

## Research Article

# Reevaluating the Mutation Classification in Genetic Studies of Bradycardia Using ACMG/AMP Variant Classification Framework

Liting Cheng <sup>1</sup>, Xiaoyan Li,<sup>1,2</sup> Lin Zhao,<sup>1</sup> Zefeng Wang,<sup>1</sup> Junmeng Zhang,<sup>1</sup> Zhuo Liang,<sup>1</sup> and Yongquan Wu <sup>1</sup>

<sup>1</sup>Beijing Anzhen Hospital, Capital Medical University, Beijing, China

<sup>2</sup>Beijing Institute of Heart, Lung & Blood Vessel Disease, Beijing, China

Correspondence should be addressed to Yongquan Wu; wuyongquan67@163.com

Liting Cheng and Xiaoyan Li contributed equally to this work.

Received 7 October 2019; Accepted 8 February 2020

Academic Editor: Byung-Hoon Jeong

Copyright © 2020 Liting Cheng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Purpose.** Next-generation sequencing (NGS) has become more accessible, leading to an increasing number of genetic studies of familial bradycardia being reported. However, most of the variants lack full evaluation. The relationship between genetic factors and bradycardia should be summarized and reevaluated. **Methods.** We summarized genetic studies published in the PubMed database from 2008/1/1 to 2019/9/1 and used the ACMG/AMP classification framework to analyze related sequence variants. **Results.** We identified 88 articles, 99 sequence variants, and 34 genes after searching the PubMed database and classified ABCC9, ACTN2, CACNA1C, DES, HCN4, KCNQ1, KCNH2, LMNA, MECP2, LAMP2, NPPA, SCN5A, and TRPM4 as high-priority genes causing familial bradycardia. Most mutated genes have been reported as having multiple clinical manifestations. **Conclusions.** For patients with familial CCD, 13 high-priority genes are recommended for evaluation. For genetic studies, variants should be carefully evaluated using the ACMG/AMP variant classification framework before publication.

## 1. Introduction

One of the inherited bradycardias that is currently being reported is inherited progressive cardiac conduction disease (IPCCD). Progressive cardiac conduction disease (PCCD) is an unidentified, heterogeneous, life-threatening disease that manifests as progressing fibrosis of the cardiac conduction system [1]. It is characterized by a decreased conduction rate, prolonged PR interval, and widened QRS wave, and it ultimately leads to complete atrioventricular block, syncope, and even sudden cardiac death [1]. Initially, patients present with only a widened QRS wave without a bundle branch block, and later, they develop complete atrioventricular block. Abnormalities in the conduction system may be related to changes in cardiac structure and function [2]. It is currently believed that the etiology of PCCD may be related to genetic factors, valvular disease, cardiomyopathy, and autoimmune disease [3]. PCCD caused by genetic factors

was originally called progressive familial heart block (PFHB) [3], and some studies directly used PCCD or IPCCD to refer to progressive conduction system diseases related to genetic factors. It is believed that PCCD is caused by the SCN5A mutation [4], and it may also be correlated with TRPM4 [5], DSP [6], and others. Genetic studies about other kinds of familial bradycardia have been published over the past decade, such as sick sinus syndrome and heart block. However, those studies have still not been summarized, and the clinical significance of the related variants is still unknown.

In 1977, Sanger et al. developed Sanger's "chain-termination" or dideoxy technique for nucleic acid sequence testing [7]. The improvement of Sanger sequencing makes DNA sequence testing for complex species available [8]. In the course of the development of next-generation sequencing (NGS), genetic testing becomes quicker, cheaper, and easier [9]. For patients who suffer from inherited cardiac disease, NGS has become a potential choice for the diagnosis,

TABLE 1: Pathogenic and benign criterion based on ACMG/AMP classification framework.

Rule	Category	Rule description
Evidence of pathogenic		
Very strong	PVSI	Null variants which caused loss of function are known to be the mechanism of diseases.
Strong	PS1	Different nucleotide change caused same amino acid change with known pathogenic variants.
	PS2	De novo (confirmed maternity and paternity) in a patient with no family history and diseases.
	PS3	Functional studies supported the effect of related pathogenic variants.
	PS4	Variants' prevalence significantly increased in affected individuals than controls.
Moderate	PM1	Mutation happened in hot spot and known function domain.
	PM2	Absent (or extremely low) in large population studies.
	PM3	With recessive disease, detected in <i>trans</i> with pathogenic variants.
	PM4	Variants (in-frame deletions/insertions in a nonrepeat region or stop-loss variants) lead to changes in protein length.
	PM5	Different missense changes at known pathogenic amino acid residue.
	PM6	De novo (without confirmation of maternity and paternity).
Supporting	PP1	Variants known to be the causes affected multiple family members.
	PP2	Missense variants in a gene that have a low rate of benign missense variation are common mechanism of disease.
	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene products.
	PP4	Phenotype specific for disease with single genetic etiology.
	PP5	Reputable source reports variants as pathogenic.
Evidence of benign		
Stand-alone	BA1	Allele frequency is > 0.5% base on population database.
Strong	BS1	Allele frequency is greater than expected for disorder.
	BS2	Recessive heredity being observed in healthy adult.
	BS3	Functional studies show no pathogenic effect.
	BS4	Without segregation.
Supporting	BP1	Missense variant in gene where only loss of function is pathogenic.
	BP2	Observed in genes with overlapping function without increased disease severity or observed in <i>cis</i> with a pathogenic variant.
	BP3	Variants (in-frame deletions/insertions in a nonrepeat region or stop-loss variants) lead to changes in a repetitive region without known function.
	BP4	Multiple lines of computational evidence suggest no impact on gene or gene product.
	BP5	Variant found in a case with alternate molecular basis for disease.
	BP6	Report as benign.
	BP7	Splicing variant predict an algorithm which predict no impact to the splice consensus sequence.



TABLE 2: Sequence variant classification.

