Research article

Phenotypic and genotypic spectrum of noonan syndrome: A retrospective analysis of 46 consecutive pediatric patients presented at a regional cardiac center in China

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A B S T R A C T

Background: Noonan syndrome (NS) is relatively common but poorly recognized. We aimed to describe the phenotypic and genotypic spectrum of NS in a Chinese cohort.
Method: The study retrospectively investigated consecutive pediatric patients who presented at the Guangdong cardiovascular institute between 2018 and 2020 with confirmed known NS-relevant mutations determined by exome sequencing. Dates of genetic testing, Age, sex, institution of genetic testing, mutated gene (related to NS) and its classification, heterozygosity, and parental origin were identified from the sequencing reports. Facial features, cardiac defect and other clinical characteristics were also assessed. Comparisons of categorical variables between groups were examined by Chi-square test or Fisher's exact test when appropriate. Intraclass correlation coefficient (ICC) was performed to evaluate the reliability of evaluation of facial features between different evaluators.
Results: The most prevalent mutated genes were PTPN11 (37.0%) and RAF1 (19.6%), and most mutations were pathogenic (67.4%) and de novo (87.0%). Most patients were with NS-relevant facial features (97.4%) and cardiac defects (92.7%), where ventricular hypertrophy, pulmonary valve stenosis, and atrial septal defect were the most prevalent. Patients with mutated RAF1 appeared to be diagnosed at an older age than those with mutated PTPN11, and with higher prevalence of mitral regurgitation, hypertrophic cardiomyopathy, and ventricular hypertrophy, but lower prevalence of pulmonary valve stenosis and pulmonary artery stenosis. Patients presented at an age ≥ 2 years appeared to be with fewer NS-relevant facial features and cardiac defects than those aged <2 years.
Conclusions: These findings indicated featured distributions of phenotypic and genotypic spectrum in Chinese pediatric patients, which might be helpful for early NS diagnosis.

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1. Introduction

Noonan syndrome (NS) is a genetically heterogeneous, mostly autosomal dominant genetic disorder caused by mutations of genes encoding proteins involved in the Ras-mitogen-activated protein kinase (Ras-MAPK) pathway, including PTPN11, SOS1, RAF1, RIT1, KRAS, NRAS, BRAF, LZTR1, SOS2 [1–4]. As the Ras-MAPK pathway plays multiple physiological roles (e.g., regulating cell proliferation, differentiation, survival, and metabolism) [5], NS is also clinically heterogeneous, which is typically characterized by (but not limited to) distinctive facial features, congenital heart disease, short stature, and developmental delay, but widely varies from asymptomatic or oligosymptomatic to life-threatening scenarios [1,6–10]. For this reason, NS tends to be poorly recognized in practice, although the incidence is estimated be 1 in 1000 to 1 in 2500 live births (which is relatively common as a genetic disorder) [11]. Diagnosis of NS mainly depends on typical clinical features, but a confirmation can be made only by genetic testing, which may further limit its early recognition in resource-restricted settings [12]. However, early diagnosis and appropriate management is obviously important for NS patients, as it will help optimize developmental and long-term outcomes [6]. In addition, the lack of consistent diagnosis criteria (without genetic testing) also hinders research on NS (e.g., comparison of epidemiology or quality of care for NS reported by different studies), which further impair progress on NS management.

Current knowledge of NS (though still very limited) mainly comes from western population in the developed regions [13–18], while it remains unknown whether the distributions of clinical features and genetic features are the same in other populations. This is actually very relevant to know, as diagnosis (or suspicion) of NS is likely to more rely on clinical features (such as facial features) due to potentially limited access to genetic testing. The Chinese population accounts for about 20% of the global population, but investigations in NS in Chinese population are rather rare, especially for genetically confirmed NS. With the availability of sequencing techniques in the very recent years, publications about NS in Chinese population appears to increase [19–25], which, however, are of limited sample size and far from enough. In this study, we aimed to describe the phenotypic and genotypic spectrum of NS in a relatively large Chinese pediatric cohort, including distributions of clinical characteristics by genetic characteristics, and patient characteristics by ages at NS diagnosis. Specifically, we also investigated the reliability of evaluating NS-relevant facial features between different evaluators according to the current recommended NS diagnostic features [6,26].

