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ORIGINAL ARTICLE

RAF1 mutation leading to hypertrophic cardiomyopathy in a Chinese family with a history of sudden cardiac death: A diagnostic insight into Noonan syndrome

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Abstract

Background: Hypertrophic cardiomyopathy (HCM) is predominantly caused by mutations in sarcomeric genes. However, a subset of cases is attributed to genetic disorders unrelated to sarcomeric genes, such as Noonan syndrome (NS) and other RASopathies. In this study, we present a family with a history of sudden cardiac death (SCD) and focus on two adults with syndromic left ventricular hypertrophy (LVH).

Methods: Clinical evaluations, including echocardiography, were conducted to assess cardiac manifestations. Whole-exome sequencing was performed to identify potential genetic variants underlying syndromic LVH in the study participants.

Results: Whole-exome sequencing revealed a missense variant in the *RAF1* gene, c.782C>T (*p*.Pro261Leu). This variant confirmed the diagnosis of NS in the affected individuals.

Conclusion: The findings of this study underscore the importance of family history investigation and genetic testing in diagnosing syndromic LVH. By identifying the underlying genetic cause, clinicians can better understand the etiology of RAS-HCM and its association with SCD in young adults.

KEYWORDS

hypertrophic cardiomyopathy, Noonan syndrome, *RAF1* gene, RAS-HCM, whole-exome sequencing

1 | INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiomyopathy (Semsarian et al., 2015), predominantly inherited in an autosomal dominant manner (Sabater-Molina et al., 2018). Approximately 50–60% of HCM cases are caused by mutations in genes encoding sarcomeric proteins (Elliott et al., 2014). Nevertheless, a subgroup (5%–10%) is caused by genetic disorders unrelated to sarcomeric genes, including inherited metabolic and

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neuromuscular diseases (Elliott et al., 2014; Limongelli et al., 2013; Medical Masterclass & Firth, 2019).

RASopathies represent a significant etiology of HCM, comprising up to 12.5% of pediatric HCM cases (Aljeaid et al., 2019; Calcagni et al., 2018; Elliott et al., 2014) and up to 33% of infantile HCM cases (Norrish et al., 2021). These conditions are distinguished by dysregulated intracellular signaling mediated by RAS and elevated mitogen-activated protein kinase (MAPK) (Calcagni et al., 2018). RAS-HCM is a frequent type of secondary HCM and often regarded as HCM phenocopies. When compared to non-syndromic, isolated primary HCM (P-HCM), RAS-HCM patients demonstrate earlier onset and a more severe clinical presentation (Wilkinson et al., 2012). In contrast to P-HCM, RAS-HCM conveys a significant risk of mortality in infancy, which may be attributable to younger age at diagnosis and development of heart failure with preserved systolic function (Alexander et al., 2018; Norrish et al., 2021).

Noonan syndrome (NS) is a RASopathy, characterized by distinctive facial features, short stature, congenital heart defects, and varying degrees of developmental delays (Gelb et al., 2015; Roberts, 1993). The majority of NS patients have a cardiovascular involvement, which can include a broad range (50%–80%) of congenital heart disease (CHD) and/or early-onset RAS-HCM (Roberts, 1993). The onset of NS generally occurs during prenatal development, although milder cases may be identified later in life. NS is primarily associated with autosomal dominant inheritance, but in recent years, it has also been linked to recessive inheritance patterns, as seen in genes like LZTR1 (Johnston et al., 2018; Yamamoto et al., 2015) and SPRED2 (Motta et al., 2021).

Identifying the causative mutation in patients with syndromic left ventricular hypertrophy (LVH) is beneficial, particularly for those with a family history of SCD. The underlying genotype can aid in the diagnosis, surveillance, and treatment of the disease, with the goal of reducing morbidity and mortality. The current study involves a clinical and genetic evaluation of two adult cases with syndromic LVH and a family history of SCD. It identifies a potential pathogenic mutation in the *RAF1* gene (OMIM 164760) and confirms the diagnosis of NS in these two patients.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The project was approved by the Ethics Committee of Sun Yat-sen University (Approval No. 2019–004), and it was carried out in strict accordance with the ethical research principle of Sun Yat-sen University.