Pathogenic	1 PVS1+ $\geq$ 1 (PS1-PS4)
	1 PVS1+ $\geq$ 2 (PM1-PM6)
	1 PVS1 + 1 (PS1-PS4) + 1 (PM1-PM6)
	1 PVS1+ $\geq$ 2 (PP1-PP5)
	$\geq$ 2 (PS1-PS4)
	1 (PS1-PS4)+ $\geq$ 3 (PM1-PM6)
	1 (PS1-PS4) + 2 (PM1-PM6)+ $\geq$ 2 (PP1-PP5)
1 (PS1-PS4) + 1 (PM1-PM6)+ $\geq$ 4 (PP1-PP5)	
Likely pathogenic	1 PVS1 + 1 (PM1-PM6)
	1 (PS1-PS4) + 1-2 (PM1-PM6)
	1 (PS1-PS4)+ $\geq$ 2 (PP1-PP5)
	$\geq$ 3 (PP1-PP5)
	2 (PM1-PM6)+ $\geq$ 2 (PP1-PP5)
1 (PM1-PM6)+ $\geq$ 4 (PP1-PP5)	
Benign	1 BA1
	$\geq$ 2 (BS1-BS4)
Likely benign	1 (BS1-BS4) + 1 (BP1-BP7)
	$\geq$ 2 (BP1-BP7)
Uncertain significant	Other criteria shown above have not met OR Criterion for benign and pathogenic is contradictory

OR: odds ratio.

information gathered in the databases and the ACMG/AMP classification framework (Tables 1 and 2), we evaluated related sequence variants and proposed a clinical judgement.

### 3. Results and Discussion

We summarized genetic studies published in the PubMed database over 11 years (Figure 1). A total 1015 articles were enrolled after searching the database. 927 articles were excluded. Finally, 88 articles fit the profile; 99 variants and 34 genes were studied in the current article.

Information in InterVar was gathered to evaluate all the sequence variants, and the relevant evidence for pathogenic and benign criteria was summarized (Table 3). For mutation cannot be defined in InterVar, we used gnomAD and ClinVar to analyze frameshift mutations (Table 4) and large fragment deletions (Table 5). We also gathered information about splicing mutations (Table 6).

We studied 88 articles, including 99 variants and 34 genes, after searching the PubMed database and identified 13 high-priority genes causing familial bradycardia, as follows: ABCC9 [18], ACTN2 [19], CACNA1C [20, 21], DES [22–27], HCN4 [28–32], KCNQ1 [33, 34], KCNH2 [35], LMNA [36, 37], MECP2 [38], LAMP2 [39], NPPA [40], SCN5A [41–45], and TRPM4 [5, 46–48] (Table 3).

We use InterVar to reevaluate APOB, CLCA2 DSG2, GJC1, GLA, GNB2, JPH2, KCNJ3, LDB3, MYBPC3, NKX2-

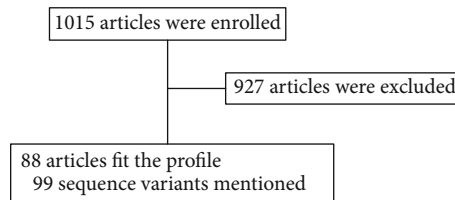


FIGURE 1: Summary of specification.

5, NXF5, PDYN, PRKAG2, and TTN, which have been published as pathogenic variants. According to the ACMG/AMP variant classification framework, those genes should be classified into uncertain significance.

For the majority of related genes, the clinical manifestations were not unique. These mutations may lead to bradycardia, arrhythmia, myopathy, and nerve system disease. LMNA mutations may present as AVB and arrhythmia; DES, GJA5, TTN, LAMP2, and MECP2 mutations may present as AVB and myopathy; GNB5 mutation may present as CCD and nerve system disease; HCN4, KCNQ1, PRKAG2, and SCN5A mutations may present as CCD, myopathy, and arrhythmia.

Genetic diagnosis has become an inalienable part of the diagnosis, treatment, and prevention of SCD. Cardiac ion channel disease, closely related to sudden cardiac death (SCD), has been discussed for decades. In contrast, the relationship between bradycardia and genetic factors is still unclear. Syncope and SCD caused by bradycardia are life-threatening diseases. If the relationship between genetic factors and bradycardia is eliminated, SCD could be prevented.

Pedigrees of bradycardia families have been reported for decades. However, those studies are lacking. Some of the studies do not include full information about related sequence variants, and some of the studies do not list the whole family tree. In addition, the methods used to evaluate sequence variants are complex, and different centers have their own experience. It is still doubtful whether those variants are pathogenic. Therefore, ACMG/AMP promotes a guideline for thorough evaluation. By analyzing the allele frequency, segregation, de novo, protein expression, functional studies, and other factors, sequencing variants can be scored into a five-tier system: pathogenic, likely pathogenic, uncertain significant, likely benign, and benign. As accurate as the guideline may be, pathogenicity has been defined as being greater than 90% of pathogenicity [15]. According to the precise classification of pathogenicity, pedigrees of familial bradycardia can be reevaluated. InterVar [16] is a tool implementing ACMG/AMP criteria that can automatically analyze sequence variants. In this article, we used InterVar to summarize 13 high-priority genes, as follows: ABCC9, ACTN2, CACNA1C, DES, HCN4, KCNQ1, KCNH2, LMNA, MECP2, LAMP2, NPPA, SCN5A, and TRPM4 (Table 3). High-throughput sequencing (next-generation sequencing) is quite expensive. In contrast, the gene panel is cheaper and easier to analyze. We recommend that patients with a family history of bradycardia have their clinical manifestations gathered and that related pathogenic genes be highly regarded.

For future reference, multicenter studies on the epidemiology of familial bradycardia should be organized. In

TABLE 3: Evaluate all sequence variants using InterVar database.

