

Research Article

Exome Sequencing Identifies Compound Heterozygous Mutations in *SCN5A* Associated with Congenital Complete Heart Block in the Thai Population

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Background. Congenital heart block is characterized by blockage of electrical impulses from the atrioventricular node (AV node) to the ventricles. This blockage can be caused by ion channel impairment that is the result of genetic variation. This study aimed to investigate the possible causative variants in a Thai family with complete heart block by using whole exome sequencing. **Methods.** Genomic DNA was collected from a family consisting of five family members in three generations in which one of three children in generation III had complete heart block. Whole exome sequencing was performed on one complete heart block affected child and one unaffected sibling. Bioinformatics was used to identify annotated and filtered variants. Candidate variants were validated and the segregation analysis of other family members was performed. **Results.** This study identified compound heterozygous variants, c.101G>A and c.3832G>A, in the *SCN5A* gene and c.28730C>T in the *TTN* gene. **Conclusions.** Compound heterozygous variants in the *SCN5A* gene were found in the complete heart block affected child but these two variants were found only in the this affected sibling and were not found in other unaffected family members. Hence, these variants in the *SCN5A* gene were the most possible disease-causing variants in this family.

1. Introduction

Congenital heart block is an uncommon disorder that occurs in about 1 in 20,000 live births [1]. It is characterized by anatomical or functional impairment in the conduction system which is caused by blockage of electrical impulses from atrioventricular node (AV node) to the ventricles [2]. The severity of heart block ranges from first-degree in which

electrical impulse to the AV node is slower than normal to third-degree or complete heart block in which electrical impulses from the atrium do not reach ventricles at all [3].

The conduction defect can be caused by a defective link between cardiomyocytes or by ion channel impairment that changes action potential shapes [4]. Inherited defects in cardiac conduction have been linked to genetic variants in several genes such as *SCN5A*, *SCN1B*, *KCNJ2*, *HCN4*,

Extracted DNA samples from III-2 and III-3 were controlled for their quality by measuring DNA concentration using Nanodrop ND1000 and measuring fragmentation of DNA by agarose gel electrophoresis. These 2 samples were prepared for whole exome sequencing. DNA samples from other family members (I-2, II-2) were prepared for segregation analysis. Written informed consent was obtained from adult family members themselves and for all children and all procedures were approved by the Institutional Review Board (MURA2012/02/SN1) of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, and EC48/364-006-3 of the Faculty of Medicine, Prince of Songkla University.

2.2. Exome Sequencing and Data Analysis. Whole exome sequencing was performed on family members III-2 and III-3. Samples were prepared following standard SOLID 5500xl (Applied Biosystems, California, USA) protocols for whole exome sequencing. Three micrograms of genomic DNA of each sample were fragmented using Covaris S2 (Covaris, Massachusetts, USA) and were captured for exome sequencing using TargetSeq Exome and Custom Enrichment System (Invitrogen, California, USA). The captured DNA was sequenced with 150 bp paired-end read on the SOLID 5500xl system according to the manufacturer's protocol. Primary analysis was performed on the sequencing machine using SOLID ICS software; then, raw sequenced data was transferred to the LifeScope Genomic Analysis server for secondary analysis. Sequence reads were mapped to the human reference genome assembly hg19 (GRCh37); then, variants calling for SNV, insertions, and deletions were performed.

Tertiary analysis was performed by using Golden Helix SVS software on identified candidate variants. Variants were filtered for minimum genotype quality of 20 and minimum coverage depths of 10 and then the qualified variants were annotated with the UCSC KnownGenes database to remove noncoding and synonymous variants. High frequency variants (minor allele frequencies greater than 2%) were excluded by annotation with allele frequencies from the 1000 Genomes Project Phase 3 [20] and an in-house exome database which consists of 172 Thai individuals. Deleterious protein functions were predicted using dbNSFP that compiled prediction scores from eleven prediction algorithms (SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, VEST3, CADD, MetaLR, MetaSVM, and PROVEAN) and 4 conservation scores, PhyloP, phastCons, GERP++, and SiPhy, and other related information [21]. Variants that were predicted not to alter protein function by any algorithm were excluded in this step. To narrow down variants, variants were focused on where these were located in the candidate genes list (Table 1). This genes list was created from combined known and suspected genes involved in the cardiovascular system from several sources and the cardiovascular defective candidate genes from the Enlis Genome Research software (Enlis, Berkeley, CA) gene panel. This genes list consists of 359 genes in total. Lastly, variants were filtered by their genotype. A genotype that was only in family member III-2 was indicated as a candidate variant for heart

block. The summarized variants filter steps are shown in Figure 3.

