kb) intron approximately 20kb from the splice acceptor site. The risk allele frequency for this SNV

TABLE 1 Demographic and clinical characteristics of Cohorts 1 and 2 stratified by the presence or absence of bifid T waves on the acute ECG

	Cohort 1			Cohort 2		
	Bifid T (+) (n = 20)	Bifid T (-) (n = 62)	*P* value	Bifid T (+) (n = 60)	Bifid T (-) (n = 161)	*P* value*
Age at the onset, years	1.8 (1.0-2.2)	4.1 (1.8-5.3)	**<.0001**	1.8 (.7-2.7)	3.8 (2.4-5.6)	**<.0001**
Sex, male, n (%)	15 (75%)	31 (49%)	NS	34 (57%)	100 (62%)	NS
Illness day, days	7 (6-7)	6 (5-9)	NS	7 (5-8)	6 (5-8)	NS
Ethnicity, n (%)						
Asian	5 (25%)	10 (16%)	NS	13 (22%)	22 (13%)	NS
Black	0 (0%)	5 (8%)		3 (5%)	8 (5%)	
White	3 (15%)	16 (25%)		8 (13%)	38 (24%)	
Hispanic	6 (30%)	14 (22%)		23 (38%)	61 (38%)	
More than one race	6 (30%)	18 (29%)		13 (22%)	31 (19%)	
IVIG resistance, n (%)	3 (15%)	15 (24%)	NS	4 (6%)	18 (11%)	NS
Coronary artery status, n (%)						
Z max <2.5	11 (55%)	47 (75%)	NS	40 (67%)	122 (76%)	NS
2.5-10	8 (40%)	14 (22%)		18 (30%)	36 (22%)	
>10	1 (5%)	2 (3%)		1 (3%)	3 (2%)	
Coronary artery Z max	2.3 (1.6-3.6)	1.7 (1.2-2.5)	NS	1.9 (1.5-2.8)	1.5 (.9-2.4)	NS
Pretreatment laboratory data						
WBC (× 10^3/ul)	16.1 (13.5-18.6)	12.9 (10.4-16.7)	NS	14.6 (10.6-18.4)	13.1 (9.6-17.4)	NS
Platelet count (x 10^3/ul)	409 (347-452)	391 (312-469)	NS	347 (296-407)	351 (274-447)	NS
ESR (mm/h)	57 (44-77)	59 (45-75)	NS	61 (40-72)	55 (41-75)	NS
CRP (mg/dl)	7.0 (4.9-8.7)	6.9 (4.1-16.4)	NS	6.2 (3.7-15.3)	6.6 (4.2-16.2)	NS
Baseline ECGs						
HR (bpm)	NA	NA	-	117 (107-150)	114 (98-130)	**.014**
PR (ms)	120 (110-125)	120 (110-140)	NS	125 (114-134)	126 (114-140)	NS
QRS (ms)	63 (60-70)	70 (60-80)	NS	70 (62-74)	72 (64-80)	**.001**
QTc (ms)	400 (396-411)	404 (400-411)	NS	401 (381-410)	394 (378-408)	NS
QTd (ms)	25 (20-30)	30 (20-30)	NS	20 (20-28)	20 (20-40)	NS
Tp-e (ms)	50 (40-63)	60 (40-70)	NS	50 (40-60)	60 (40-60)	NS
Baseline echocardiography						
LVEF (%)	69 (63-73)	64 (60-68)	NS	68 (64-73)	67 (63-72)	NS
LAD Z score	1.4 (.5-2.2)	1.1 (.6-1.9)	NS	1.6 (1.0-2.4)	1.0 (.4-1.9)	**.044**
RCA Z score	1.1 (.5-2.5)	.8 (.1-1.8)	NS	1.2 (.5-2.0)	.8 (−.1 to −1.4)	**.034**

Continuous variables shown as median, interquartile range. IVIG resistance: persistent or recrudescent fever (oral or rectal T ≥ 38.0˚C) at least 36 h to 7 days following the end of the IVIG infusion.

Abbreviations: CRP, C-reactive protein; ECG, electrocardiogram; ESR, erythrocyte sedimentation rate; HR, heart rate; IVIG, intravenous immunoglobulin; LAD, left anterior descending coronary artery; LVEF, left ventricular ejection fraction; NA, not applicable; NS, not significant; PR, PR interval; QRS, QRS duration; QTc, corrected QT interval; QTd; QT dispersion; RCA, right coronary artery; Tp-e, peak to end interval of the T wave; WBC, white blood cells.