Chr	Position	Ref	Alt	Gene	Criterion	Clinical manifest	Authors
12	21882785	G	A	ABCC9	Likely pathogenic	PCCD; SSS	Celestino-Soper et al. [18]
1	236731300	T	C	ACTN2	Likely pathogenic	AVB; AF	Girolami et al. [19]
2	21006288	A	T	APOB	Uncertain significance	PCCD; SSS	Celestino-Soper et al. [18]
12	2567685	G	A	CACNA1C	Likely pathogenic	SSS	Zhu et al. [20]
12	2567694	G	A	CACNA1C	Likely pathogenic	SSS	Zhu et al. [20]
12	2448997	C	T	CACNA1C	Likely pathogenic	PCCD	Gao et al. [21]
12	2504538	G	A	CACNA1C	Pathogenic	AVB; Timothy syndrome 1 (TS1)	Sepp et al. [49]
1	86447519	G	T	CLCA2	Uncertain significance	AVB; PCCD	Mao et al. [50] Tan et al. [51]
2	219425671	C	A	DES	Uncertain significance	AVB; AF	Jurcu et al. [52]
2	219418500	C	T	DES	Pathogenic	AVB	van Tintelen et al. [22]
18	31524751	A	G	DSG2	Benign/likely benign	AVB	Castellana et al. [53]
17	44805594	G	T	GJC1	Uncertain significance	AVB	Seki et al. [54]
X	101398869	A	C	GLA	Uncertain significance	HCM; AVB	Csanyi et al. [55]
7	100676751	G	T	GNB2	Uncertain significance	SSS; AVB	Stallmeyer et al. [56]
15	73329719	C	T	HGN4	Pathogenic/likely pathogenic	SSS; LVNC	Milano et al. [28]
15	73343416	A	T	HGN4	Uncertain significance	SSS; AF; LVNC	Ishikawa et al. [31]
15	73329719	C	T	HGN4	Pathogenic/likely pathogenic	SSS	Ishikawa et al. [31]
15	73323745	G	C	HGN4	Likely benign	SSS	Schweizer et al. [29]
15	73322804	C	A	HGN4	Uncertain significance	AVB	Zhou et al. [57]
20	44160305	A	T	JPH2	Uncertain significance	HCM; AVB	Vanninen et al. [58]
7	150951555	C	A	KCNH2	Pathogenic	AVB; LQT	Priest et al. [35]
2	155555534	A	C	KCNJ3	Uncertain significance	SSS; AF	Yamada et al. [59]
11	2549192	G	A	KCNQ1	Pathogenic/likely pathogenic	SSS; AF	Righi et al. [34]
X	119589315	C	T	LAMP2	Pathogenic	AVB; WPW; Danon disease	Miani et al. [39]
10	88446830	G	A	LDB3	Benign	PCCD; SSS	Celestino-Soper et al. [18]
1	156104224	C	T	LMNA	Pathogenic	AVB; VT; SCD	Glockhofer et al. [36]
1	156104281	A	G	LMNA	Uncertain significance	AVB; HF	Petillo et al. [60]
1	156106186	G	C	LMNA	Uncertain significance	AVB; HF	Petillo et al. [60]
1	156084953	G	A	LMNA	Pathogenic	AVB; DCM	Wu et al. [61]
1	156104629	C	T	LMNA	Pathogenic	AVB; VT; SCD	Saga et al. [62]
1	156104755	T	C	LMNA	Pathogenic/likely pathogenic	AVB; muscular dystrophy; cardiomyopathy	Romeike et al. [63]
1	156084787	C	T	LMNA	Likely benign	AVB; AF	Saj et al. [37]
1	156108298	C	T	LMNA	Likely pathogenic	AVB; HCM	Francisco et al. [64]
X	153297719	G	A	MECP2	Pathogenic/likely pathogenic	SSS	Shioda et al. [38]
11	47354497	G	A	MYBPC3	Uncertain significance	AVB	Kouakam et al. [65]

TABLE 3: Continued.

Chr	Position	Ref	Alt	Gene	Criterion	Clinical manifest	Authors
5	172660006	G	A	NKX2-5	Uncertain significance	AVB; AF; DCM	Yuan et al. [66]
5	172661762	C	A	NKX2-5	Uncertain significance	AVB; congenital cardiovascular diseases (CCVD)	Pabst et al. [67]
5	172660110	G	C	NKX2-5	Uncertain significance	AVB; ASD	Xie et al. [68]
1	11907171	C	T	NPPA	Pathogenic	SSS; atrial dilatation (AD)	Disertori et al. [69]
X	101096287	G	A	NXF5	Uncertain significance	AVB; focal segmental glomerulosclerosis (FSGS)	Esposito et al. [70]
20	1961153	T	A	PDYN	Uncertain significance	PCCD	Su et al. [71]
20	1961154	C	G	PDYN	Uncertain significance	PCCD	Su et al. [71]
7	151560613	A	G	PRKAG2	Uncertain significance	HCM; AVB	Thevenon et al. [72]
3	38550326	G	T	SCN5A	Uncertain significance	SSS	Chen et al. [73]
3	38603929	G	C	SCN5A	Uncertain significance	AVB	Nikulina et al. [74]
3	38556532	T	C	SCN5A	Uncertain significance	SSS	Hothi et al. [41]
3	38550734	A	C	SCN5A	Uncertain significance	SSS	Asadi et al. [75]
3	38613790	C	T	SCN5A	Likely pathogenic	SSS	Abe et al. [76]
3	38566426	C	T	SCN5A	Pathogenic	SSS	Abe et al. [76]
3	38550899	T	A	SCN5A	Uncertain significance	AVB; DCM	Watanabe et al. [77]
3	38581137	G	A	SCN5A	Likely benign	SSS	Ishikawa et al. [31]
3	38581002	C	T	SCN5A	Uncertain significance	AVB	Hu et al. [78]
3	38633207	G	T	SCN5A	Uncertain significance	SSS; AFL; AF	Moreau et al. [79]
3	38613787	G	A	SCN5A	Uncertain significance	AVB	Thongnak et al. [80]
3	38597787	C	A	SCN5A	Likely pathogenic	PCCD; SSS	Baskar et al. [81]
3	38630342	T	A	SCN5A	Pathogenic/likely pathogenic	SSS; AFL	Celestino-Soper et al. [18]
3	38575424	C	A	SCN5A	Uncertain significance	SSS; AFL; VT	Selly et al. [82]
3	38551477	A	T	SCN5A	Likely pathogenic	AVB; DCM	Holst et al. [43]
3	38560398	G	A	SCN5A	Pathogenic	SSS; AVB	Ge et al. [83]
3	38550968	C	A	SCN5A	Uncertain significance	AVB	Robyns et al. [84]
19	49196760	G	A	TRPM4	Uncertain significance	SSS	Thongnak et al. [80]
19	49157885	G	A	TRPM4	Pathogenic	PCCD	Abe et al. [76]
19	49167950	G	A	TRPM4	Benign	PCCD; SSS	Liu et al. [47]
19	49196790	A	G	TRPM4	Likely benign	AVB; VT	Kruse et al. [48]
19	49202140	A	T	TRPM4	Uncertain significance	PCCD	Bianchi et al. [46]
19	49171597	A	G	TRPM4	Uncertain significance	AVB; VT	Daumy et al. [5]
19	49200395	A	G	TRPM4	Pathogenic	AVB	Bianchi et al. [46]
19	49168301	C	T	TRPM4	Pathogenic	AVB	Stallmeyer et al. [85]
19	49182608	G	A	TRPM4	Uncertain significance	PCCD	Stallmeyer et al. [85]
						AVB	Liu et al. [47]
						AVB	Syam et al. [86]