Additionally, pathogenic variants which were related to other diseases followed were explored in The American College of Medical Genetics and Genomics (ACMG) recommendations for reporting of incidental findings in clinical exome and genome sequencing [22]. Known pathogenic variants were identified by using The Human Gene Mutation Database (HGMD) [23].

2.3. Variant Validations and Segregation Analysis. Sanger sequencing was used to validate the candidate variants found in whole exome sequencing and segregation analyses were performed on the family members. Primers were designed using the Primer3 version 0.4.0 web-based tool [24]. Sequencing reactions were performed using Applied Biosystems 3130 DNA Analyzer (Life Technologies, Carlsbad, CA, USA).

3. Results

A family with complete heart block in only one of the 3rd generation family members was explored. By whole exome sequencing, a total of 99,834 variants in family members III-2 and III-3 were detected with an average depth over 60x coverage. After removal of low quality, noncoding and synonymous variants, 14,284 variants remained. Subsequently, variants were reduced by a filtering pipeline that included variants with minor allele frequencies, inheritance models, and a candidate gene list which reduced the number of variants to 36 variants in 28 genes. Finally, variants were prioritized and selected as candidate variants by annotated information from the deleterious protein function prediction database that resulted in 21 heart defective candidate variants in 18 genes. A list of candidate variants is shown in Table 2. All 21 candidate variants were then investigated for validation and segregation analysis. Six variants successfully passed this step while the other variants were dropped for 2 reasons: (1) a discordance between whole exome sequencing and Sanger sequencing or (2) variants found in the affected child being found in other unaffected family members except for two or more variants that were found in the same gene which indicated compound heterozygous inheritance. These 6 variants in 5 genes were identified as candidate variants for heart defects in this family. The list of final candidate variants is shown in Table 3.

Two nonsynonymous missense variants in the *SCN5A* gene in this study, c.101G>A and c.3832G>A (NM_000335), were likely to be present in a compound heterozygous fashion because 2 heterozygous variants were found in same gene in the affected case but only one or none of them were found in unaffected family members. Heterozygous c.101G>A was found in III-2 (index) and II-2 (mother) while heterozygous c.3832G>A was found in III-2 (index) and III-3 (brother) (Figure 2). Chromatograms of both variants in all available subjects are shown in Figure 4.

All other 4 variants were nonsynonymous missense variants in which their genotypes were heterozygous. These variants consisted of c.28730C>T (NM_133378) in the *TTN* gene, c.449C>T (NM_153240) in the *NPHP3* gene,

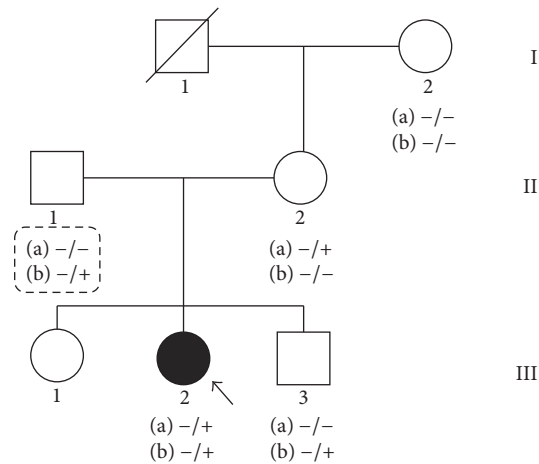


FIGURE 2: The lineage of the family with complete heart block indicated by the dark symbol, females by circles, and males by squares. Letters (a) and (b) indicate genotypes of 2 variants in the *SCN5A* gene for c.101G>A and c.3832G>A. The homozygous reference genotypes are indicated by (-/-) and heterozygous alternate genotypes by (-/+). The genotypes of II-1 are presumed genotypes which are inferred from his children.

