Clinical and mutation profile of pediatric patients with RASopathy-associated hypertrophic cardiomyopathy: results from a Chinese cohort

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Abstract

Background: The RASopathies are a class of developmental disorders caused by germline mutations in the RAS-mitogen-activated protein kinase (MAPK) pathway. Hypertrophic cardiomyopathy (HCM) has been frequently described in children with RASopathy, but only a minority of patients have received formal genotyping. The purpose of this study was to evaluate the genetic basis and clinical outcome of pediatric patients with RASopathy-associated HCM.

Methods: We retrospectively reviewed the mutation spectrum and clinical outcome of all the patients with RASopathy derived from 168 pediatric HCM cases referred to our institution between January 2012 and July 2018.

Results: A heterozygous missense mutation in one of known RASopathy genes was identified in 46 unrelated children with HCM. Mutations in the PTPN11 gene were the most prevalent (19/46); this was followed by mutations in RAF1 (11/46), KRAS (5/46), RIT1 (4/46), BRAF (3/46), SOS1 (2/46), HRAS (1/46), and SHOC2 (1/46). Moreover, two compound heterozygous missense mutations in the LZTR1 gene were identified in one patient with the Noonan syndrome phenotype and HCM. The median age at the diagnosis of HCM was 3.0 months (range 0 months to 8.1 years). Twenty-one of the patients had significant left ventricular outflow tract obstruction and 32 had concomitant congenital heart disease. Three patients with a mutation in exon 13 of the PTPN11 gene died of cardiac failure at the ages of 3.0, 3.5, and 6.0 months. The remaining 44 patients were alive after an average follow-up time of 3.9 years (0.5 to 17.1 years, median 2.9 years) from the initial diagnosis of HCM, including 5 patients with spontaneous regression of their cardiac hypertrophy.

Conclusions: RASopathy-associated HCM is a heterogeneous genetic condition characterized by early-onset cardiac hypertrophy and a high prevalence of co-existing congenital heart disease, which is most frequently related to specific mutations in the PTPN11 gene. Rapidly progressive HCM, resulting in an early death, is uncommon in RASopathy patients except those with specific mutations in exon 13 of the PTPN11 gene.

Keywords: RAS-MAPK pathway, Hypertrophic cardiomyopathy, RASopathy
Background
Noonan syndrome (NS) is a relatively common genetic condition with an incidence of 1 in every 1000–2500 live births [1]. Hypertrophic cardiomyopathy (HCM) is present in approximately 20 to 30% of individuals with NS and represents a major determinant of outcome in these patients. Several genetic disorders phenotypically related to NS are also frequently associated with HCM, such as Noonan syndrome with multiple lentigines (NSML), cardiofaciocutaneous syndrome (CFCS), Costello syndrome (CS), and Noonan syndrome-like disorder with loose anagen hair (NS/LAH) [2].

NS and related syndromes, also known as the RASopathies, are caused by germline mutations in the RAS-mitogen-activated protein kinase (MAPK) pathway. More than twenty genes have been reported to be associated with RASopathies, including A2ML1, BRAF, CBL, HRAS, KRAS, LZTR1, MAP2K1, MAP2K2, NF1, NRAS, PTPN11, RAF1, RASA1, RASA2, RIT1, RRAS, SHOC2, SOS1, SOS2, and SPRED1 [3]. Owing to their similar pathogenic mechanisms, these syndromes share many clinical features such as distinct facial features, congenital heart defects, cardiomyopathy, skin anomalies, and growth retardation. In the majority of cases, the diagnosis of RASopathy can be made clinically by the identification of the typical clinical features. However, the syndromic manifestations can be subtle or even absent for some patients, making RASopathy-associated HCM difficult to distinguish from non-syndromic HCM on clinical grounds alone.

HCM has been frequently described in children with RASopathy, but only a minority of study patients have received formal genotyping in previous studies [4, 5]. The genetic basis and genotype-phenotype correlation of pediatric HCM associated with RASopathy still remain to be elucidated. In the current study, we present the genetic basis and genotype-phenotype correlation of pediatric HCM associated with RASopathy.