TABLE 3: Continued.

Chr	Position	Ref	Alt	Gene	Criterion	Clinical manifest	Authors
19	49188641	G	A	TRPM4	Uncertain significance	AVB	Syam et al. [86]
19	49183108	C	T	TRPM4	Uncertain significance	PCCD	Liu et al. [47]
19	49196597	T	C	TRPM4	Uncertain significance	AVB	Stallmeyer et al. [85]
2	178569522	G	T	TTN	Uncertain significance	SSS	Zhu et al. [20]



TABLE 4: Using ClinVar to analysis frameshift mutation.

Genome AD	Chr	dbSNP	Gene	Variant	Functional study	Criterion
—	—	—	ALG13	c.383+2821_383+2822delinsTT	—	—
—	Chr2:219418955-219418982	rs1114167332	DES	c.493_520del28insGCGT	—	Pathogenic
—	—	—	DSC2	c.2688_2688delinsGAA	—	—
—	—	—	EXT2	c.1101_1102delAG (E368Kfs*18)	—	—
—	Chr1:156130627-156130629	rs794728597	LMNA	c.367_369delAAG	Pathogenic	Likely pathogenic
—	—	—	LMNA	c.364_366AAG	—	—
—	—	—	LMNA	c.103-105del CTG	—	—
—	—	—	LMNA	815_818delinsCCAGAC	—	—
—	—	—	MYL4	c.234delC	—	—
—	Chr5:173232761	rs587784067	NKX2.5	c.959delC	—	Conflicting interpretations of pathogenicity
—	—	—	SCN5A	c.2401_2409delinsTCC	—	Uncertain significant
—	—	—	SCN5A	c.5355_5354delCT	—	Uncertain significant
—	—	—	SCN5A	c.5368 GNA	—	—
—	—	—	SCN5A	c.3142_3153de-112ins11 delE933	—	—
—	—	—	MYH6	delE933	—	—
—	—	—	MYL4	c.234delC	—	—



TABLE 5: Using InterVal to analysis large fragment deletion.

Genome AD	Chr	dbSNP	Gene	Variant	Functional study
—	—	—	DES	Deletion-insertion mutation (c.1045-1063 del/G ins), deleting 7 amino acids (Met349-Arg355) and inserting 1 amino acid (Gly349)	—

TABLE 6: Analyzing splicing mutation.

Genome AD	Chr	dbSNP	Gene	Variant	Functional study
—	—	—	HCN4	c.1737+1G>T	—
—	Chr:1:156130615	—	LMNA	c.357-2A>G	—
—	—	—	LMNA	c.357-1G>T	—
—	—	—	LMNA	IVS9-3C>G	—
G = 0.00001	Chr3:38562413	rs397514447	SCN5A	c.3963+2T>C	—
—	—	—	SCN5A	c.1141-2A>G	—
—	—	—	SCN5A	c.-225-820T>C	—
—	—	—	TGF beta 1	c.4246-2A>G	—
—	—	—	MYH6	c.2292+2T>C	—

addition, detailed information about sequence variants should be addressed in related articles and should be evaluated under the ACMG/AMP classification framework. The relationship between bradycardia and genomic variants remains unknown, and epigenetics and modifier genes should be used to investigate the relationship between genes and diseases.

#### 4. Limitation

We summarized sequence variants published in only the PubMed database. There should be more pathogenic genes studied related to bradycardia.

#### 5. Conclusion and Future Direction

Only 13 pathogenic genes (99 sequence variants and 34 genes being studied) were identified after using the ACMG/AMP variant classification framework to reevaluate. For future reference, pedigree studies should be fully evaluated before being published.

For patients with familial CCD, 13 high-priority genes are recommended for evaluation. Compared to whole genome sequencing, this will increase the clinical utility of genetic testing.

#### Data Availability

There are no restrictions on data access of this paper. All works have been provided in this paper

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

#### Authors' Contributions

Liting Cheng and Xiaoyan Li contribute the same to this article.

#### Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 81570220).