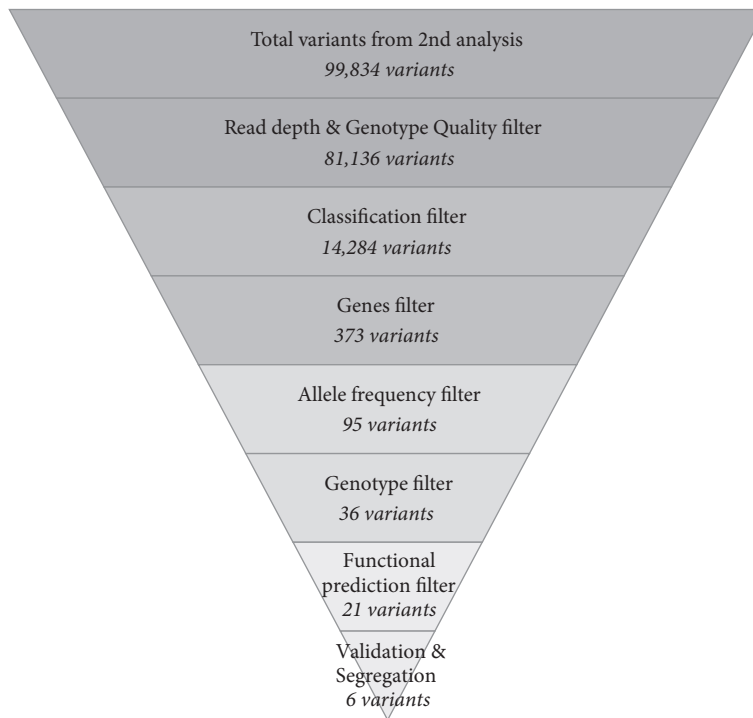


FIGURE 3: Filtering procedure of variants obtained by whole exome sequencing 2nd data analysis.

c.1133A>T (NM_012144) in the *DNAI1* gene, and c.13481A>C (NM_002458) in the *MUC5B* gene. *TTN* encoded Titin or connectin, a giant muscle protein, is expressed in the cardiac and skeletal muscles. *NPHP3* encodes a protein that is required for normal ciliary development. *DNAI1* encodes a member of the dynein intermediate chain family. Lastly, *MUC5B* encodes a protein member of the mucin family.

Finally, incidental findings in the whole exome data in this family following ACMG recommendations were explored. Aside from variants in the *SCN5A* gene that were assigned in the ACMG panel as known/expected pathogenic

variants for Romano-Ward Long QT syndromes Types 1, 2, and 3 and Brugada syndrome, which were key variants in this family, a variant in the *MYBPC3* gene that was assigned in the ACMG panel for hypertrophic/dilated cardiomyopathy was explored and validated.

4. Discussion

Although a congenital heart defect was found in this autistic patient, this was unlike the Timothy syndrome which is a rare disorder that affects heart, nervous system, and fingers/toes.