2.2 | Whole-exome sequencing

Peripheral blood samples were collected from patients and genomic DNA was extracted using standard techniques. The quality of the DNA was evaluated using Nanodrop 2000. The coding exons were captured using the Agilent SureSelect Human All Exon 51M. The captured fragments were then sequenced on the Illumina Hiseq2000 sequencer to an average depth over 50 reads per target base. The sequence reads were aligned to the human reference genome (UCSC NCBI37.1/hg19) using the SOAPaligner (version 2.21, Beijing Genomics Institute), and high-quality variants were functionally annotated using SOAPsnp software (Beijing Genomics Institute) for the single-nucleotide variation.

2.3 | Variant screening

To identify putatively pathogenic variants, we used the following screening strategies. First, variants were excluded based on the following criteria: (1) Variants with a minor allele frequency (MAF) greater than 1% in the gnomAD database (http://www.gnomad-sg.org/); (2) non-coding and synonymous variants. Subsequently, the remaining variants were evaluated for their clinical relevance with hypertrophic cardiomyopathy based on information from OMIM (https://www.omim.org/), ClinGen (https://www.clinicalgenome.org/), and ClinVar (https://ncbi.nlm.nih.gov/clinvar/) and then subjected into in silico pathogenicity prediction analysis using SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationAssessor (http://mutationassessor.org), or CONDEL (http://bg.upf.edu/fannsdb/). Finally, the likely causative variants identified through whole-exome sequencing (WES) were validated using Sanger sequencing.

3 | RESULTS

3.1 | Clinical findings

The proband is a 22-year-old young-adult male (III-4) of Han Chinese descent with apparently healthy non-consanguineous parents (Figure 1a). He had normal psychophysical development. After experiencing several episodes of palpitation, dizziness, and syncope, he sought medical attention at the First Affiliated Hospital of Sun Yat-sen University and underwent a comprehensive clinical evaluation. During the physical examination, he was found to have a stature of 157 cm and slightly curly hair, as well as prominent nasolabial folds. No other signs of cardiovascular disease were