2. Materials and methods

2.1. Study population and data sources

The study retrospectively investigated consecutive pediatric patients who presented at the Guangdong cardiovascular institute between 2018 and 2020 with confirmed known NS-relevant mutations determined by exome sequencing (see below). The institute is a regional cardiac center of the Guangdong province (with about 120 million inhabitants) in China, to which patients with complex heart diseases including congenital heart diseases are commonly referred, either for diagnosis or treatment [27]. Since 2017, exome sequencing has been gradually available in clinical practice in the institute and patients who visited the Department of pediatric cardiology of the institute with suspected NS would be advised to receive a genetic testing together with their parents if possible. There were no prespecified procedures/criteria for the responsible physicians (who were at least an attending physician) to identify a suspected NS, which could be due to typical facial features, cardiac defect determined by echocardiography, or other NS-relevant phenotypes, but remained a decision independently made by the responsible physicians. For those patients with a positive finding (i.e., with identified NS-relevant mutations), they would be included into an ongoing registry conducted in the same department for pediatric patients with congenital heart diseases if informed consent was provided. The current study secondly used data from this ongoing registry to identify the study population, including detailed reports of the exome sequencing and facial photos (if available). In addition, we retrieved reports of echocardiography for the studied patients (if available) from the hospital information system.

All subjects gave their informed consent for inclusion in writing before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (No. GDRECKY2020-033-01).

2.2. Demographic and genetic characteristics

When an exome sequencing was ordered, peripheral blood sample of the patient (and the parents, if applicable) were collected by a nurse in the institute; then together with the basic information of the patient (including reason(s) for the examination), the sample was sent to a commercial company, which performed deoxyribonucleic acid (DNA) extraction, exome sequencing (including relevant quality control), and data analysis including variant annotation and reporting. For the patients we included, two commercial companies were involved (i.e., JiaJian Medicine, and KingMed Diagnostics). The detailed multigene panel for sequencing differed between patients and commercial companies, but for all the patients the commercial companies were clearly informed that the patients were suspicious of NS and the multigene panel for sequencing should cover known RASopathy genes. We identified the below information from the sequencing reports: dates of genetic testing, age, sex, institution of genetic testing, mutated gene (related to NS) and its classification (based on the American College of Medical Genetics and Genomics (ACMG) classification) [28], heterozygosity, and parental origin.

2.3. Clinical characteristics

2.3.1. Facial features

To identify facial features, a list of NS-relevant facial features reported in literature [6,29] was first made and translated into Chinese (Table S1). For the patients with available facial photos, three researchers were asked to independently evaluate presence of NS-relevant facial features according to the list. The evaluation was not blinded, in which the evaluators knew the patients were with NS but other information was not provided (except for age and the facial photos). As some facial features were reported for specific age groups, the suggestion was also provided on the list, but the evaluators were informed that it is not necessary to strictly follow the suggestion. After the evaluations, those facial features that were identified as present by ≥ 2 evaluators were considered as present for

Table 1
Demographic, genetic, and clinical characteristics of the patients.

Patient characteristics	N = 46
Calendar year of genetic testing	
2018	11 (23.9%)
2019	11 (23.9%)
2020	14 (30.4%)
2021	10 (21.7%)
Age (years)	1.5 (0.7–4.3)
<2	26 (56.5%)
2-12	17 (37.0%)
12-18	3 (6.5%)
Sex	
Male	22 (47.8%)
Female	24 (52.2%)
Institution of genetic testing	
JiaJian Medicine	44 (95.7%)
KingMed Diagnostics	2 (4.3%)
Mutated gene	
PTPN11	17 (37.0%)
RAF1	9 (19.6%)
RIT1	6 (13.0%)
LZTR1	5 (10.9%)
SOS1	3 (6.5%)
BRAF	3 (6.5%)
SHOC2	2 (4.3%)
SOS2	1 (2.2%)
ACMG classification[a]	
Pathogenic	31 (67.4%)
Likely pathogenic	11 (23.9%)
Uncertain significance	4 (8.7%)
Heterozygous	46 (100%)
Parental origin	
De novo	40 (87.0%)
Paternal	3 (6.5%)
Maternal	3 (6.5%)
Presence of facial feature[b]	37 (97.4%)
Presence of cardiac defect[c]	38 (92.7%)
Short stature/Developmental delay	11 (23.9%)
Cryptorchidism[d]	1 (4.5%)
Other clinical features[e]	3 (6.5%)