#### References

- [1] S. G. Priori, A. A. Wilde, M. Horie et al., "HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHS in May 2013 and by ACCF, AHA, PACES, and AEP in June 2013," *Heart Rhythm*, vol. 10, no. 12, pp. 1932–1963, 2013.
- [2] S. N. Barra, R. Providencia, L. Paiva, J. Nascimento, and A. L. Marques, "A review on advanced atrioventricular block in young or middle-aged adults," *Pacing and Clinical Electrophysiology*, vol. 35, no. 11, pp. 1395–1405, 2012.
- [3] A. J. Brink and M. Torrington, "Progressive familial heart block, two types," *South African Medical Journal*, vol. 52, no. 2, pp. 53–59, 1977.
- [4] C. A. Martin, C. L. Huang, and A. A. Grace, "Progressive conduction diseases," *Cardiac Electrophysiology Clinics*, vol. 2, no. 4, pp. 509–519, 2010.
- [5] X. Daumy, M. Y. Amarouch, P. Lindenbaum et al., "Targeted resequencing identifies *TRPM4* as a major gene predisposing to progressive familial heart block type I," *International Journal of Cardiology*, vol. 207, pp. 349–358, 2016.
- [6] A. Kiselev, E. Mikhaylov, E. Parmon et al., "Progressive cardiac conduction disease associated with a *DSP* gene mutation," *International Journal of Cardiology*, vol. 216, pp. 188–189, 2016.

- [7] F. Sanger, S. Nicklen, and A. R. Coulson, "DNA sequencing with chain-terminating inhibitors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 12, pp. 5463–5467, 1977.
- [8] E. L. van Dijk, H. Auger, Y. Jaszczyszyn, and C. Thermes, "Ten years of next-generation sequencing technology," *Trends in Genetics*, vol. 30, no. 9, pp. 418–426, 2014.
- [9] V. N. Parikh and E. A. Ashley, "Next-generation sequencing in cardiovascular disease: present clinical applications and the horizon of precision medicine," *Circulation*, vol. 135, no. 5, pp. 406–409, 2017.
- [10] J. R. Giudicessi, D. M. Roden, A. A. M. Wilde, and M. J. Ackerman, "Classification and reporting of potentially proarrhythmic common genetic variation in long QT syndrome genetic testing," *Circulation*, vol. 137, no. 6, pp. 619–630, 2018.
- [11] J. Brugada, O. Campuzano, E. Arbelo, G. Sarquella-Brugada, and R. Brugada, "Present status of Brugada syndrome: JACC state-of-the-art review," *Journal of the American College of Cardiology*, vol. 72, no. 9, pp. 1046–1059, 2018.
- [12] R. G. Weintraub, C. Semsarian, and P. Macdonald, "Dilated cardiomyopathy," *The Lancet*, vol. 390, no. 10092, pp. 400–414, 2017.
- [13] J. B. Geske, S. R. Ommen, and B. J. Gersh, "Hypertrophic cardiomyopathy: clinical update," *JACC: Heart Failure*, vol. 6, no. 5, pp. 364–375, 2018.
- [14] E. Gandjbakhch, A. Redheuil, F. Pousset, P. Charron, and R. Frank, "Clinical diagnosis, imaging, and genetics of arrhythmogenic right ventricular cardiomyopathy/dysplasia: JACC state-of-the-art review," *Journal of the American College of Cardiology*, vol. 72, no. 7, pp. 784–804, 2018.
- [15] S. Richards, N. Aziz, S. Bale et al., "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology," *Genetics in Medicine*, vol. 17, no. 5, pp. 405–424, 2015.
- [16] Q. Li and K. Wang, "InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines," *The American Journal of Human Genetics*, vol. 100, no. 2, pp. 267–280, 2017.
- [17] A. Allot, Y. Peng, C. H. Wei, K. Lee, L. Phan, and Z. Lu, "LitVar: a semantic search engine for linking genomic variant data in PubMed and PMC," *Nucleic Acids Research*, vol. 46, no. W1, pp. W530–W536, 2018.
- [18] P. B. Celestino-Soper, A. Doytchinova, H. A. Steiner et al., "Evaluation of the genetic basis of familial aggregation of pacemaker implantation by a large next generation sequencing panel," *PLoS One*, vol. 10, no. 12, article e0143588, 2015.
- [19] F. Girolami, M. Iascone, B. Tomberli et al., "Novel  $\alpha$ -actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: a massively parallel sequencing study," *Circulation: Cardiovascular Genetics*, vol. 7, no. 6, pp. 741–750, 2014.