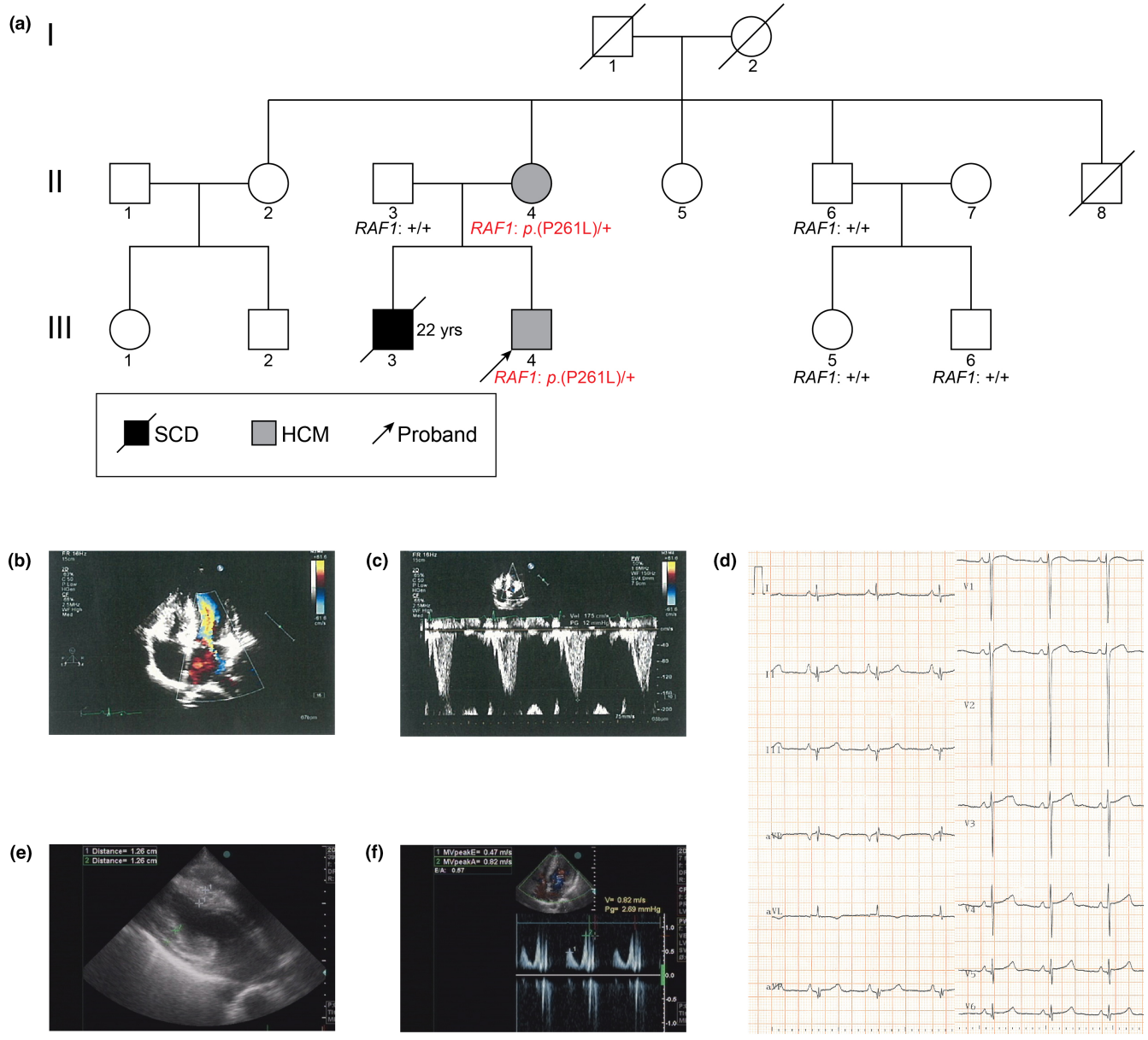

FIGURE 1 Pedigree, echocardiograph, and ECG of patients. (a) The pedigree shows co-segregation of the mutation *RAF1*-P261L (NM_002880.4: c.782C>T) with disease in affected family members. (b) Four-chamber echocardiographic view of III-4 showing interventricular septal hypertrophy (17 mm), color doppler flow imaging show mild mitral regurgitation. (c) Pulsed wave Doppler imaging of III-4 showing an increased blood flow velocity of left ventricular outflow tract (Vmax = 1.7 m/s, PG = 16 mmHg). (d) A 12-lead ECG of III-4 showing P pulmonale; J point elevation in II, III, aVF, V3-6; and Q wave in II, III, and aVF. (e) Four-chamber echocardiographic view of II-4 showing left ventricular hypertrophy (with an interventricular septal thickness of 13 mm, a left ventricular posterior wall thickness of 13 mm). (f) M-mode echocardiography of II-4 showing reduced left ventricular diastolic function. E/A = 0.57.

detected. An echocardiogram revealed asymmetric LVH with an increased interventricular septal thickness of 17 mm (normal range: <12 mm) (Figure 1b), a slightly increased right ventricular anterior wall thickness of 5.2 mm (normal range: 3–4 mm), a hyperdynamic left ventricle with an ejection fraction of 78% (> 60%), an elevated blood flow velocity in the left ventricular outflow tract (Figure 1c), and mild mitral regurgitation (Figure 1b) and no evidence of pulmonary valve stenosis. A 12-lead electrocardiogram showed possible right atrial enlargement and early repolarization: P pulmonale; J point elevation in II, III, aVF, V3-6; and Q wave in II, III, and aVF (Figure 1d).