Methods

Patients and clinical evaluation
The study cohort comprised all the patients with RASopathy derived from 168 pediatric HCM cases referred to our institution between January 2012 and July 2018. Children with cardiac hypertrophy secondary to inborn errors of metabolism were excluded. HCM was defined as left ventricular posterior and/or septal wall thickness > 2 standard deviations above the normal mean for body surface area in the absence of hemodynamic stresses sufficient to account for the degree of hypertrophy [6]. A left ventricular (LV) outflow tract gradient ≥ 30 mmHg was considered to represent significant left ventricular outflow tract obstruction (LVOTO). All patients were clinically assessed by experienced clinical geneticists and pediatric cardiologists. Cardiovascular evaluation was performed on all the patients, including baseline electrocardiogram and echocardiography at the time of clinical diagnosis and during follow-up.

Molecular genetic analysis
Peripheral blood was collected, and genomic DNA was extracted according to standard procedures. Genetic testing was performed by next-generation sequencing (NGS) using either an expanded cardiomyopathy panel (58 genes, including 12 known RASopathy genes: BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NF1, NRAS, PTPN11, RAF1, SHOC2, and SOS1) or a whole-exome sequencing approach (Illumina HiSeq2500). Alignment of sequence reads to a reference human genome (Human 37.3, SNP135) was performed using the NextGEnet software (SoftGenetics, State College, Pennsylvania, USA). All single nucleotide variants and indels were saved as VCF format files, and uploaded to Ingenuity® Variant Analysis™ (Ingenuity Systems, Redwood City, California, USA) for variation filtering and interpretation. All the variations were classified according to the recommended method of the American College of Medical Genetics and Genomics [7]. Pathogenic and potentially pathogenic mutations were confirmed by Sanger sequencing, where possible, and validated by parental testing and segregation analysis. Variants classified as likely benign or benign were not selected to validate using Sanger sequencing.

Analysis of the pathogenicity of novel missense variants
To determine the potential pathogenicity of novel missense variants, we used three in silico prediction methods: MutationTaster (http://www.mutationtaster.org), Sorting Tolerant from Intolerant (SIFT, http://sift.jcvi.org/), and Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/).