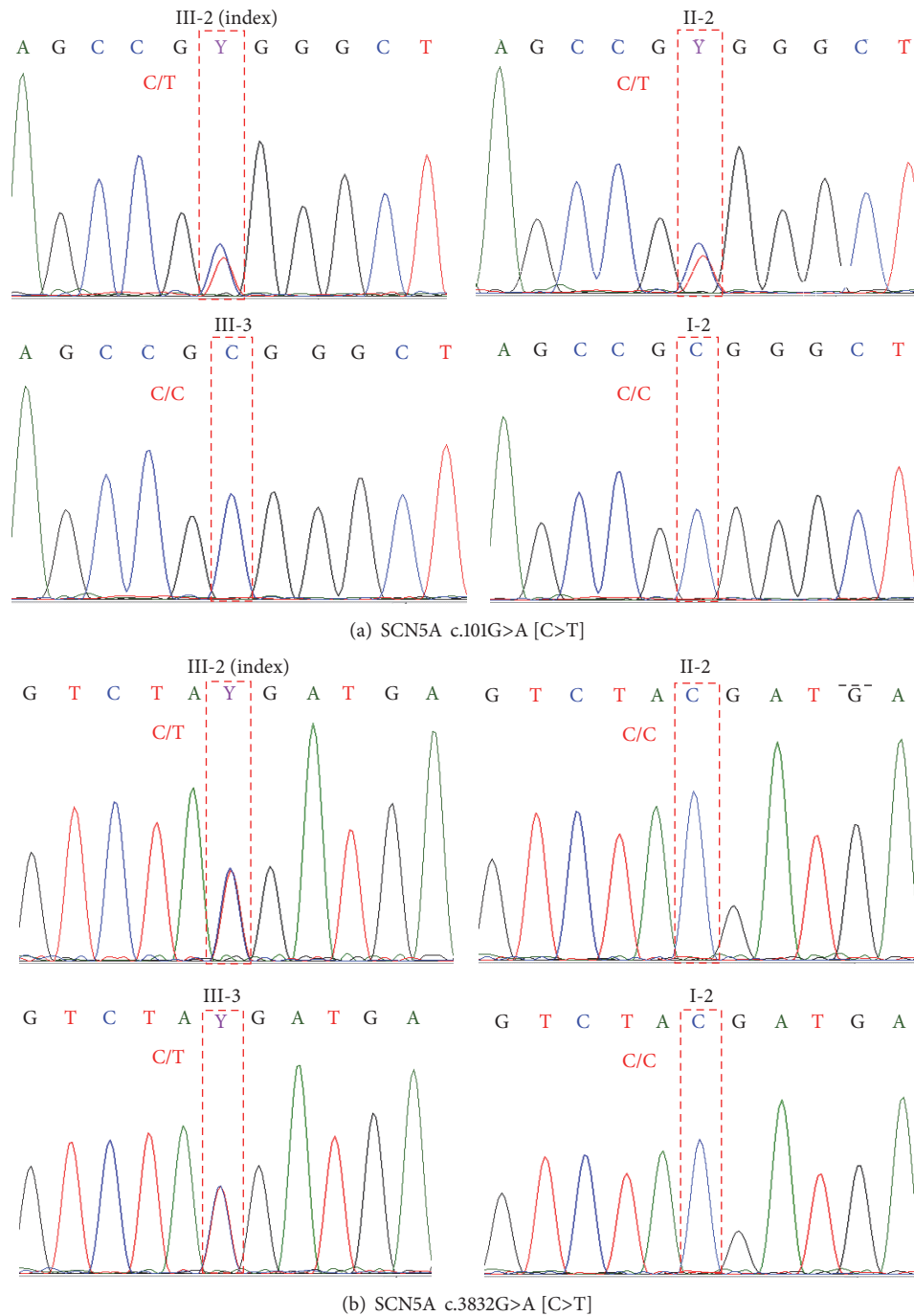


FIGURE 4: Chromatograms of 2 heterozygous missense variants in SCN5A gene: c.101G>A (a) and c.3832G>A (b). Letters in [] indicate complementary (FWD) alleles.

Syndactyly, the webbing of fingers and toes, one of Timothy syndrome signs, was not found in this case. Moreover, by direct sequencing and whole exome sequencing, variants in the CACNA1C gene, which are the common causes of both classical and atypical Timothy syndrome, were not detected [25]. Therefore, it was inferred that heart block and autism in this family are a coincidence.

SCN5A, a cardiac sodium channel gene, is important in generation and transmission of electrical impulses by its role

in controlling the flow of sodium ions into cells [11]. Several variants in the SCN5A that are located in four homologous domains of alpha subunit of NaV1.5, including 6 segments of each domain and linkers, N-terminal, and C-terminal, have been reported to be associated with various phenotypes of cardiac diseases [26]. The mechanism of SCN5A mutations associated with cardiac conduction disease has been explained by loss of function in NaV1.5 channels. Loss of NaV1.5 function leads to a reduction of inward sodium flow in

TABLE 1: List of genes involved in cardiovascular system defects.