Abbreviation: ACMG, The American College of Medical Genetics and Genomics.

[a] According to the ACMG recommendation published in 2015.

[b] Presence of facial feature was defined as ≥2 different categories of facial features (i.e., head, hair, eyes, nose, lip, ears, facial skin, chin/neck). There were eight cases without facial photos which were excluded from the analysis.

[c] Presence of cardiac defect was defined as ≥1 abnormal findings in echocardiography (i.e., tricuspid regurgitation, mitral regurgitation, tricuspid valve stenosis, atrial septal defect, patent foramen ovale, ventricular septal defect, hypertrophic cardiomyopathy, ventricular hypertrophy, pulmonary valve stenosis, pulmonary regurgitation, aortic stenosis, pulmonary artery stenosis, patent ductus arteriosus). There were five cases without echocardiography which were excluded from the analysis.

[d] The analysis was performed in male patients only.

[e] There was one case with alpha thalassemias, one case with laryngeal dysplasia, and one case with brown papules and lymphatic dysplasia.

the final analysis. In addition, the various facial features were categorized as eight categories (i.e., head, hair, eyes, nose, lip, ears, facial skin, and chin/neck), and a patient was considered with presence of (typical NS-relevant) facial feature only when he/she presented ≥2 different categories of facial features. Patients without available facial photos were not included in the analysis of facial features.

2.3.2. Cardiac defect

To identify cardiac defect, reports of echocardiography were retrieved and manually read to identify whether a patient was with cardiac defect including ventricular hypertrophy, pulmonary valve stenosis, atrial septal defect, patent foramen ovale, pulmonary artery stenosis, tricuspid regurgitation, hypertrophic cardiomyopathy, mitral regurgitation, ventricular septal defect, patent ductus arteriosus, pulmonary regurgitation, tricuspid valve stenosis, and aortic stenosis. A cardiac defect was considered present only when it was mentioned directly in the description and conclusion (in text) presented in the reports of echocardiography. We did not evaluate whether the descriptions/conclusions presented in the reports were correct or not according to the ultrasound parameters/images. Patients without echocardiography were not included in the analysis of cardiac defect.

2.3.3. Other clinical characteristics

From the sequencing reports other conditions mentioned as reasons for the examination (such as short stature, developmental delay, and cryptorchidism) were also retrieved as other clinical characteristics, but this information was not further validated.

2.4. Statistical analysis

Continuous variables were presented as mean ± standard deviation or median (25th-75th percentiles) when appropriate, and categorical variables were presented as frequency and percentage. Comparisons of categorical variables between groups were examined by Chi-square test or Fisher's exact test when appropriate. Intraclass correlation coefficient (ICC) to evaluate the reliability of evaluation of facial features between different evaluators. A P value less than 0.05 was considered statistically significant. All the analyses were performed by SPSS 25 for Windows (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Patient characteristics

A total of 46 pediatric patients with confirmed NS-relevant mutations were included. As presented in Table 1, the sizes of patients presented in different calendar years were rather stable. The median age of the patients were 1.5 years (25-75th percentile 0.7–4.3 years), and 56.5% were below 2 years old. Female patients accounted for a slightly higher proportion (52.2%) than the male.

The most prevalent mutated genes were PTPN11 (37.0%), followed by RAF1 (19.6%), RIT1 (13.0%), and LZTR1 (10.9%). Most mutations were pathogenic (67.4%) and de novo (87.0%), and all were heterozygous. Detailed genetic characteristics of the patients were presented in Table S2, which showed c.922A > G was the most frequent site of PTPN11 mutation (5 in 17 patients) and c.770C >

Table 2
Detailed facial feature of the patients.