Treatment and follow-up
β-Blocker therapy had been prescribed in 14 subjects with LVOTO. Balloon pulmonary valvuloplasty for treatment of pulmonary valve stenosis (PVS) was undertaken in one patient (patient 20 in Table 1). Six patients (patients 09, 23, 24, 37, 38, and 45 in Table 1) received surgical therapy for concomitant congenital heart disease (CHD), such as atrial septal defect (ASD), PVS, ventricular septal defect, and patent ductal arteriosus. Three patients (patient 18, 21, and 29 in Table 1) received surgical myectomy as a treatment for HCM. All patients were followed up by either telephone interview or outpatient clinic visit.
| Case | Sex | Age (months) | HCM Diagnosed | Clinical phenotype | LVOTO | Associated cardiac defect | Gene Mutation | Origin of mutation | ACMG classification | Follow-up time (years) |
|------|-----|--------------|----------------|-------------------|-------|---------------------------|---------------|--------------------|----------------------|-----------------------|
| 01   | M   | 1.7          | NSML           | +                 | ASD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 7.1                  |                       |
| 02   | M   | 12           | NSML           | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 15.2                 |                       |
| 03   | M   | 1.0          | NSML           | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 1.4                  |                       |
| 04   | F   | 2 d          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 1.2                  |                       |
| 05   | M   | 6.0          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 0.5                  |                       |
| 06   | F   | 10           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         | 1.6                  |                       |
| 07   | M   | 3 d          | –              | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 08   | M   | 10           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 09   | M   | Fetal        | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 10   | F   | 8 d          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 11   | M   | 17 d         | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 12   | F   | Fetal        | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 13   | F   | 2.5          | –              | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 14   | M   | 1.0          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 15   | F   | 1.5          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 16   | M   | 1 d          | NS             | +                 | VSD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 17   | M   | 1 d          | NS             | +                 | VSD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 18   | M   | 3 d          | NS             | +                 | VSD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 19   | F   | 1.3          | NS             | +                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 20   | F   | 9.0          | NS             | +                 | VSD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 21   | F   | 8.0          | NSML           | +                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 22   | M   | 12           | NS             | –                 | VSD, ASD | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 23   | M   | 2 d          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 24   | F   | 30           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 25   | M   | 6.0          | NS             | –                 | VSD, ASD | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 26   | F   | 4.0          | NS             | –                 | DORV, VSD, ASD, PVS | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 27   | F   | 5.5          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 28   | F   | 12           | NS             | +                 | ASD   | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 29   | M   | 17           | NS             | +                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 30   | M   | 8.1y         | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 31   | F   | 16 d         | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 32   | M   | 34           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 33   | M   | 2.0          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 34   | M   | 15 d         | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 35   | F   | 16           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 36   | F   | 3.0          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 37   | F   | 17           | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 38   | F   | 6.8          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 39   | M   | 2.4          | NS             | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
| 40   | M   | 3.5          | CFCS           | –                 | –     | PTPN11, c.836A > G (p.Y279C) | De novo       | Pathogenic         |                      |                       |
Three known mutations (p.P34L, p.G60S, and p.D153V) in the RAF1 gene were detected in three subjects with the NS phenotype. Another three known mutations (p.P261T, p.T491I, and p.L613V) in the RAF1 gene were identified in five individuals with NSML and five children with the NS phenotype. Another recurrent mutation p.S548R and p.E846K) in the SOS1 gene were identified in two patients with typical facial appearance of NS, short stature, and severe HCM. The recurrent mutation p.S548R was found in an individual with BVH but without overt clinical features at the time of inclusion. One mutation in exon 8 (p.F285S) was detected in another infant with the NS phenotype and focal septal hypertrophy.

Six different variants in the RAF1 gene were identified in this series, which included four in the conserved region two (CR2) domain, one in the activation segment of the kinase domain, and one at the C-terminus of RAF1. The recurrent mutation p.S257L was found in one girl with NSML and five children with the NS phenotype. Another two known mutations (p.N262K and p.V263G) were identified in two patients with typical cardiac defects and PVS. The clinical phenotypes and genetic features pertinent to the index cases are described below and summarized in Table 1.

**Results**

**Mutation analysis and clinical phenotype**

Germline mutations in one of known RASopathy genes were identified in 47 unrelated children (27 males, 20 females), including 36 patients with the NS phenotype, four with NSML, three with CFCS, one with CS, one with NS/LAH, and two infants without typical syndromic features (e.g., short stature, typical facial dysmorphism, and developmental delay) at the time of inclusion. The majority of patients (30/47, 64%) were diagnosed with HCM before the age of 6 months, including two with prenatal onset and 10 with neonatal onset. The median age at the diagnosis of HCM was 3.0 months (range 0 month to 8.1 years). Of the 47 patients, 21 (45%) had significant LVOTO at initial diagnosis of HCM. Concomitant CHD was observed in 32 of 47 (68%) patients, frequently in the form of septal defects and PVS. The clinical phenotypes and genetic features pertinent to the index cases are described below and summarized in Table 1.

Six different known mutations in the PTPN11 gene were identified in this series. Mutations in exon 13 were the most common variants, and were found in 12 individuals with early-onset biventricular hypertrophy (BVH). The recurrent mutation p.Q510E was identified in eight patients with biventricular outflow tract obstruction. Seven of these patients presented with the NS phenotype, whereas the other one did not have overt clinical syndromic features at the time of inclusion. Meanwhile, two other missense mutations in exon 13 (p.R498W and p.Q506P) were detected in four individuals with the NS phenotype, including two patients with significant LVOTO. Moreover, one recurrent mutation in exon 7 (p.Y279C) was found in three patients with NSML and two with the NS phenotype. Three of these five patients also had LVOTO. One mutation in exon 12 (p.G464A) was identified in an infant with BVH but without overt clinical features at the time of inclusion. One mutation in exon 8 (p.F285S) was detected in another infant with the NS phenotype and focal septal hypertrophy.