*P values by Mann-Whitney U test for continuous variables and chi-square test for categorical variables. Significant *P* values are bolded.

was higher in individuals of Asian compared to European descent (rs1409207: .94 for Asian and .61 for European descent). Although Asians comprised 15/83 (18%) and 35/221 (16%) subjects in Cohorts 1 and 2, respectively, they made up 5/20 (25%) and 13/60 (22%) subjects with a bifid T. Thus, there was a trend toward overrepresentation of bifid T waves among Asian subjects. The association between the risk allele of rs1409207 and bifid T waves among White (n = 46) and Asian (n=35) subjects in Cohort 2 showed a similar trend, but did not reach significance with these small cohorts (Table 2). Thus, it does not appear that the Asian subjects were solely responsible for driving the association with bifid T waves.

3.4 | Association of CACNB2 SNV with KD susceptibility and bifid T waves

We accessed published European descent KD GWAS data to test the association of CACNB2 rs1409207 with KD susceptibility (Figure 1 and Table 2) and found association (*P* = .009, OR 1.21) (Table 2).

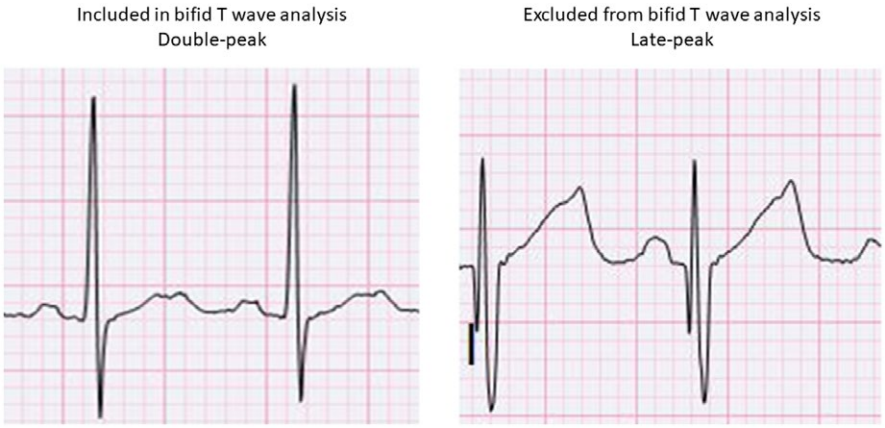

FIGURE 2 Representative variation in bifid T wave morphology seen in lead I in ECGs from acute KD subjects. Only subjects with an acute ECG showing the double-peak T wave in Leads I or II were included for the bifid T wave analysis and association with risk alleles in calcium channel genes

3.5 | Longitudinal assessment of bifid T waves

To determine whether the bifid T waves were associated only with the acute inflammatory phase of KD, we examined ECGs from the late convalescent phase (1-3 years postonset of KD) in 31 subjects from Cohort 2 who had bifid T waves on their acute ECG. Bifid T waves were identified in only 5/31 (16%) subjects (Figure 3). The interval between KD onset and the convalescent ECG had no relationship to the presence or absence of bifid T waves in the convalescent study. However, age at KD onset and age at the time of the late convalescent ECG were significantly younger in subjects with bifid T waves (median age at acute ECG: .4 (IQR .3-.6) years vs 2.1 (1.5-2.5) years; median age at convalescent ECG: 1.5 (1.2-1.8) years vs 3.3 (3.0-4.0) years, for subjects with and without bifid T waves, respectively, $P < .05$ for both comparisons). Age at the time of the late convalescent ECG was <2 years in 4/5 subjects with bifid T waves compared to only 3/26 subjects without bifid T waves. There were no significant differences in risk allele frequency for the SNV in *CACNB2* between subjects with and without bifid T waves in the late convalescent ECG (data not shown).

3.6 | Characteristics of KD subjects with or without bifid T wave

We compared demographic and clinical characteristics between subjects with and without bifid T waves. In both Cohorts 1 and 2, the median age at KD onset was younger in the subjects with bifid T waves compared to those without bifid T (Cohort 1: 1.8 vs 4.1 years, and Cohort 2: 1.8 vs 3.8 years, respectively, both $P < .0001$) (Table 1). However, the risk allele in *CACNB2* SNV remained significantly associated with bifid T waves even in older subjects (Table 3). There were no significant differences in clinical laboratory values between two groups. Both LAD and RCA Z scores were higher in subjects with bifid T (Table 1).