Category	Detailed features	N = 38[a]
Head	Large head compared to face, tall forehead with narrow temples	10 (26.3%)
	Wide forehead	30 (78.9%)
	Triangle-shaped head	6 (15.8%)
Hair	Wispy hair	8 (21.1%)
	Curly or wooly hair	2 (5.3%)
	High anterior hairline	27 (71.1%)
Eyes	Wide-spaced eyes (hypertelorism)	27 (71.1%)
	Downward slant of palpebral fissures	8 (21.1%)
	Thickly hooded, prominent eyes	10 (26.3%)
	palpebral ptosis	14 (36.8%)
Nose	Short, broad nose with depressed root and full tip	16 (42.1%)
	Wide-based, depressed nose with bulbous, upturned tip	17 (44.7%)
Lip	Full lips with high, wide peaks to the vermilion border of upper lip	17 (44.7%)
	Cupid bow appearance of upper lip	15 (39.5%)
Ears	Low-set posteriorly rotated ears	4 (10.5%)
Facial skin	Epicanthal folds	9 (23.7%)
	Deeply grooved philtrum	15 (39.5%)
	Transparent, wrinkled skin	0 (0.0%)
	Prominent nasolabial folds	1 (2.6%)
Chin/neck	Small chin	25 (65.8%)
	Short neck, excess nuchal skin	3 (7.9%)
	Neck skin webbing	0 (0.0%)

[a] Among the 46 cases, there were eight cases without facial photos which were excluded from the analysis.

T was the most frequent site of RAF1 (3 in 9 patients). Mutations of LZTR1 appeared to be less likely to be de novo (only 1 in 5 patients).

Overall, the majority of the patients were with NS-relevant facial features (≥2 categories, 97.4%) and ≥1 cardiac defect (92.7%), and 23.9% of the patients were with short stature/developmental delay.

Among the investigated facial features, features of eyes, nose, head, and hair were the most prevalent categories. In detail, wide forehead, high anterior hairline, and wide-spaced eyes (hypertelorism) were the most prevalent facial features (Table 2). However, poor reliability (ICC <0.5) of the evaluation of facial features between the three evaluators was found for most prespecified facial features (Table S3), and only the facial features short neck, excess nuchal skin (ICC = 0.606), wide-spaced eyes (hypertelorism, ICC = 0.56), and wispy hair (ICC = 0.513) showed a relatively better reliability.

Among the investigated cardiac defects, where ventricular hypertrophy (51.2%), pulmonary valve stenosis (51.2%), and atrial septal defect (41.5%) were the most prevalent (Table 3).

3.2. Distribution of phenotypes by genotypes

When the patients were stratified by mutated genes (i.e., PTPN11, RAF1, and others), difference in distribution of some patient characteristics could be observed (Table 4). Patients with mutated RAF1 appeared to be diagnosed at an older age than the others (about 80% ≥ 2 years, versus less than 40% in other mutated genes). For facial features, lip features were more likely to present in those with mutated RAF1 than the others (85.7% versus 66.7% in mutated PTPN11, and 31.3% in other mutated genes), but features of chin/neck was less prevalent (28.6% versus 86.7% and 68.8% respectively). For cardiac defects, higher prevalence of mitral regurgitation, hypertrophic cardiomyopathy, and ventricular hypertrophy was observed in patients with mutated RAF1, while it was lower for pulmonary valve stenosis and pulmonary artery stenosis when compared to other mutated genes. Short stature or developmental delay was also less likely to be present in patients with mutated RAF1.

3.3. Distribution of patient characteristics by age at diagnosis

When the patients were stratified by ranges of age at diagnosis (i.e., <2 or ≥2 years), some patient characteristics showed different distributions between the two groups (Table 5). Compared to those diagnosed at an age <2 years, mutated RAF1 other than PTPN11 was more likely to present in patients diagnosed at an age ≥2 years, and the mutations were less likely to be pathogenic (55.0% versus 76.9%). Overall, those aged ≥2 years appeared to be with fewer NS-relevant facial features and cardiac defects, but lip features and hypertrophic cardiomyopathy were more prevalent in those aged ≥2 years.