Six different variants in the RAF1 gene were identified in this series, which included four in the conserved region two (CR2) domain, one in the activation segment of the kinase domain, and one at the C-terminus of RAF1. The recurrent mutation p.S257L was found in one girl with NSML and five children with the NS phenotype. Another two known mutations (p.N262K and p.V263G) were identified in two patients with typical cardiac defects and PVS. The clinical phenotypes and genetic features pertinent to the index cases are described below and summarized in Table 1.

**Table 1 The cardiac manifestations and mutation profiles of 47 pediatric patients with RASopathy and HCM (Continued)**

| Case | Sex | Age (months) | HCM Diagnosis | LVOTO | Associated cardiac defect | Gene Mutation | Origin of mutation | ACMG classification | Follow-up time (years) |
|------|-----|-------------|---------------|-------|--------------------------|---------------|-------------------|---------------------|-----------------------|
| 41   | F   | 1.5         | CFCS          | –     | –                        | BRAF, c.1502A > G (p.E501G) | Determined       | Pathogenic         | 0.6                  |
| 42   | F   | 5.0         | CFCS          | –     | –                        | BRAF, c.1796C > T (p.T599I) * | De novo           | Pathogenic         | 3.7                  |
| 43   | M   | 6.0y        | NS            | +     | –                        | SOS1, c.1644 T > G (p.S548R) | De novo           | Pathogenic         | 7.4                  |
| 44   | M   | 3.0         | NS            | +     | ASD                      | SOS1, c.2536G > A (p.E846K) | De novo           | Pathogenic         | 17.1                 |
| 45   | M   | 3.0         | CS            | –     | ASD, PVS                 | HRAS, c.179G > A (p.G660D) | De novo           | Pathogenic         | 3.8                  |
| 46   | M   | 3.0         | NS/LAH        | –     | –                        | SHOC2, c.4A > G (p.S2G) | De novo           | Pathogenic         | 2.7                  |
| 47   | M   | 1.0         | NS            | +     | ASD                      | LZTR1, c.509G > C(p.R170P) * LZTR1, c.2374 T > G(p.C792G) * | De novo           | Pathogenic         | 4.2                  |

HCM hypertrophic cardiomyopathy, LVOTO left ventricular outflow tract obstruction, ACMG American College of Medical Genetics and Genomics, NSML Noonan syndrome with multiple lentigines, ASD atrial septal defect, PVS pulmonary valve stenosis, NS Noonan syndrome, PDA patent duct arteriosus, DORV double outlet right ventricle, VSD ventricular septal defect, NA not available, LSVC left superior vena cava, CFCS cardiofaciocutaneous syndrome, CS Costello syndrome, NS/LAH Noonan syndrome with loose anagen hair.

*The mutation identified in this study is in boldface.
(p.G60D) in HRAS was detected in a patient with CS and severe LV hypertrophy; and one mutation (p.S2G) in SHOC2 was detected in a patient with NS/LAH and moderate LV hypertrophy.

Moreover, two novel compound heterogeneous variants in the LZTR1 gene (p.R170P and p.C792G) were identified in one patient with the typical facial appearance of NS, short stature, and severe LV hypertrophy. Family screening for the variants revealed that each of his parents was the carrier of one of these variants, his brother was heterozygote carrier for the p.R170P variant, and his maternal half-sister was wild-type for both variants. Neither his parents nor his siblings were clinically affected.

Analysis of the pathogenicity of novel missense variants

Five novel variants were identified in this study, including two de novo variants (p.N262K and p.V263G) in the RAF1 gene, one de novo variant (p.T599I) in the BRAF gene, and two compound heterogeneous variants (p.R170P and p.C792G) in the LZTR1 gene. All affected residues were evolutionarily conserved, and no change had been reported in Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org). Moreover, all five variants were consistently predicted to be deleterious by three bioinformatics tools (Table 2).

