Research Article

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

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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 | PVS1 | 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 trans 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 cis 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. |
2. Materials and Methods

2.1. Database Search. We searched the PubMed database by using the term “heart block” or “sick sinus syndrome” associated with “pedigree” and “2008/1/1” [PDAT]: ‘2009/9/1’ [PDAT] [We used the term of (((((((((((((((((((Heart Block) OR Block, Heart) OR Blocks, Heart) OR Heart Blocks) OR Auriculo-Ventricular Dissociation) OR Auriculo-Ventricular Dissociations) OR Dissociation, Auriculo-Ventricular) OR Dissociations, Auriculo-Ventricular) OR Atrioventricular Dissociation) OR Atrioventricular Dissociations) OR Dissociation, Atrioventricular (version: hg38) to evaluate sequence variants directly. According to the ACMG/AMP classification framework, we used InterVar (http://wintervar.wglab.org) (version: hg38) to evaluate sequence variants directly. With those variants that could not be defined in InterVar, we used The Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org) to complete detailed information on each variant.

2.2. Study Selection. The aim of this study was to evaluate genetic studies of bradycardia, in addition to the inclusion criteria and exclusion criteria, as follows:

Inclusion criterion:

(i) Article published in English or have an abstract written in English
(ii) Pedigree studies with at least one family member with bradycardia (include both sick sinus syndrome and atrioventricular block)

Exclusion criteria:

(i) Functional studies that demonstrate the main function of the sequence variants that are not focused on bradycardia
(ii) Studies that have not demonstrated the specific mutation sites

2.3. Sequence Variants Analyze

2.3.1. Organization of Relevant Sequence Variants. After a thorough evaluation of the related articles by two researchers, we gathered basic information about relevant sequence variants. The information included the chromosome position of the sequence variant (version: GRCh38), genomic sequence, protein sequence, dbSNP, gene, clinical manifestations, and so on.

2.3.2. Clarification of Sequence Variants. The variants were named after different versions of genomics, so we used The National Center for Biotechnology Information’s ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), Online Mendelian Inheritance in Man (OMIM, https://www.omim.org), and The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) to complete detailed information on each variant.

2.3.3. Use of the ACMG/AMP Classification Framework to Evaluate. According to the ACMG/AMP classification framework, we used InterVar (http://wintervar.wglab.org) (version: hg38) to evaluate sequence variants directly. With those variants that could not be defined in InterVar, we used The Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org) to complete detailed information on each variant.
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 variant classification. InterVar [16] is a tool implementing ACMG/AMP criterion 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; GNBS5 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 re-evaluated. 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 [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 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).
Table 3: Evaluate all sequence variants using InterVar database.