- [20] Y. B. Zhu, J. W. Luo, F. Jiang, and G. Liu, "Genetic analysis of sick sinus syndrome in a family harboring compound CACNA1C and TTN mutations," *Molecular Medicine Reports*, vol. 17, no. 5, pp. 7073–7080, 2018.
- [21] Y. Gao, X. Xue, D. Hu et al., "Inhibition of late sodium current by mexiletine: a novel pharmacotherapeutical approach in timothy syndrome," *Circulation: Arrhythmia and Electrophysiology*, vol. 6, no. 3, pp. 614–622, 2013.
- [22] J. P. van Tintelen, I. C. van Gelder, A. Asimaki et al., "Severe cardiac phenotype with right ventricular predominance in a large cohort of patients with a single missense mutation in the DES gene," *Heart Rhythm*, vol. 6, no. 11, pp. 1574–1583, 2009.
- [23] E. Arbustini, M. Pasotti, A. Pilotto et al., "Desmin accumulation restrictive cardiomyopathy and atrioventricular block associated with desmin gene defects," *European Journal of Heart Failure*, vol. 8, no. 5, pp. 477–483, 2006.
- [24] L. Cao, D. Hong, M. Zhu, X. Li, H. Wan, and K. Hong, "A novel heterozygous deletion-insertion mutation in the desmin gene causes complete atrioventricular block and mild myopathy," *Clinical Neuropathology*, vol. 32, no. 1, pp. 9–15, 2013.
- [25] I. Schirmer, M. Dieding, B. Klauke et al., "A novel desmin (DES) indel mutation causes severe atypical cardiomyopathy in combination with atrioventricular block and skeletal myopathy," *Molecular Genetics & Genomic Medicine*, vol. 6, no. 2, pp. 288–293, 2018.
- [26] C. Scuderi, E. Borgione, F. Castello et al., "The in cis T251I and P587L POLG1 base changes: description of a new family and literature review," *Neuromuscular Disorders*, vol. 25, no. 4, pp. 333–339, 2015.
- [27] D. Q. Chen, X. B. Shen, S. H. Zhang, G. Y. Ye, and S. H. Xu, "Malignant arrhythmia with variants of desmocollin-2 and desmoplakin genes," *International Heart Journal*, vol. 60, no. 5, pp. 1196–1200, 2019.
- [28] A. Milano, A. M. Vermeer, E. M. Lodder et al., "HCN4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy," *Journal of the American College of Cardiology*, vol. 64, no. 8, pp. 745–756, 2014.
- [29] P. A. Schweizer, J. Schröter, S. Greiner et al., "The symptom complex of familial sinus node dysfunction and myocardial noncompaction is associated with mutations in the HCN4 channel," *Journal of the American College of Cardiology*, vol. 64, no. 8, pp. 757–767, 2014.
- [30] L. Hategan, B. Csányi, B. Ördög et al., "A novel 'splice site' HCN4 Gene mutation, c.1737+1 G>T, causes familial bradycardia, reduced heart rate response, impaired chronotropic competence and increased short-term heart rate variability," *International Journal of Cardiology*, vol. 241, pp. 364–372, 2017.
- [31] T. Ishikawa, S. Ohno, T. Murakami et al., "Sick sinus syndrome with HCN4 mutations shows early onset and frequent association with atrial fibrillation and left ventricular noncompaction," *Heart Rhythm*, vol. 14, no. 5, pp. 717–724, 2017.
- [32] R. Yokoyama, K. Kinoshita, Y. Hata et al., "A mutant HCN4 channel in a family with bradycardia, left bundle branch block, and left ventricular noncompaction," *Heart and Vessels*, vol. 33, no. 7, pp. 802–819, 2018.
- [33] P. F. Aziz and M. J. Shah, "Efficacy of ventricular pacing in the treatment of an arrhythmic storm associated with a congenital long QT mutation," *Congenital Heart Disease*, vol. 8, no. 6, pp. E165–E167, 2013.
- [34] D. Righi, M. S. Silvetti, and F. Drago, "Sinus bradycardia, junctional rhythm, and low-rate atrial fibrillation in short QT syndrome during 20 years of follow-up: three faces of the same genetic problem," *Cardiology in the Young*, vol. 26, no. 3, pp. 589–592, 2016.
- [35] J. R. Priest, S. R. Ceresnak, F. E. Dewey et al., "Molecular diagnosis of long QT syndrome at 10 days of life by rapid whole genome sequencing," *Heart Rhythm*, vol. 11, no. 10, pp. 1707–1713, 2014.