Follow-up and survival

Three patients with a mutation in exon 13 of the PTPN11 gene had rapidly progressive HCM and died of cardiac failure at the age of 3.0, 3.5, and 6.0 months, respectively. The remaining 44 patients were alive and received an average follow-up time of 3.9 years (0.5 to 17.1 years, median 2.9 years) from the initial diagnosis of HCM. As assessed by serial echocardiographic follow-up, five patients had spontaneous regression of ventricular wall thickness over an average of 5.7 years (0.5 to 15.4 years, median 5.7 years), including two with mutations in PTPN11, two with mutations in KRAS, and one with a mutation in SOS1. Progression of HCM was seen in 4 patients with mutations in PTPN11, one with a mutation in SOS1, and one with mutations in LZTR1. HCM was stabilized in the remaining 33 patients, including three patients who have received surgical myectomy for HCM.

Discussion

Germline PTPN11 mutations are major causes for both NS and NSML, but the point mutations are almost mutually exclusive between these two conditions [8]. Of the six different PTPN11 mutations identified in the study, only the p.F285S variant has been regarded as an NS-associated PTPN11 mutation, while the other five mutations appeared to be specific for NSML according to previous studies [9]. However, among the 18 patients with an NSML-associated PTPN11 mutation, only three patients presented with the NSML phenotype at the time of inclusion, while the other 15 patients did not fulfill the criteria for a clinical diagnosis of NSML according to Voron et al. [10]. These findings indicated that NSML-associated PTPN11 mutations were the most common causes of HCM associated with RASopathy, whereas the differential diagnosis between NS and NSML may be difficult on clinical grounds alone due to their overlapping clinical features. Our cohort also included two infants without overt clinical syndromic features at the first time. The novel variant p.N262K was caused by a c.786 T > G substitution; a different base substitution (c.786 T > A) resulting in the same amino acid substitution (p.S2G) has been described in a patient with a CS phenotype [14]. The novel variant p.V263G was caused by a c.788 T > G substitution; a different amino acid change at the same position (p.V263A) has been described in a patient with NS and HCM [15]. Both of the novel variants affected a highly conserved residue within the CR2 domain and were

| Gene | DNA change | Protein change | Mutation taster | PolyPhen-2 | SIFT | MAF (gnomAD) |
|------|------------|----------------|----------------|------------|------|--------------|
| RAF1 | c.786 T > G | p.N262K | Disease-causing | Probably damaging | Deleterious | – |
| RAF1 | c.788 T > G | p.V263G | Disease-causing | Probably damaging | Deleterious | – |
| BRAF | c.1796C > T | p.T599I | Disease-causing | Probably damaging | Deleterious | – |
| LZTR1 | c.509G > C | p.R170P | Disease-causing | Probably damaging | Deleterious | – |
| LZTR1 | c.2374T > G | p.C792G | Disease-causing | Probably damaging | Deleterious | – |
identified as occurring de novo. Both of them were absent in the SNP database and were consistently predicted to be deleterious by three bioinformatics tools. These findings, along with the typical clinical features in the affected patients, indicate that these two variants are pathogenic.

Patients harboring RIT1 mutations display a high incidence of HCM, similar to those with RAF1 mutations [16]. PVS and ASD are also prevalent among patients harboring RIT1 mutations according to previous studies [16]. We identified two different RIT1 mutations in four patients with NS and HCM, all of whom had co-existing PVS and ASD. Our findings, along with previous studies, indicated that the combination of PVS and ASD with HCM seemed to be frequent among individuals with NS due to RIT1 mutations [2, 16].