| Chr | Position | Ref | Alt | Gene       | Criterion             | Clinical manifest | Authors                          |
|-----|----------|-----|-----|------------|-----------------------|-------------------|----------------------------------|
| 12  | 21882785 | G   | A   | ABC9       | Likely pathogenic     | PCCD; SSS         | Celestino-Soper et al. [18]     |
| 1   | 23673100 | 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]                 |
| 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   | 10067651  | G   | T   | GN2B      | Uncertain significance| SSS; AVB          | Stallmeyer et al. [56]          |
| 15  | 73329719  | C   | T   | HCN4      | Pathogenic/likely pathogenic| SSS; LVNC        | Milano et al. [28]             |
| 15  | 73343416  | A   | T   | HCN4      | Uncertain significance| SSS; AF; LVNC     | Ishikawa et al. [31]           |
| 15  | 73329719  | C   | T   | HCN4      | Pathogenic/likely pathogenic| SSS             | Ishikawa et al. [31]           |
| 15  | 73323745  | G   | C   | HCN4      | Likely benign          | SSS               | Schweizer et al. [29]          |
| 15  | 73322804  | C   | A   | HCN4      | 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   | 15555534  | 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      | Gocklihofer 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]            |
| Chr | Position | Ref | Alt | Gene | Criterion               | Clinical manifest                                      | Authors |
|-----|----------|-----|-----|------|-------------------------|--------------------------------------------------------|---------|
| 5   | 172250006 | G   | A   | NKX2-5 | Uncertain significance | AVB; AF; DCM                                           | Yuan et al. [66] |
| 5   | 172261762 | C   | A   | NKX2-5 | Uncertain significance | AVB; congenital cardiovascular diseases (CCVD)          | Palbst et al. [67] |
| 5   | 172260110 | C   | 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                                                   | Abe et al. [75]  |
| 3   | 38613790  | C   | T   | SCN5A  | Likely pathogenic       | SSS                                                   | Abe et al. [76]  |
| 3   | 38566266  | C   | T   | SCN5A  | Pathogenic              | AVB; DCM                                               | Watanabe et al. [77] |
| 3   | 38550899  | T   | A   | SCN5A  | Uncertain significance | SSS                                                   | Ishikawa et al. [31] |
| 3   | 38581137  | G   | A   | SCN5A  | Likely benign           | AVB                                                   | Hu et al. [78]   |
| 3   | 38581002  | C   | T   | SCN5A  | Uncertain significance | SSS; AFL; AF                                           | Moreau et al. [79] |
| 3   | 38633207  | G   | T   | SCN5A  | Uncertain significance | AVB                                                   | Thongnak et al. [80] |
| 3   | 38613787  | G   | A   | SCN5A  | Uncertain significance | PCCD; SSS                                              | Baskar et al. [81] |
| 3   | 38597787  | C   | A   | SCN5A  | Likely pathogenic       | SSS; AFL                                               | Selly et al. [82] |
| 3   | 38630342  | T   | A   | SCN5A  | Pathogenic/likely pathogenic | SSS; AFL; VT                                        | Holst et al. [43] |
| 3   | 38575424  | C   | A   | SCN5A  | Uncertain significance | AVB; DCM                                               | Ge et al. [83]   |
| 3   | 38551477  | A   | T   | SCN5A  | Likely pathogenic       | SSS; AVB                                               | Robyns et al. [84] |
| 3   | 38560398  | G   | A   | SCN5A  | Pathogenic              | AVB                                                   | Thongnak et al. [80] |
| 3   | 38550968  | C   | A   | SCN5A  | Uncertain significance | SSS                                                   | Abe et al. [76]  |
| 19  | 49196760  | G   | A   | TRPM4  | Uncertain significance | PCCD                                                   | Liu et al. [47] |
| 19  | 49157855  | G   | A   | TRPM4  | Pathogenic              | PCCD; SSS                                              | Kruse et al. [48] |
| 19  | 49167950  | G   | A   | TRPM4  | Pathogenic              | AVB; VT                                                | Bianchi et al. [46] |
| 19  | 49167990  | A   | G   | TRPM4  | Likely benign           | AVB                                                   | Dauny et al. [5] |
| 19  | 49201240  | A   | T   | TRPM4  | Uncertain significance | AVB; VT                                                | Bianchi et al. [46] |
| 19  | 49173597  | A   | G   | TRPM4  | Uncertain significance | AVB                                                   | Stallmeyer et al. [85] |
| 19  | 49200395  | A   | G   | TRPM4  | Pathogenic              | AVB                                                   | Stallmeyer et al. [85] |
| 19  | 49168301  | C   | T   | TRPM4  | Pathogenic              | PCCD                                                   | Liu et al. [47] |
| 19  | 49182608  | G   | A   | TRPM4  | Uncertain significance | AVB                                                   | Syam et al. [86] |
| 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 analyze frameshift mutations.

| 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.234ddC                        | -                | -                                  |
| -         | Chr5:173232761 | rs587784067 | NKK2.5 | 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_3153del112ins11          | -                | -                                  |
| MYH6      |     |          |      | delE933                         | -                | -                                  |
| MYL4      |     |          |      | c.234ddC                        | -                | -                                  |
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.

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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 APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC 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. Mikhailov, E. Parmon et al., “Progressive cardiac conduction disease associated with a DSP gene mutation,” International Journal of Cardiology, vol. 216, pp. 188-189, 2016.
[36] C. R. Glöckelhofer, 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 Europe, 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. Hotli, F. Ara, and J. Timperley, “p.Y1449C SCN5A mutation associated with overlap disorder comprising cardiac 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 pathological consequences of the novel mutation R878C in a highly conserved pore residue in the cardiac Na+ 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. Ozthahil, 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, pp. 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 endothytosis 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. Bacci 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. Mikyama 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.(Thr161Iys) is a monogenic cause for HCM with heart failure,” PLoS One, vol. 13, no. 9, article 0203422, 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. 454–458, 2006.

[61] B. Bianchi, L. C. Ozthahil, 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, pp. 177, 2018.

[62] 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.

[63] M. Kruse, E. Schulze-Bahr, V. Corfield et al., “Impaired endothytosis 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.

[64] R. Sepp, L. Hategan, A. Bacci 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.

[65] 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.
[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 arrhythmia,” *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 starndstill 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 NFXF 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. Adet al., “High prevalence of arrhythmic and myocardial complications in patients with cardiac glycosenosis due to PRKAG2 mutations,” *Europeace*, 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 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. Tangviriyaipaiboon 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 Ca2+–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. Ozhatih 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.