4. Discussion

NS is estimated to be the second cause of congenital heart disease next to trisomy 21 [12], which is not rare but poorly recognized in practice. In this study, by including a Chinese cohort consisting of pediatric patients with confirmed NS-relevant mutations, we investigated the distributions of genetic and clinical characteristics (especially facial features and cardiac defects) among the NS patients. Our main findings are: (1) about half of the patients were diagnosed at an age greater than 2 years, and patients with greater disease burden tends to be diagnosed at an earlier age; (2) PTPN11 is the most frequent NS-relevant mutations, which is followed by RAF1, while there is difference in distributions of some facial features and cardiac defects between patients with different mutated genes. (3) there is rather great variation of evaluation of NS-relevant facial features between (untrained) evaluators. These findings provide new information about patient characteristics of NS in the Chinese population. The identified prevalent genetic and clinical characteristics and featured distributions might, to some extent, represent the typical characteristics of NS in Chinese population, and therefore would benefit early recognition of NS. In addition, the inconsistent evaluation of NS-relevant facial features between evaluators suggest efforts should be put into the relevant education and training to improve NS recognition. It also raises a concern about whether the typical NS-relevant facial features identified in the western populations could be directly used for the Chinese

Table 3
Detailed cardiac defect of the patients.

Detailed cardiac defect	N = 41[a]
Ventricular hypertrophy	21 (51.2%)
Pulmonary valve stenosis	21 (51.2%)
Atrial septal defect	17 (41.5%)
Patent foramen ovale	11 (26.8%)
Pulmonary artery stenosis	8 (19.5%)
Tricuspid regurgitation	7 (17.1%)
Hypertrophic cardiomyopathy	7 (17.1%)
Mitral regurgitation	6 (14.6%)
Ventricular septal defect	5 (12.2%)
Patent ductus arteriosus	4 (9.8%)
Pulmonary regurgitation	2 (4.9%)
Tricuspid valve stenosis	1 (2.4%)

[a] Among the 46 cases, there were five cases without echocardiography which were excluded from the analysis.

Table 4
Distribution of demographic and clinical features between mutated genes.