HCM is also a frequent cardiovascular manifestation in patients with CFCS, which was documented in our three patients with CFCS due to a BRAF mutation. The novel variant p.T599I affected a highly conserved residue within the activation segment of the CR3 domain, and was identified as occurring de novo. This variant was absent in the SNP database and was consistently predicted to be deleterious by three bioinformatics tools. The same mutation was predicted to be deleterious by three bioinformatics tools. Moreover, a different variant p.T599R has been described in a patient with CFCS due to a BRAF mutation. The detailed mechanisms are unclear, but may be attributed to the fact that germline KRAS mutations cause only a moderate up-regulation of the RAS-MAPK pathway [19]. Germline SOS1 mutations are the second most common causes of NS, but the risk of HCM in individuals with SOS1 mutations is low [20, 21]. Only two patients in our study population were found to harbor a mutation in the SOS1 gene. HCM is frequently seen in patients with CS caused by HRAS mutations and NS/LAH caused by SHOC2 mutations. However, both of these diseases are rare syndromes within the RASopathy group [2, 22]. Only one patient with an HRAS mutation and another patient with a SHOC2 mutation were found in our study.

Germline LZTR1 mutations have been shown to be associated with NS in recent studies, which can follow either recessive or dominant inheritance patterns [23, 24]. While a limited number of patients with germline LZTR1 mutations have been described, it appears that a high percentage of HCM is associated with this condition [24, 25]. In the current study, two novel compound heterogeneous variants in the LZTR1 gene (p.R170P and p.C792G) were identified in one patient with a typical NS phenotype and HCM. Both of the variants were absent in the SNP database and were consistently predicted to be deleterious by three bioinformatics tools. Moreover, they were found to co-segregate with the disease phenotype in an autosomal recessive fashion in the affected family. This implies that the two variants may be the pathogenic mutations responsible for the NS phenotype in this patient.

An early onset of cardiac hypertrophy was observed in our study population, with a median age of 3.0 months at the diagnosis of HCM. Meanwhile, LVOTO was detected at a high frequency in our cohort, especially in patients with NSM1-associated PTEN mutations (13/18, 72%). Furthermore, a high prevalence of co-existing CHD was observed in our study population. These results are consistent with several previous reports [26, 27].

The clinical outcomes of our patients were markedly variable. The disease can be rapidly progressive in infancy in some patients, or remain asymptomatic, or regress in other patients. The varied severity of HCM and clinical outcome may be related to the different gene mutations or other factors. The correlation of specific genetic mutations and clinical outcomes of patients with RASopathy-associated HCM is warranted.

Conclusions

RASopathies are one of the most common causes of pediatric HCM, characterized by early-onset cardiac hypertrophy as well as a higher prevalence of CHD and
LVOTO. RASopathy-associated HCM is a genetically heterogeneous condition involving diverse genes within the RAS-MAPK pathway, but frequently related to NSML-associated PTPN11 mutations. The clinical course and prognosis of patients with this condition are markedly variable and seemed to be related to specific gene mutations. However, rapidly progressive HCM, resulting in an early death, was uncommon in RASopathy patients except those with specific mutations in exon 13 of the PTPN11 gene.

### Abbreviations
- ASD: Atrial septal defect; BHV: Biventricular hypertrophy; CFCS: Cardiofaciocutaneous syndrome; CHD: Congenital heart disease; CR: Conserved region; CS: Costello syndrome; gnomAD: Genome Aggregation Database; HCM: Hypertrophic cardiomyopathy; LV: Left ventricular; LVOTO: Left ventricular outflow tract obstruction; MAPK: Mitogen-activated protein kinase; NGS: Next-generation sequencing; NSML: Noonan syndrome; NS/LAH: Noonan syndrome-like disorder with loose anagen hair; NSML: Noonan syndrome with multiple lentigines; PTP: Protein tyrosine phosphatase; PVS: Pulmonary valve stenosis

### Ethics approval and consent to participate
This study was approved by the Institutional Review Boards of Shanghai Children’s Medical Center and carried out in accordance with the ethical principles of the Declaration of Helsinki. For gene studies, written informed consents were obtained from the parents. All authors read and approved the final manuscript.

### Consent for publication
Not applicable.

### Competing interests
The authors declare that they have no competing interests.

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