AARS2	ATP6V0A2	CSRP3	FASTKD2	HAND1	LMF1	MYH11	PLN	SFTPA1	TGFB2
ABCA1	ATRX	CTF1	FBLN5	HAND2	LMNA	MYH6	PLOD1	SFTPA2	TGFB3
ABCA3	B3GAT3	CTNNA3	FBN1	HCN4	LPIN1	MYH7	PNPLA3	SFTPB	TGFBR1
ABCC6	BAG3	DES	FBN2	HERG	LPL	MYL2	PPARA	SFTPC	TGFBR2
ABCC9	BCOR	DHCR24	FGD1	HFE	LRP5	MYL3	PPARG	SFTPD	THEMIS
ABCG5	BMPR2	DHCR7	FGFR2	HOXA1	LTBP4	MYLK	PPP1R17	SGCD	TL1
ABCG8	BRAF	DLL3	FHL1	HRAS	MAP2K1	MYLK2	PRDM16	SGCG	TMEM43
ACADVL	CACNA1B	DMD	FHL2	IGBP1	MAP2K2	MYO6	PRKAB2	SHOC2	TMEM70
ACTA1	CACNA1C	DMPK	FKTN	ILK	MED12	MYOCD	PRKAG2	SKI	TMPO
ACTA2	CACNA1D	DNAH11	FLNA	IRX4	MEF2C	MYOM1	PRKARIA	SLC25A3	TNNC1
ACTC	CACNA2D1	DNAH5	FLNB	JAG1	MHY11	MYOT	PRKG1	SLC25A4	TNNI3
ACTC1	CACNB2	DNAI1	FOXC2	JPH2	MIB1	MYOZ2	PSEN1	SLC2A10	TNNT2
ACTN2	CALM1	DNAJC19	FOXH1	JUP	MID1	MYPN	PSEN2	SLMAP	TNXB
ACVR2B	CALM2	DNMIL	FOXRED1	KCNA5	MKKS	NEBL	PTPN11	SMAD3	TOPBP1
ACVRL1	CALR3	DOLK	FRYL	KCND3	MKS1	NEXN	PTRF	SMAD4	TPM1
ADCK3	CASQ2	DPP6	FXN	KCNE1	MOG1	NF1	RAF1	SMAD9	TPM2
ADRB1	CAV1	DSC2	GAA	KCNEIL	MRPL3	NIPBL	RAI1	SNTA1	TRDN
AGL	CAV3	DSG2	GATA4	KCNE2	MTND1	NKX2.5	RANGRF	SNX3	TRIM63
AKAP9	CBL	DSP	GATA5	KCNE3	MTND5	NKX2.6	RASA1	SOS1	TRPM4
AKT3	CBS	DTNA	GATA6	KCNH2	MTND6	NKX2-5	RBM10	SOX2	TFSM
ALMS1	CFC1	EFEMP2	GATAD1	KCNJ2	MTTD	NODAL	RBM20	SOX7	TTN
ALPK3	CHD7	EIF2AK4	GATT6	KCNJ5	MTTG	NOS1AP	RET	SPEG	TTR
ANGPTL3	CHST14	ELMOD2	GDF1	KCNJ8	MTTH	NOTCH1	RPL4	SPRED1	TWIST1
ANGPTL4	COA5	ELN	GDF2	KCNK3	MTTI	NOTCH2	RPSA	SURF1	TXNRD2
ANK2	COL18A1	EMD	GJA1	KCNQ1	MTTK	NOTCH3	RYR1	SYNE1	UQCRB
ANKRD1	COL1A1	ENG	GJA5	KRAS	MTTL1	NPC1	RYR2	SYNE2	USF1
ANO5	COL1A2	EPHX2	GLA	LAMA4	MTTL2	NPHP3	SALL1	TAZ	VCL
APOA1	COL2A1	ESCO2	GLB1	LAMP2	MTTM	NPPA	SALL4	TBX1	VCP
APOA2	COL3A1	EVC	GLI3	LBR	MTTP	NRAS	SCN1B	TBX20	VHL
APOA5	COL4A1	EVC2	GNAI2	LCAT	MTTQ	NSDHL	SCN2B	TBX3	XK
APOB	COL5A1	EYA1	GPC3	LDB3	MTTS1	NUBPL	SCN3B	TBX5	ZASP
APOC2	COL5A2	EYA4	GPD1L	LDLR	MTTS2	PCSK9	SCN4B	TCAP	ZFPM2
APOE	CREBBP	FANCA	GPIHBP1	LDLRAP1	MUC5B	PDLIM3	SCN5A	TCTN3	ZIC3
ARHGAP31	CRELD1	FANCC	GSN	LEFTY2	MYBPC3	PEX7	SCO2	TERC	ZMPSTE24
ARX	CRYAB	FANCD2	GUSB	LIPC	MYCN	PKP2	SDHA	TERT	ZNF469
ATP5E	CSF2RA	FANCE	HADH	LIPI	MYF6	PKP4	SEMA5A	TFAP2B	