Parental examination found the proband's mother (II-4) also displayed symptoms of palpitations, shortness of breath, and edema of both lower limbs at the age of 54. She was 147 cm tall and had prominent nasolabial fold facial features with the proband. Her echocardiographic

evaluation showed LVH, with an interventricular septal thickness of 13 mm, a left ventricular posterior wall thickness of 13 mm (Figure 1e), and decreased left ventricular diastolic function (E/A = 0.57, Figure 1f). Although her blood pressure and aortic valve were normal, she was suspected of having RAS-HCM. On the other hand, the proband's father showed no signs of facial dysmorphism and had no evidence of cardiovascular disease.

Family history investigation revealed that the proband's elder brother (III-3) passed away unexpectedly at the age of 22 while sleeping in his factory dormitory at night. Further investigation, including a death-scene examination, toxicology tests, and forensic autopsy ruled out any possibility of murder or suicide. The autopsy results, which included gross examination and histological analysis, indicated that cardiac hypertrophy-caused arrhythmias might be the probable cause of death.

3.2 | Genetic findings

The proband and his mother underwent WES based on their clinical and echocardiographic features. Unfortunately, the samples of the proband's elder brother were unavailable.

The WES revealed a heterozygous mutation in the *RAF1* gene (NM_002880.4: c.782C>T, *p*.Pro261Leu) in both patients (III-4 and II-4), which has previously been reported as a causative factor for NS (Kobayashi et al., 2010; Pandit et al., 2007). The variant identified by WES was also validated by Sanger sequencing (Figure 2). No pathogenic mutations were detected in other genes associated with NS.

All the family members underwent a comprehensive cardiological evaluation and no signs of cardiovascular disease were observed. The proband's father (II-4), uncle on his mother's side (II6), and two cousins (III5 and III6) underwent mutation confirmation for the *RAF1 p*.P261L variant, with negative results. The family pedigree is shown in Figure 1a.

4 | DISCUSSION

NS is a hereditary multisystem disorder that affects approximately 1 in every 1000–2500 individuals in the general population. It is recognized by its dominant autosomal inheritance pattern (Mendez & Opitz, 1985; Noonan, 1968; Roberts, 1993). This condition presents with various features, including short stature, craniofacial dysmorphism, CHD, skeletal abnormalities, developmental delay, hematologic disorders, and other abnormalities (Roberts, 1993).

CHD is a major concern in the long-term management of NS patients. It is estimated that CHD affects between 50% and 80% of individuals with this condition (Roberts, 1993). The most commonly observed CHD in NS is pulmonary valve stenosis, often accompanied by dysplasia, which occurs in 25%–71% of affected individuals (Roberts, 1993). Hypertrophic cardiomyopathy is present in 10%–29% of NS patients and typically manifests at an early age, impacting their survival (Gelb et al., 2015; Hickey et al., 2011; Wilkinson et al., 2012).

NS is a genetically heterogeneous disorder caused by mutations in genes that are involved in the RAS/MAPK pathway (Baban et al., 2019; Calcagni et al., 2018). The most frequently mutated gene in NS is PTPN11, accounting for approximately 50% of cases, with individuals carrying PTPN11 mutations often presenting with pulmonary valve stenosis (Tartaglia et al., 2002). Other genes involved in NS include SOS1 (present in 10%–13% of cases) (Roberts et al., 2007; Tartaglia et al., 2007), LZTR1 (present in ~8% of cases) (Chen et al., 2014; Yamamoto et al., 2015), RAF1 (present in 5%–15% of cases) (Pandit et al., 2007; Razzaque et al., 2007), and RIT1 (present in ~5% of cases) (Aoki et al., 2013; Gelb et al., 2015). Among the genes involved in NS, RAF1 is the major gene associated with RAS-HCM in NS. Approximately 95% of individuals with NS who harbor pathogenic *RAF1* variants present with RAS-HCM (Kobayashi et al., 2010; Pandit et al., 2007; Razzaque et al., 2007).