- [36] C. R. Glöckhofer, J. Steinfurt, G. Franke et al., “A novel *LMNA* nonsense mutation causes two distinct phenotypes of cardiomyopathy with high risk of sudden cardiac death in a large five-generation family,” *EP Europace*, vol. 20, no. 12, pp. 2003–2013, 2018.
- [37] M. Saj, R. Dabrowski, S. Labib et al., “Variants of the lamin A/C (*LMNA*) gene in non-valvular atrial fibrillation patients: a possible pathogenic role of the Thr528Met mutation,” *Molecular Diagnosis & Therapy*, vol. 16, no. 2, pp. 99–107, 2012.
- [38] T. Shioda, S. Takahashi, T. Kaname, T. Yamauchi, and T. Fukuoka, “*MECP2* mutation in a boy with severe apnea and sick sinus syndrome,” *Brain and Development*, vol. 40, no. 8, pp. 714–718, 2018.
- [39] D. Miani, M. Taylor, L. Mestroni et al., “Sudden death associated with danon disease in women,” *The American Journal of Cardiology*, vol. 109, no. 3, pp. 406–411, 2012.
- [40] H. J. Kee and H. Kook, “Krüppel-like factor 4 mediates histone deacetylase inhibitor-induced prevention of cardiac hypertrophy,” *Journal of Molecular and Cellular Cardiology*, vol. 47, no. 6, pp. 770–780, 2009.
- [41] S. S. Hothi, F. Ara, and J. Timperley, “p.Y1449C *SCN5A* mutation associated with overlap disorder comprising conduction disease, Brugada syndrome, and atrial flutter,” *Journal of Cardiovascular Electrophysiology*, vol. 26, no. 1, pp. 93–97, 2015.
- [42] A. Neu, M. Eiselt, M. Paul et al., “A homozygous *SCN5A* mutation in a severe, recessive type of cardiac conduction disease,” *Human Mutation*, vol. 31, no. 8, pp. E1609–E1621, 2010.
- [43] A. G. Holst, B. Liang, T. Jespersen et al., “Sick sinus syndrome, progressive cardiac conduction disease, atrial flutter and ventricular tachycardia caused by a novel *SCN5A* mutation,” *Cardiology*, vol. 115, no. 4, pp. 311–316, 2010.
- [44] Y. Zhang, T. Wang, A. Ma et al., “Correlations between clinical and physiological consequences of the novel mutation R878C in a highly conserved pore residue in the cardiac Na<sup>+</sup> channel,” *Acta Physiologica*, vol. 194, no. 4, pp. 311–323, 2008.
- [45] P. J. Laitinen-Forsblom, P. Makynen, H. Makynen et al., “*SCN5A* mutation associated with cardiac conduction defect and atrial arrhythmias,” *Journal of Cardiovascular Electrophysiology*, vol. 17, no. 5, pp. 480–485, 2006.
- [46] B. Bianchi, L. C. Ozhatil, A. Medeiros-Domingo, M. H. Gollob, and H. Abriel, “Four *TRPM4* cation channel mutations found in cardiac conduction diseases lead to altered protein stability,” *Frontiers in Physiology*, vol. 9, p. 177, 2018.
- [47] H. Liu, L. el Zein, M. Kruse et al., “Gain-of-function mutations in *TRPM4* cause autosomal dominant isolated cardiac conduction disease,” *Circulation: Cardiovascular Genetics*, vol. 3, no. 4, pp. 374–385, 2010.
- [48] M. Kruse, E. Schulze-Bahr, V. Corfield et al., “Impaired endocytosis of the ion channel *TRPM4* is associated with human progressive familial heart block type I,” *The Journal of Clinical Investigation*, vol. 119, no. 9, pp. 2737–2744, 2009.
- [49] R. Sepp, L. Hategan, A. Bacsi et al., “Timothy syndrome 1 genotype without syndactyly and major extracardiac manifestations,” *American Journal of Medical Genetics Part A*, vol. 173, no. 3, pp. 784–789, 2017.
- [50] Z. Mao, Y. Wang, H. Peng et al., “A newly identified missense mutation in *CLCA2* is associated with autosomal dominant cardiac conduction block,” *Gene*, vol. 714, article 143990, 2019.
- [51] X. J. Tan, H. Huang, F. He et al., “Mutation screening for the causative gene in a four-generation Chinese pedigree with progressive cardiac conduction defect,” *Zhonghua Xin Xue Guan Bing Za Zhi*, vol. 44, no. 5, pp. 411–415, 2016.
- [52] T. R. Jurcu, A. E. Bastian, S. Militaru et al., “Discovery of a new mutation in the desmin gene in a young patient with cardiomyopathy and muscular weakness,” *Romanian Journal of Morphology and Embryology*, vol. 58, no. 1, pp. 225–230, 2017.
- [53] S. Castellana, S. Mastroianno, P. Palumbo et al., “Sudden death in mild hypertrophic cardiomyopathy with compound *DSG2/DSC2/MYH6* mutations: Revisiting phenotype after genetic assessment in a master runner athlete,” *Journal of Electrocardiology*, vol. 53, pp. 95–99, 2019.
- [54] A. Seki, T. Ishikawa, X. Daumy et al., “Progressive atrial conduction defects associated with bone malformation caused by a connexin-45 mutation,” *Journal of the American College of Cardiology*, vol. 70, no. 3, pp. 358–370, 2017.
- [55] B. Csányi, L. Hategan, V. Nagy et al., “Identification of a novel *GLA* gene mutation, p.Ile239Met, in fabry disease with a predominant cardiac phenotype,” *International Heart Journal*, vol. 58, no. 3, pp. 454–458, 2017.
- [56] B. Stallmeyer, J. Kuß, S. Kotthoff et al., “A mutation in the G-protein Gene *GNB2* Causes familial sinus node and atrioventricular conduction dysfunction,” *Circulation Research*, vol. 120, no. 10, pp. e33–e44, 2017.
- [57] J. Zhou, W. G. Ding, T. Makiyama et al., “A novel *HCN4* mutation, G1097W, is associated with atrioventricular block,” *Circulation Journal*, vol. 78, no. 4, pp. 938–942, 2014.
- [58] S. U. M. Vanninen, K. Leivo, E. H. Seppälä et al., “Heterozygous junctophilin-2 (*JPH2*) p.(Thr161Lys) is a monogenic cause for HCM with heart failure,” *PLoS One*, vol. 13, no. 9, article e0203422, 2018.
- [59] N. Yamada, Y. Asano, M. Fujita et al., “Mutant *KCNJ3* and *KCNJ5* potassium channels as novel molecular targets in bradyarrhythmias and atrial fibrillation,” *Circulation*, vol. 139, no. 18, pp. 2157–2169, 2019.
- [60] R. Petillo, P. D’Ambrosio, A. Torella et al., “Novel mutations in *LMNA* A/C gene and associated phenotypes,” *Acta Myologica*, vol. 34, no. 2-3, pp. 116–119, 2015.
- [61] X. Wu, Q. K. Wang, L. Gui et al., “Identification of a new lamin A/C mutation in a Chinese family affected with atrioventricular block as the prominent phenotype,” *Journal of Huazhong University of Science and Technology [Medical Sciences]*, vol. 30, no. 1, pp. 103–107, 2010.
- [62] A. Saga, A. Karibe, J. Otomo et al., “Lamin A/C gene mutations in familial cardiomyopathy with advanced atrioventricular block and arrhythmia,” *The Tohoku Journal of Experimental Medicine*, vol. 218, no. 4, pp. 309–316, 2009.
- [63] B. F. M. Romeike, K. Becker, J. Grosskreutz, S. Schulz, J. Weis, and S. Cirak, “A family with limb girdle muscular dystrophy type 1B and multiple exostoses,” *Clinical Neuropathology*, vol. 38, no. 9, pp. 225–232, 2019.
- [64] A. R. G. Francisco, I. Santos Goncalves, F. Veiga, M. Mendes Pedro, F. J. Pinto, and D. Brito, “Fenótipo complexo associado a uma mutação no exão 11 do gene da lâmina A/C: miocardiopatia hipertrófica, bloqueio auriculoventricular, dislipidemia grave e diabetes mellitus,” *Revista Portuguesa de Cardiologia*, vol. 36, no. 9, pp. 669.e1–669.e4, 2017.
- [65] C. Kouakam, S. Boule, and F. Brigadeau, “Bloc auriculo-ventriculaire de haut degré révélant une cardiomyopathie hypertrophique liée à une mutation du gène *MYBPC3*,” *La Presse Médicale*, vol. 48, no. 1, pp. 68–71, 2019.