the cells that results in difficulty producing and transmitting electrical signals. Conduction defects, however, could occur apart from transmission of inward sodium. The mutations on *SCN5A*, p.R219H, p.R222Q, and p.R225W which express alternative pathways, through a cation leak through NaV1.5, have been reported to be associated with mixed arrhythmias and dilated cardiomyopathy [27, 28]. In this study, 2 missense variants in the *SCN5A* gene in a patient who had complete heart block (III-2) were identified. The first variant, c.101G>A, results in replacement of arginine by histidine (p.R34H) in exon 2 whereas the second variant, c.3832G>A, results in replacement of valine by isoleucine (p.V1278I) in exon 21. Both variants were rare variants in which alternate alleles were not found in the Asian population from 1000 genome databases and the Thai population from the Thai in-house exome database. Functional prediction results show that

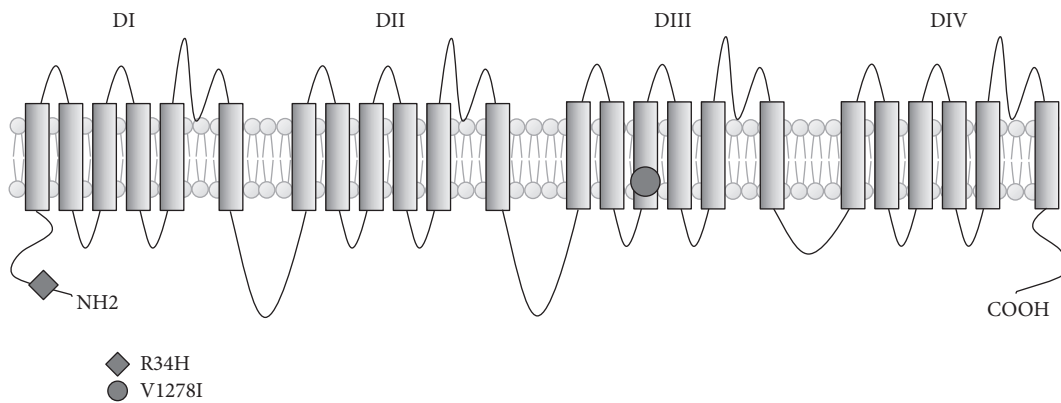
both variants were predicted to alter protein function which indicates that these variants have a high possibility to damage function of the sodium channel and cause the conduction defect in this case (Table 3).

c.101G>A (p.R34H) is a novel variant which is located in the N-terminal cytoplasmic domain of the sodium channel alpha subunit (Figure 5). A variant (c.80G>A) in the same region, the N-terminal cytoplasmic domain of sodium channel, has been reported to be associated with Brugada syndrome by Priori et al. [29]. Makita et al. have reported a mutation, which resulted in a stop codon (p.Q55X), presented in a Brugada syndrome affected patient with 1st-degree AV block history [30].

c.3832G>A (p.V1278I) has been reported as a “disease causing mutation” of dilated cardiomyopathy in the HGMD database [31–33]. This variant is located in the S3

TABLE 2: Candidate variants from whole exome sequencing.