4 | DISCUSSION

Bifid T waves consistent with a short delay in cardiomyocyte repolarization were associated with a risk allele in a calcium channel gene that influences KD susceptibility. The nominal association of genetic variation in *CACN2B* with bifid T waves was detected in two independent cohorts. While the link between variants in a calcium channel gene and cardiomyocyte repolarization is clear, the biologic effect influencing KD susceptibility is less straightforward. Bifid T waves may be a marker for changes in calcium flux, but this variation may have its greatest influence on disease pathogenesis by affecting calcium flux in immune cells that mediate inflammation and thus influence KD susceptibility. *CACN2B* joins the growing list of calcium signaling and calcium channel genes harboring variants that affect KD susceptibility perhaps through increased immune cell activation. Our hypothesis would be that some non-KD children with fever/inflammation would also carry this same risk allele in *CACN2B* and might manifest bifid T waves in leads I/II. However, the KD population is enriched for these variants because (1) they are also risk alleles for KD susceptibility and (2) these children have high levels of cardiac inflammation. Thus, CACN2B variants contribute to KD susceptibility but are not sufficient.

4.1 | ECG abnormalities associated with KD

Over 30 years ago, a variation in T wave morphology including a "notched T wave" was reported in Japanese children with acute KD.[10] To our knowledge, this finding was never subsequently noted in association with KD. Ichida and colleagues reported a flattened T wave in 28/44 (64%) of subjects studied within the first two weeks after fever onset, but bifid T waves were not described.[11] Others reported electrophysiologic findings related to ventricular repolarization in KD patients.[12-15] These subtle signs may be related to myocardial inflammation coupled with differences in repolarization that may be affected by genotype.

TABLE 2 Association between *CACNB2* rs1409207 and bifid T waves or KD susceptibility

Association	Cohort		Group	n	Genotype			G allele frequency	OR	*P*
					AA	AG	GG			
Bifid T	Csohort 1[a]	All	Bifid T (+)	20	1	8	11	.75	2.24	.04
			Bifid T (-)	61	13	25	23	.58		
	Cohort 2	All	Bifid T (+)	60	3	23	34	.76	2.38	.003
			Bifid T (-)	161	38	63	60	.57		
		Asian	Bifid T (+)	13	0	0	13	1.00	NA	.08
			Bifid T (-)	22	1	4	17	.00		
		White	Bifid T (+)	8	1	4	3	.63	NA	.4
			Bifid T (-)	38	8	22	8	.50		
KD susceptibility	GWAS		KD	405	76	178	151	.59	1.21	.009
			Control	6217	1258	3134	1825	.54		

[a]Genotype was undetermined in one subject. Abbreviations: GWAS, genome-wide association studies; OR, odd's ratio; NA, not applicable.

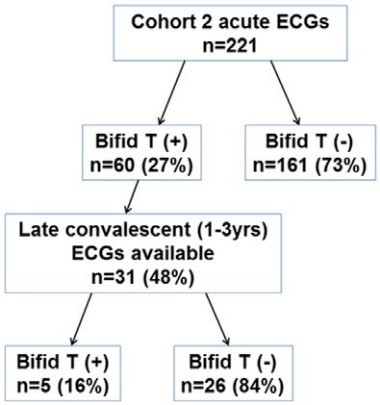

FIGURE 3 Bifid T waves in acute and late convalescent KD subjects

4.2 | CACNB2 encoded proteins in the heart

CACNB2 is one of four homologous genes coding for the auxiliary calcium channel, voltage-dependent beta subunits, which are important modulators of calcium channel activity.[16] Recent studies revealed the association of *CACNB2* variants with three major cardiovascular diseases: hypertension, heart failure, and ventricular fibrillation causing sudden death including Brugada syndrome.[17-19] The SNV in *CACNB2* associated with bifid T waves is located in the middle of a large intron and its function is unknown. No relevant effect of expression quantitative trait loci (eQTL) was reported in the available databases (Ensembl genome browser 90: https://www.ensembl.org/index.html). We found no eQTL effect using our whole blood transcriptome data from acute and convalescent KD subjects (data not shown).[20]

4.3 | Bifid T waves in children

Bifid T waves in leads V2 and V3 in healthy children tend to disappear with age due to changes in the ventricular repolarization process. In healthy children (mean age 7.3 ± 3.2 years) without heart disease, 18.3% of children showed bifid T waves in leads V2 and V3 but not in the limb leads.[9] We found that the presence of bifid T waves in the limb leads was also influenced by age with younger subjects having a higher incidence of bifid T waves. Other age-dependent presentations of cardiac arrhythmias and conduction abnormalities are well described, however, the mechanisms are unknown.[21] Whether these age-related conduction abnormalities are due to epigenetic factors, hormonal changes, or other influences remains unknown.