Mutated genes	PTPN11 (N = 17)	RAF1 (N = 9)	Others (N = 20)	P value
Calendar year of genetic testing				0.071
2018	1 (5.9%)	5 (55.6%)	5 (25.0%)	
2019	4 (23.5%)	0 (0.0%)	7 (35.0%)	
2020	7 (41.2%)	3 (33.3%)	4 (20.0%)	
2021	5 (29.4%)	1 (11.1%)	4 (20.0%)	
Age (years)				0.077
<2	12 (70.6%)	2 (22.2%)	12 (60.0%)	
2-12	5 (29.4%)	5 (55.6%)	7 (35.0%)	
12-18	0 (0.0%)	2 (22.2%)	1 (5.0%)	
Sex				0.928
Male	8 (47.1%)	5 (55.6%)	9 (45.0%)	
Female	9 (52.9%)	4 (44.4%)	11 (55.0%)	
Presence of facial feature[a]	15 (100.0%)	7 (100.0%)	15 (93.8%)	1.000
Head	13 (86.7%)	6 (85.7%)	11 (68.8%)	0.510
Hair	13 (86.7%)	5 (71.4%)	12 (75.0%)	0.663
Eyes	14 (93.3%)	7 (100.0%)	12 (75.0%)	0.234
Nose	13 (86.7%)	4 (57.1%)	14 (87.5%)	0.192
Lip	10 (66.7%)	6 (85.7%)	5 (31.3%)	0.034
Ears	2 (13.3%)	1 (14.3%)	1 (6.3%)	0.659
Facial skin	9 (60.0%)	4 (57.1%)	9 (56.3%)	1.000
Chin/neck	13 (86.7%)	2 (28.6%)	11 (68.8%)	0.028
Presence of cardiac defect[b]	14 (93.3%)	7 (100.0%)	17 (89.5%)	1.000
Tricuspid regurgitation	3 (20.0%)	1 (14.3%)	3 (15.8%)	1.000
Mitral regurgitation	0 (0.0%)	3 (42.9%)	3 (15.8%)	0.034
Tricuspid valve stenosis	1 (6.7%)	0 (0.0%)	0 (0.0%)	0.537
Atrial septal defect	7 (46.7%)	2 (28.6%)	8 (42.1%)	0.838
Patent foramen ovale	5 (33.3%)	1 (14.3%)	5 (26.3%)	0.730
Ventricular septal defect	3 (20.0%)	0 (0.0%)	2 (10.5%)	0.561
Hypertrophic cardiomyopathy	0 (0.0%)	5 (71.4%)	2 (10.5%)	<0.001
Ventricular hypertrophy	5 (33.3%)	6 (85.7%)	10 (52.6%)	0.076
Pulmonary valve stenosis	8 (53.3%)	0 (0.0%)	13 (68.4%)	0.006
Pulmonary regurgitation	0 (0.0%)	0 (0.0%)	2 (10.5%)	0.652
Aortic stenosis	1 (6.7%)	0 (0.0%)	0 (0.0%)	0.537
Pulmonary artery stenosis	6 (40.0%)	0 (0.0%)	2 (10.5%)	0.061
Patent ductus arteriosus	2 (13.3%)	0 (0.0%)	2 (10.5%)	1.000
Short stature/Developmental delay	3 (17.6%)	0 (0.0%)	8 (40.0%)	0.054
Cryptorchidism[c]	0 (0.0%)	1 (20.0%)	0 (0.0%)	0.227
Other clinical features[d]	0 (0.0%)	1 (11.1%)	2 (10.0%)	0.406

Abbreviation: ACMG, The American College of Medical Genetics and Genomics.

[a] Presence of facial feature was defined as ≥2 different categories of facial features (i.e., head, hair, eyes, nose, lip, ears, facial skin, chin/neck). There were 8 cases without facial photos which were excluded from the analysis.

[b] Presence of cardiac defect was defined as ≥1 abnormal findings in echocardiography (i.e., tricuspid regurgitation, mitral regurgitation, tricuspid valve stenosis, atrial septal defect, patent foramen ovale, ventricular septal defect, hypertrophic cardiomyopathy, ventricular hypertrophy, pulmonary valve stenosis, pulmonary regurgitation, aortic stenosis, pulmonary artery stenosis, patent ductus arteriosus). There were 5 cases without echocardiography which were excluded from the analysis.

[c] The analysis was performed in male patients only.

[d] There was one case with alpha thalassemias, one case with laryngeal dysplasia, and one case with brown papules and lymphatic dysplasia.

population, a topic which was rarely mentioned before but does warrant more investigations.

Our findings about patient characteristics of NS are overall consistent with most other studies. For genetic characteristics, NS is nearly always autosomal-dominant and most cases are with de novo pathogenic variant [30], which we also observed. Family history (i.e., first-degree relative with NS) is included in the current diagnostic features of NS [6], but in our study none of the parents of the pediatric NS patients had NS-relevant clinical features, although this may also due to underdiagnosis. PTPN11 was previously reported to account for about 50 percent of NS patients [31], which was higher than what we observed, but in a recent study by Athota et al. [32], among 363 clinically diagnosed NS patients (from Indian population), 107 (about 30%) were with PTPN11 mutations, which is closer to our findings (i.e., 37.0%). For clinical features, we found high prevalence of NS-relevant facial features and cardiac defect (i. e., about 80%), and this is also consistent with findings from other studies [33–35].Among the detailed clinical features, widely spaced eyes, low-set ears (>80 percent), short stature (>70 percent), and pulmonic stenosis (about 50 percent) were reported as the most frequent clinical features consistently between studies) [33–35]. Compared to these reports, we found a lower prevalence of ear feature and short stature, which may be because of the limited quality of facial photos we used for identifying NS-relevant facial features (which usually did not present the ears clearly) and data on short stature/developmental delay were merely obtained according to the recorded reasons for the genetic testing (instead of a formal and strict evaluation).