The gene *RAF1*, located on Chr3p25.2, encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) that acts as a downstream effector of the RAS signaling pathway. This gene is a member of the *RAF* proto-oncogene family, which includes three conserved regions (CR1, CR2, and CR3). The CR2 domain, located in exon 7 and composed of residues 251–266, serves as a site of regulatory phosphorylation and interacts with the 14-3-3 protein that includes the residues Arg256, Ser257, Ser259, and Pro261 (Thompson et al., 2017). According to other studies (Table S1), we found that the clinical heterogeneity of carriers of *RAF1* mutations varied, with carriers of CR2 mutations having a younger median age than carriers of mutations in other domains (6 [1.17, 10] years old vs. 14.5 [6.25, 17.5] years old, $p = 0.0284$). This difference may be due to the fact that variants clustered in the CR2 domain of *RAF1* are more strongly associated with RAS-HCM in NS (CR2: 84.2% vs. Not CR2: 25%), and RAS-HCM in NS typically presents at an early age (Pierpont & Digilio, 2018).

The present study aimed to identify the causative gene variant underlying syndromic LVH in a family with a history of SCD. Our investigation led to the identification of a mutation, *p*.P261L (c.782C>T), located in the CR2 domain of the *RAF1* gene. This particular mutation has been

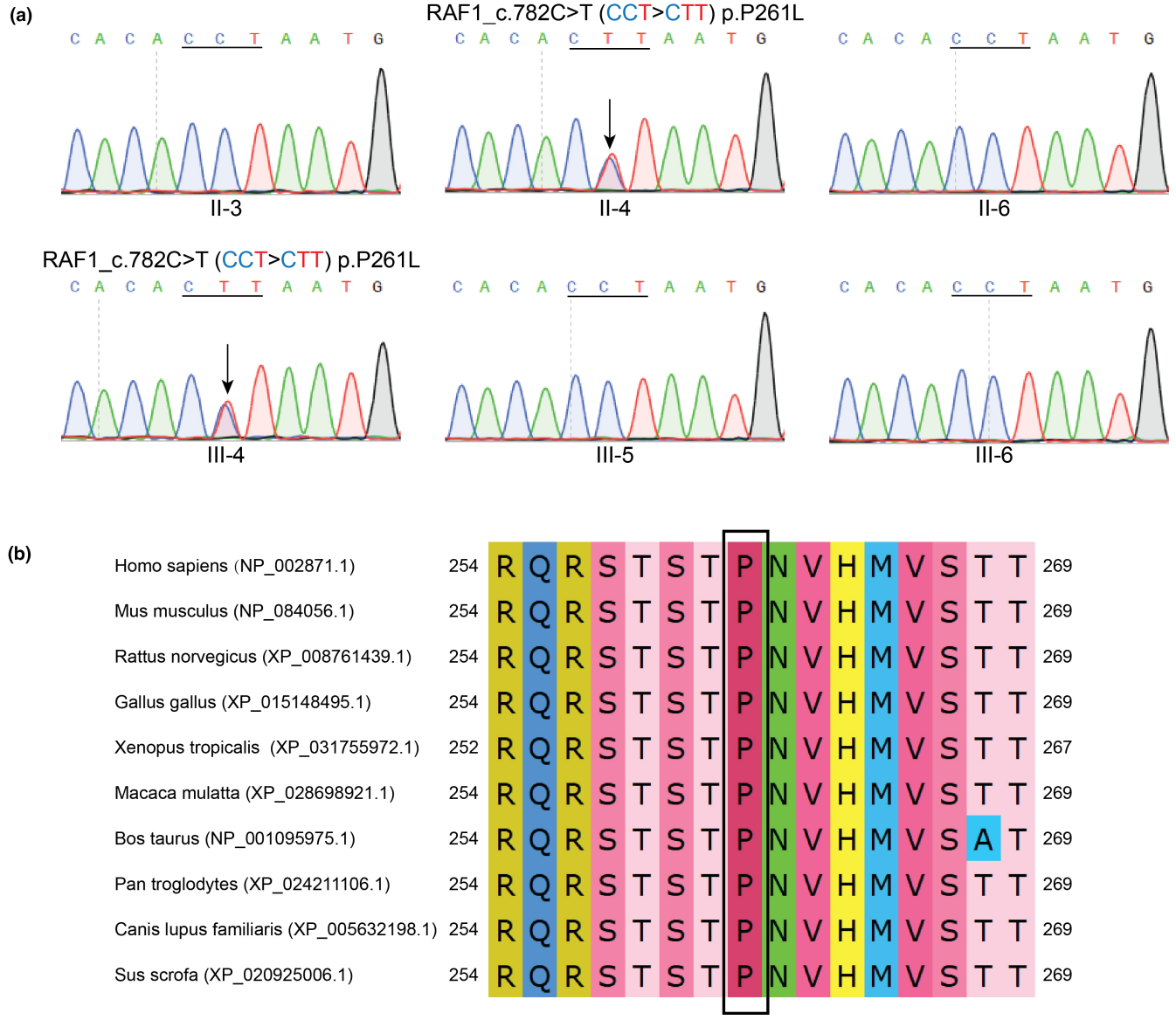

FIGURE 2 *RAF1* missense substitution. (a) Sequence analyses of *RAF1* exon 7 shows a heterozygous base substitution of proline c.782 by leucine in II-4 and III-4. II-3, II-6, III-5 and III-6 show as wild-type. The black line under the nucleotide sequence highlights codon 261 (NM_002880.4: c.782C>T). (b) Amino acid sequence alignment of the human *RAF1* CR2 domain (amino acid residues 254–269) with the orthologues from different species (Software: Unipro Ugene 45.1).