Position	Gene	Classification	Transcript	HGVS coding	dbSNP
Chr1: 13036736	<i>PRAMEF22</i>	Nonsyn SNV	NM_001100631	c.808T>A	rs202011965
Chr2: 179402104	<i>TTN</i>	Nonsyn SNV	NM_003319	c.72635G>A	—
Chr2: 179542464	<i>TTN</i>	Nonsyn SNV	NM_133378	c.30443C>T	—
Chr2: 179549988	<i>TTN</i>	Nonsyn SNV	NM_133378	c.28730C>T	rs146400809
Chr2: 203395591	<i>BMP2</i>	Nonsyn SNV	NM_001204	c.1042G>A	rs201067849
Chr3: 38607905	<i>SCN5A</i>	Nonsyn SNV	NM_000335	c.3832G>A	rs199473341
Chr3: 38674698	<i>SCN5A</i>	Nonsyn SNV	NM_000335	c.101G>A	rs199473046
Chr3: 132438619	<i>NPHP3</i>	Nonsyn SNV	NM_153240	c.449C>T	rs142663818
Chr7: 42064927	<i>GLI3</i>	Nonsyn SNV	NM_000168	c.1292A>G	—
Chr9: 34506694	<i>DNAI1</i>	Nonsyn SNV	NM_012144	c.1133A>T	—
Chr9: 97080945	<i>FAM22F</i>	Del	NM_017561	c.2071_2073delTCT	rs150455117
Chr11: 1267969	<i>MUC5B</i>	Nonsyn SNV	NM_002458	c.9859A>C	—
Chr11: 1271591	<i>MUC5B</i>	Nonsyn SNV	NM_002458	c.13481A>C	rs201038498
Chr11: 47356616	<i>MYBPC3</i>	Unknown	NM_000256	c.2883C>T	—
Chr11: 126143258	<i>FOXRED1</i>	Frameshift Del	NM_017547	c.445delC	—
Chr12: 58177067	<i>TSMF</i>	Splicing	NM_001172695	c.231+1_231+2delGT	—
Chr12: 112892433	<i>PTPN11</i>	Stop-gain	NM_002834	c.591T>G	rs76982592
Chr16: 1245007	<i>CACNA1H</i>	Nonsyn SNV	NM_001005407	c.335T>C	—
Chr16: 71061495	<i>HYDIN</i>	Stop-loss	NM_017558	c.3052T>C	rs146649547
Chr16: 89815152	<i>FANCA</i>	Nonsyn SNV	NM_000135	c.3263C>T	rs17233497
Chr21: 35821734	<i>KCNE1</i>	Nonsyn SNV	NM_001127670	c.199C>T	rs199473645

FIGURE 5: Schematic of the transmembrane topology of the *SCN5A* protein. The location of the variants R34H (c.101G>A) and V1278I (c.3832G>A) is shown.

transmembrane segment of domain III (DIII) of the sodium channel alpha subunit. McNair et al. have reported that heterozygous c.3823G>A variant, which is located near c.3832G>A, was associated with dilated conduction disorder, cardiomyopathy, and arrhythmia [34]. In the same region, an association between atrial standstill and p.D1275N with polymorphisms in other gap junction protein, connexin40, has been reported by Groenewegen et al. [35]. Electrophysiological studies in xenopus oocytes showed that the c.3823G>A mutation results in an activation curve shift of the sodium channel conductance [35]. This activation curve shift toward more positive voltages may result in reduced excitability of myocytes. According to studies of Gosselin-Badaroudine et

al. and Moreau et al., this electrical disturbance could be the result from positive charge leakages of mutated NaV1.5 channels that could lead to a Na⁺ leak into cardiac myocytes [27, 28].