In addition to investigate the distribution of patient characteristics among the overall NS patients, we also investigated it after stratifying by types of mutated genes and age at diagnosis, as information about genotype-phenotype correlations in NS patients is still

Table 5
Distribution of clinical and genetic features by age at diagnosis.

Age (years)	<2 (N = 26)	≥2 (N = 20)	P value
Sex			0.149
Male	15 (57.7%)	7 (35.0%)	
Female	11 (42.3%)	13 (65.0%)	
Mutated gene			0.056
PTPN11	12 (46.2%)	5 (25.0%)	
RAF1	2 (7.7%)	7 (35.0%)	
Others	12 (46.2%)	8 (40.0%)	
ACMG classification[a]			0.231
Pathogenic	20 (76.9%)	11 (55.0%)	
Likely pathogenic	5 (19.2%)	6 (30.0%)	
Uncertain significance	1 (3.8%)	3 (15.0%)	
Presence of facial feature[b]	23 (100.0%)	14 (93.3%)	0.395
Head	19 (82.6%)	11 (73.3%)	0.687
Hair	19 (82.6%)	11 (73.3%)	0.687
Eyes	19 (82.6%)	14 (93.3%)	0.630
Nose	21 (91.3%)	10 (66.7%)	0.089
Lip	9 (39.1%)	12 (80.0%)	0.013
Ears	4 (17.4%)	0 (0.0%)	0.138
Facial skin	14 (60.9%)	8 (53.3%)	0.743
Chin/neck	20 (87.0%)	6 (40.0%)	0.004
Presence of cardiac defect[c]	23 (95.8%)	15 (88.2%)	0.560
Tricuspid regurgitation	3 (12.5%)	4 (23.5%)	0.421
Mitral regurgitation	2 (8.3%)	4 (23.5%)	0.212
Tricuspid valve stenosis	0 (0.0%)	1 (5.9%)	0.415
Atrial septal defect	12 (50.0%)	5 (29.4%)	0.217
Patent foramen ovale	8 (33.3%)	3 (17.6%)	0.309
Ventricular septal defect	3 (12.5%)	2 (11.8%)	1.000
Hypertrophic cardiomyopathy	1 (4.2%)	6 (35.3%)	0.014
Ventricular hypertrophy	12 (50.0%)	9 (52.9%)	1.000
Pulmonary valve stenosis	16 (66.7%)	5 (29.4%)	0.028
Pulmonary regurgitation	1 (4.2%)	1 (5.9%)	1.000
Aortic stenosis	1 (4.2%)	0 (0.0%)	1.000
Pulmonary artery stenosis	7 (29.2%)	1 (5.9%)	0.110
Patent ductus arteriosus	3 (12.5%)	1 (5.9%)	0.629
Short stature/Developmental delay	7 (26.9%)	4 (20.0%)	0.732
Cryptorchidism[d]	1 (6.7%)	0 (0.0%)	1.000
Other clinical features[e]	2 (7.7%)	1 (5.0%)	1.000

Abbreviation: ACMG, The American College of Medical Genetics and Genomics.

[a] According to the ACMG recommendation published in 2015.

[b] Presence of facial feature was defined as ≥2 different categories of facial features (i.e., head, hair, eyes, nose, lip, ears, facial skin, chin/neck). There were 8 cases without facial photos which were excluded from the analysis.

[c] Presence of cardiac defect was defined as ≥1 abnormal findings in echocardiography (i.e., tricuspid regurgitation, mitral regurgitation, tricuspid valve stenosis, atrial septal defect, patent foramen ovale, ventricular septal defect, hypertrophic cardiomyopathy, ventricular hypertrophy, pulmonary valve stenosis, pulmonary regurgitation, aortic stenosis, pulmonary artery stenosis, patent ductus arteriosus). There were 5 cases without echocardiography which were excluded from the analysis.