widely described as a pathogenic variant for NS in previous studies (Kobayashi et al., 2010; Pandit et al., 2007). It was first reported in a 6-year-old girl with clinical symptoms such as short stature, webbed neck, pectus anomalies, and dysmorphic facial features, including macrocephaly, downslanting palpebral fissures, hypertelorism, epicanthal folds, palpebral ptosis, a flat nasal bridge, and low-set ears with a thickened helix. An echocardiogram also showed the presence of RAS-HCM and a mitral valve anomaly (Pandit et al., 2007). In the current study, the proband and his mother only presented with LVH, curly hair and prominent nasolabial folds, but no significant facial dysmorphism such as a tall forehead and hypertelorism, which can decrease with age. Both patients had short stature, but this cannot be solely attributed to NS as other healthy individuals in the family also had similar height.

The majority of variants are clustered on the Ser257 residue. However, only one type of missense variant, *p*.Ser257Leu, has been reported at this residue (Kobayashi et al., 2010; Pandit et al., 2007; Razzaque et al., 2007; Xu et al., 2017). In contrast to the Ser257 residue, the Pro261 residue, where the *p*.P261L variant identified in this study is located, exhibits various missense mutations (see Table S2). Among these, *p*.P261S is the most common, although no cases resulting in mortality have been reported. While *p*.P261R has only been reported in two cases (Ratola et al., 2015; Thompson et al., 2017), both individuals succumbed during infancy, representing the only two

documented fatalities associated with Pro261 residue mutations, suggesting a high risk of mortality associated with this mutation. As for the *p*.P261L variant identified in this study, a total of four cases have been reported, including the two cases presented in this study, of which three involve adult individuals. Another gene, *RIT1*, is also frequently associated with the development of RAS-HCM in patients with NS (Aoki et al., 2013). *RIT1* (Ras-like without CAAX 1), which belongs to the RAS subfamily of small GTPases and is ubiquitously expressed, has been found to be responsible for 56%–70% of RAS-HCM cases, higher than the incidence caused by mutations in *PTPN11* and *SOS1* (Aoki et al., 2013; Yaoita et al., 2016; Zha et al., 2022). The majority of the *RIT1* mutations occur in the switch II region, which is homologous to the Ras gene (Yaoita et al., 2016).