Results from segregation analysis indicated that both variants in the *SCN5A* gene were most possibly inherited in a compound heterozygous manner. Heterozygous c.101G>A was present in family members III-2 (index) and II-2 but was absent in family members I-2 and III-3. Likewise, heterozygous c.3832G>A was present in family members III-2 (index) and III-3 but was absent in family members I-2 and II-2 while only III-2 was the heart block affected family member. Although most of reported variants in the *SCN5A*

TABLE 3: List of final candidate variants.

Genomic coordinates	Genotype*			AA change	Gene	1kG (ASN)	MAF	Thai	SIFT	Functional prediction		Mutation taster
	III-2	III-3	II2							I2	I2	
Chr2: 179549988	A/G	G/G	G/G	G/G	<i>TTN</i>	0.01	0	0	Damaging	Probably damaging	Disease causing	
Chr3: 38607905	C/T	C/T	C/C	C/C	SCN5A	0	0	0	Damaging	Probably damaging	Disease causing	
Chr3: 38674698	C/T	C/C	C/T	C/C	SCN5A	0	0	0	Damaging	Possibly damaging	Disease causing	
Chr3: 132438619	A/G	G/G	G/G	G/G	<i>NPHP3</i>	0.02	0.0067	0	Damaging	Probably damaging	Disease causing	
Chr9: 34506694	A/T	A/A	A/A	A/A	<i>DNAIL</i>	0	0	0	Damaging	Benign	Disease causing	
Chr11: 1271591	A/G	A/A	A/A	A/A	<i>MUC5B</i>	0	0.0067	0	Damaging	Benign	Polymorphism	

* Genotypes in this table are FWD genotype while genotypes in HGVS are REV genotype.

gene were autosomal dominant, however, variants that were inherited in compound heterozygous individuals, similar to the current case finding, have been previously reported.

For instance, Bezzina et al. described compound heterozygous inheritance of 2 variants in the *SCN5A* gene which was associated with severe cardiac conduction disturbances and degenerative changes in the conduction system [36]. A nonsense p.W156X, which is located in the S1-S2 linker of domain I, was inherited from the father and a missense p.R225W, which was located in S4 segment of domain I, was inherited from the mother [36].

Benson et al. studied compound heterozygous variants in *SCN5A* in three families with congenital sick sinus syndrome [13]. A heterozygous missense p. P1298L, which is located in S4 segment of domain III, and p.G1408R, which is located in S5-S6 linker of domain III, were found in three siblings of the first family. Heterozygous missense p.T220I and p.R1623X were found in an individual of the second family. These two variants were located in the S4 segment of domain I and S4 segment of domain IV. The third family presented heterozygous deletions p.delF1617 in S3-S4 linker of domain IV and the missense p.R1632H in S4 segment of domain IV [13].

Beside the variants in *SCN5A* gene, 4 heterozygous missense variants in *TTN*, *NPHP3*, *DNAI1*, and *MUC5B* genes were found in the present case. All variants were found in only the index case, family member III-2. Among these 4 genes, *TTN* was the most likely cardiac disease associated gene while lack of evidence for *NPHP3*, *DNAI1*, and *MUC5B* genes existed. Mutations in *TTN* have been reported in about 18% of sporadic dilated cardiomyopathies and 25% of familial autosomal dominant cardiomyopathies and rarely caused hypertrophic cardiomyopathies [37].

Since this study only focused on the coding variants in the exon by whole sequencing, noncoding variants and structural variants were not explored. Moreover, it should be noted that novel variants in the study were found by the bioinformatics process so determination of biological functions will be needed in further studies.

5. Conclusion

In conclusion, this study demonstrated the potential of whole exome sequencing and a bioinformatics pipeline to identify the possible causative variants of complete heart block in a Thai family. The investigation found compound heterozygous variants in the *SCN5A*, cardiac sodium channel subunit gene, of which one was a novel variant and another one was a known pathogenic variant. Additionally, a heterozygous missense variant in the *TTN*, titin or connectin gene, has also been identified.

Competing Interests

The authors have no relevant affiliations or financial involvements with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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