[d] The analysis was performed in male patients only.

[e] There was one case with alpha thalassemias, one case with laryngeal dysplasia, and one case with brown papules and lymphatic dysplasia.

rather limited. The different distribution of cardiac defect we observed between patients with mutated PTPN11 and RAF1 was consistent with previous studies, which reported PTPN11 and SOS1 mutations were associated with pulmonary valve stenosis, but RAF1 and RIT1 mutations were more commonly with hypertrophic cardiomyopathy [36,37]. As we observed patients with RAF1 appeared to be diagnosed at an older age than PTPN11, it remains unknown whether the different distribution of cardiac defects was related to the different ages at diagnosis. Nevertheless, this information would be helpful for early diagnosis of NS when a pediatric patient presents with cardiac defect in a clinic.

The current diagnosis criteria of NS greatly rely on clinical features including facial features, while it is known that facial features differ even in healthy populations of different ethnic background. We therefore compared the evaluation of a list of prespecified facial features conducted by three different evaluators using the same facial photos for the same patients. Surprisingly, we found great variations in the evaluation between evaluators, although there is much room for improvement in the design of this investigation (i.e., blinding, introducing non-NS controls, training before evaluation, etc). This topic is rarely explored before as far as we know. The study conducted by Paul Kruszka et al. [35] was the first one to examine potential difference in phenotypes of NS (including facial features) in diverse populations. Among the 21 clinical features (including seven facial features), difference in ptosis and webbed neck was found to be statistically significant between populations, while other clinical features were consistent. However, the studied NS patients in this study was much older (i.e., mean age about 10 years), while it has been known that clinical characteristics especially facial features change over time [6]. Moreover, the study used facial analysis technology for the identification of facial features, which is

different from our study and should be interpreted differently. Considering NS patients may first present to a variety of healthcare professionals, our findings (i.e., the great variation of evaluation of facial features between evaluators) provide a direction for improving NS recognition.

Our study has some strengths. First, we included a relatively large NS cohort, and they were from the consecutive pediatric patients presented at a regional cardiac center in recent years (2018–2020). Second, NS was confirmed by genetic testing, and the facial features and cardiac defects we investigated were identified by multiple evaluators or directly obtained from echocardiography. However, there are also some limitations. First, the study population was selected from a single cardiac center, which may limit generalizability of our findings. Second, all the information was retrospectively collected, and there were a few patients without facial photos or echocardiography, who might be likely to be without typical facial features and signs of cardiac defect. This would lead to an overestimated prevalence of the clinical characteristics we investigated. Third, the facial photos we used for evaluation were not taken in a standard position or of similar image quality. This may also explain the variation in evaluation of facial features between evaluators. Fourth, the exome sequencing was conducted by two commercial companies over a four-year time period (i.e., 2018–2021), and hence it could be expected that there were variations in the detailed processes. Even for subjects tested by the same commercial companies, the examined gene panels might have changed over time, and we therefore could not provide the exact technical details on the exome sequencing as well as quality control and examined gene panels. It remains unknown whether these issues might bias our findings, although we thought this might not be a severe concern since most features we observed seemed similar to literature (as we mentioned above). A prospective study using the same and well-planned exome sequencing processes as well as prespecified gene panels would be preferred to address this limitation. Last, although the sample size in our study was still larger than most studied that investigated NS patients (especially from Chinese population), the absolute size is still limited, and variations by chance cannot be ruled out. These limitations should be considered for future studies.

Data and code availability

Due to privacy reasons the authors cannot share the data used for this study.

Ethics declarations

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (No. GDRECKY2020-033-01). All subjects gave their informed consent for inclusion in writing before they participated in the study.

CRediT authorship contribution statement

Qinchang Chen: Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Dian Hong:** Writing – review & editing, Validation, Methodology. **Yulu Huang:** Writing – review & editing, Validation, Investigation. **Zhiwei Zhang:** Writing – review & editing, Supervision, Resources, Project administration, Data curation, Conceptualization. **Shushui Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27038.

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