RAS-HCM carries a significant risk of mortality in infancy and a higher incidence of SCD during adolescence compared to primary hypertrophic cardiomyopathy (P-HCM) (Lynch et al., 2023). In RAS-HCM patients, the presence of sustained ventricular tachycardia (VT) is common and correlated with an increased risk of SCD (Aydin et al., 2011; Eichhorn et al., 2019; Petrin et al., 2019). Factors such as a history of VT or cardiac arrest, unexplained syncope, nonsustained VT, or extensive LVH are associated with a higher SCD risk and may warrant consideration of implantable cardioverter-defibrillator (ICD) implantation (Ommen et al., 2020). Interestingly, in a cohort study comparing pediatric RAS-HCM patients and P-HCM patients, the 10-year cumulative incidence of ICD insertions was five times lower in RAS-HCM than in P-HCM (Lynch et al., 2023). Better risk stratification is needed to guide ICD practices in RAS-HCM along with monitoring for heart failure and timely consideration of advanced heart failure therapies. Utilizing risk stratification tools, such as the Swedish HCMRisk-Kids score (Ostman-Smith et al., 2021), can help assess the individual risk of SCD in RAS-HCM patients.

For families with a history of SCD, a clinical and genetic diagnosis is of critical importance, as it helps identify at-risk family members and reduces the anxiety that can accompany such a condition. In the current study, genetic testing provided reassurance to the uncle and his children that they did not carry the pathogenic variant identified in the proband and his mother. Genetic counseling and testing for the RAF1 variant were recommended for the maternal family members who were at risk. Cardiomyopathies and arrhythmia-related disorders can both contribute to SCD. Unfortunately, our inability to access the autopsy report and genetic information of the deceased brother prevents us from pinpointing the precise cause of his passing, whether it resulted from RAS-HCM or another hereditary cardiac condition predisposing to SCD. Moreover, we solely confirmed the absence of the RAF1-*p*.P261L mutation in the proband's father through Sanger sequencing. However, the absence of this mutation in paternal family members does not guarantee a complete absence of SCD risk. For this reason, ongoing follow-up and regular evaluations for the proband's father are strongly recommended.

5 | CONCLUSION

In conclusion, this study underscores the pivotal role of comprehensive clinical evaluation, incorporating family history assessment and genetic testing, in the diagnosis and management of RAS-HCM. By identifying pathogenic variants within the RAF1 gene in this family, we have not only confirmed diagnoses but also highlighted the significance of genetic screening for at-risk family members. Our analysis, combined with existing literature, suggests that individuals carrying different variants at the *RAF1*-Pro261 residue may exhibit varying clinical phenotypes. Further elucidation of these distinctions will depend on additional clinical case reports and research efforts. Future research should continue to explore the diverse clinical presentations associated with RAS-HCM, enhancing our comprehension and advancing patient care.

AUTHOR CONTRIBUTIONS

Erwen Huang, Jianding Cheng, and Qianhao Zhao designed the project. Longyun Peng, Zhiyan Li, and Jianjie Xie conducted detailed examination on the whole family. Jingjing Zheng and Erwen Huang collected all the data and blood samples. Ruofei Cheng extracted the genomic DNA and conducted the Sanger sequencing. Jingjing Zheng, Longyun Peng, and Qianhao Zhao analyzed and discussed the data. Jingjing Zheng, Qianhao Zhao, and Jianding Cheng wrote the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article.

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SUPPORTING INFORMATION

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