4.4 | Generation of bifid T waves

It has been suggested that the difference in time course of repolarization in the three predominant myocardial cell types (epi-myocardial, endo-myocardial, and mid-myocardial M cells) and the voltage gradients between them contribute to inscription of the T wave.[22] β-adrenergic stimulation has been shown to abbreviate the action potential duration of epicardial and endocardial but not M cells,

TABLE 3 Risk allele frequency distribution in subjects <2 and ≥2 years at the time of KD onset

SNVs (risk allele of bifid T wave)	Age at the onset (years)	Bifid T (+)	Bifid T(-)	Combined *P* value
CACNB2 rs1409207 (G allele)	<2	72/98 (73%)	58/98 (59%)	.034
	≥2	49/62 (79%)	198/348 (57%)	.001

leading to accentuation of transmural dispersion of repolarization. These repolarization differences in myocardial layers and the different reaction times may explain the presence of bifid T waves.[23,24]

Given these observations, the presence of bifid T waves in acute KD suggests heterogeneity of ventricular repolarization. Histological studies of RV endomyocardial biopsies and autopsies in acute KD patients have demonstrated that some degree of myocardial inflammation is universal.[2,25,26] Clinically, LV dysfunction presumably related to myocardial inflammation has been described in series of patients with "KD shock syndrome."[27] One case report showed findings of myocardial edema and decreased LV function by cardiac magnetic resonance imaging.[28] Numano et al reported the existence of RV dysfunction in acute KD documented by echocardiography.[29] Therefore, myocardial inflammation in both the right and left heart in acute KD may lead to dispersion of ventricular repolarization and bifid T waves.

The clinical implications of bifid T waves and CACNB2 variants are unclear. While arrhythmia has been reported as the cause of death in 10.8% of young adults following KD in one series, it was not clear if ischemia was the trigger for the arrhythmia in these patients.[30,31] The long-term outcomes in adults post-KD are still being defined and the incidence of arrhythmias that could be related to genetic variation in CACNB2 in this population is currently unknown.

4.5 | Limitations

We recognize both strengths and limitations to the present study. The unique association of bifid T waves with a SNV in a calcium channel gene was detected in two independent cohorts and suggests a direct genotype/phenotype relationship. However, the function of the SNV in *CACNB2* is unclear as no eQTL function could be assigned. Nor does this SNV reside in any known noncoding RNA sequence. It is possible that these variants function as eQTLs with respect to cardiomyocytes, but there is no transcriptome database of acute KD myocardium in which to test this hypothesis. Borderline statistical significance and limited sample size were further limitations. We used a nominal *P* value of < .05 in the analysis of Cohort 1 that consisted of a small number of subjects. However, in the analysis of Cohort 2, the SNV in *CACNB2* was significant after Bonferroni correction for multiple comparisons. Another limitation is that the 42 SNVs that were nominally associated with bifid T and KD susceptibility in Cohort 1 were never tested in Cohort 2 because the loci were not included on the genotyping platform. Only 9 of the 42 SNVs (21%) were in linkage disequilibrium ($r^2 > .6$) with the 40 SNVs tested in Cohort 2. Thus, further genotyping might reveal additional variants that also influence cardiomyocyte repolarization in acute KD.

5 | CONCLUSION

A calcium channel genetic variant associated with KD susceptibility may influence myocardial repolarization in the setting of acute inflammation and results in bifid T waves. These findings add to the growing list of variants in calcium channel and calcium signaling pathway genes that influence KD pathogenesis.

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CONFLICT OF INTEREST

None

AUTHOR CONTRIBUTIONS

Data analysis/interpretation, *Concept/Design*, *Drafting article*, *Statistics*: Jun Oyamada

Data analysis/interpretation, *Concept/Design*, *Drafting article*: *Chisato Shimizu*

Data analysis/interpretation, *Concept/Design*, *Statistics*, *Approval of article*: Jihoon Kim

Data analysis/interpretation, *Concept/Design*, *Approval of article*: Matthew R. Williams

Data collection, *Approval of article*: Eileen Png

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Data analysis/interpretation, *Concept/Design*, *Approval of article*: James C Perry

Data analysis/interpretation, *Concept/Design*, *Drafting article*: Jane C Burns

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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