- [66] F. Yuan, X. B. Qiu, R. G. Li et al., "A novel NKX2-5 loss-of-function mutation predisposes to familial dilated cardiomyopathy and arrhythmias," *International Journal of Molecular Medicine*, vol. 35, no. 2, pp. 478–486, 2015.
- [67] S. Pabst, B. Wollnik, E. Rohmann et al., "A novel stop mutation truncating critical regions of the cardiac transcription factor NKX2-5 in a large family with autosomal-dominant inherited congenital heart disease," *Clinical Research in Cardiology*, vol. 97, no. 1, pp. 39–42, 2008.
- [68] W. H. Xie, C. Chang, Y. J. Xu et al., "Prevalence and spectrum of Nkx2.5 mutations associated with idiopathic atrial fibrillation," *Clinics*, vol. 68, no. 6, pp. 777–784, 2013.
- [69] M. Disertori, S. Quintarelli, M. Grasso et al., "Autosomal recessive atrial dilated cardiomyopathy with standstill evolution associated with mutation of *natriuretic peptide precursor A*," *Circulation: Cardiovascular Genetics*, vol. 6, no. 1, pp. 27–36, 2013.
- [70] T. Esposito, R. A. Lea, B. H. Maher et al., "Unique X-linked familial FSGS with co-segregating heart block disorder is associated with a mutation in the *NXF5* gene," *Human Molecular Genetics*, vol. 22, no. 18, pp. 3654–3666, 2013.
- [71] J. Y. Su, R. F. Zhang, Y. X. Dong et al., "Preprodynorphin gene mutation causes progressive cardiac conduction disease: a whole-exome analysis of a pedigree," *Life Sciences*, vol. 219, pp. 74–81, 2019.
- [72] J. Thevenon, G. Laurent, F. Ader et al., "High prevalence of arrhythmic and myocardial complications in patients with cardiac glycogenosis due to *PRKAG2* mutations," *Europace*, vol. 19, no. 4, pp. 651–659, 2017.
- [73] J. Chen, T. Makiyama, Y. Wuriyanghai et al., "Cardiac sodium channel mutation associated with epinephrine-induced QT prolongation and sinus node dysfunction," *Heart Rhythm*, vol. 13, no. 1, pp. 289–298, 2016.
- [74] S. Y. Nikulina, A. A. Chernova, V. A. Shulman et al., "An investigation of the association of the H558R polymorphism of the *SCN5A* gene with idiopathic cardiac conduction disorders," *Genetic Testing and Molecular Biomarkers*, vol. 19, no. 6, pp. 288–294, 2015.
- [75] M. Asadi, R. Foo, M. R. Samienasab et al., "Genetic analysis of Iranian family with hereditary cardiac arrhythmias by next generation sequencing," *Advanced Biomedical Research*, vol. 5, no. 1, article 178801, p. 55, 2016.
- [76] K. Abe, T. Machida, N. Sumitomo et al., "Sodium channelopathy underlying familial sick sinus syndrome with early onset and predominantly male characteristics," *Circulation: Arrhythmia and Electrophysiology*, vol. 7, no. 3, pp. 511–517, 2014.
- [77] H. Watanabe, T. Yang, D. M. Stroud et al., "Striking in vivo phenotype of a disease-associated human *SCN5A* mutation producing minimal changes in vitro," *Circulation*, vol. 124, no. 9, pp. 1001–1011, 2011.
- [78] D. Hu, H. Barajas-Martinez, V. V. Nesterenko et al., "Dual variation in *SCN5A* and *CACNB2b* underlies the development of cardiac conduction disease without Brugada syndrome," *Pacing and Clinical Electrophysiology*, vol. 33, no. 3, pp. 274–285, 2010.
- [79] A. Moreau, A. Janin, G. Millat, and P. Chevalier, "Cardiac voltage-gated sodium channel mutations associated with left atrial dysfunction and stroke in children," *EP Europace*, vol. 20, no. 10, pp. 1692–1698, 2018.
- [80] C. Thongnak, P. Limprasert, D. Tangviriyapaiboon et al., "Exome sequencing identifies compound heterozygous mutations in *SCN5A* associated with congenital complete heart block in the Thai population," *Disease Markers*, vol. 2016, Article ID 3684965, 10 pages, 2016.
- [81] S. Baskar, M. J. Ackerman, D. Clements, K. A. Mayuga, and P. F. Aziz, "Compound heterozygous mutations in the *SCN5A*-encoded Nav1.5 cardiac sodium channel resulting in atrial standstill and His-Purkinje system disease," *The Journal of Pediatrics*, vol. 165, no. 5, pp. 1050–1052, 2014.
- [82] J.-B. Selly, B. Boumahni, A. Edmar et al., "Cardiac sinus node dysfunction due to a new mutation of the *SCN5A* gene," *Archives de Pédiatrie*, vol. 19, no. 8, pp. 837–841, 2012.
- [83] J. Ge, A. Sun, V. Paajanen et al., "Molecular and clinical characterization of a novel *SCN5A* mutation associated with atrioventricular block and dilated cardiomyopathy," *Circulation: Arrhythmia and Electrophysiology*, vol. 1, no. 2, pp. 83–92, 2008.
- [84] T. Robyns, D. Nuyens, L. Van Casteren et al., "Reduced penetrance and variable expression of *SCN5A* mutations and the importance of co-inherited genetic variants: case report and review of the literature," *Indian Pacing and Electrophysiology Journal*, vol. 14, no. 3, pp. 133–149, 2014.
- [85] B. Stallmeyer, S. Zumhagen, I. Denjoy et al., "Mutational spectrum in the  $Ca^{2+}$ -activated cation channel gene *TRPM4* in patients with cardiac conductance disturbances," *Human Mutation*, vol. 33, no. 1, pp. 109–117, 2012.
- [86] N. Syam, S. Chatel, L. C. Ozhatil et al., "Variants of transient receptor potential melastatin member 4 in childhood atrioventricular block," *Journal of the American Heart Association*, vol. 5, no. 5, 2016.