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Prevalence of Noonan spectrum disorders in a pediatric population with valvar pulmonary stenosis

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Abstract

Objective: To evaluate the prevalence of Noonan spectrum disorders (NSD) in a pediatric population with valvar pulmonary stenosis (vPS) and identify the clinical characteristics that differentiate those with NSD from those without NSD.

Design: A retrospective chart review of 204 patients diagnosed with vPS between 9/1/2012 and 12/1/2016 at a pediatric medical center was performed. The quantitative features of vPS, genetic diagnosis information, and phenotypic characteristics of Noonan syndrome were collected. Chi-square test, Fisher's exact test, *t* test, Wilcoxon rank-sum test, and ANOVA were used for comparisons among the groups. Logistic regression was used to test for the association between the clinical characteristics and the presence of NSD.

Results: Syndromic diagnoses were made in 10% of the children with vPS, with NSD accounting for 6%. Hypertrophic cardiomyopathy ($P < .0001$), short stature ($P < .0001$), developmental delay ($P < .0001$), ophthalmological abnormalities ($P < .0001$), pectus carinatum/excavatum ($P = .01$), neurological abnormalities ($P = .022$), and aortic stenosis ($P = .031$) were present more often in individuals with NSD compared to nonsyndromic vPS. A logistic regression analysis showed a 4.8-fold increase in odds for NSD for each additional characteristic ($P < .0001$).

Conclusions: At least 6% of the children with vPS have an underlying NSD. Individuals with vPS and NSD were significantly more likely to have additional features known to be associated with NSD than those with vPS without NSD. We conclude that vPS in the presence of one or more significant characteristics should prompt referral for genetic evaluation as a guide to ascertain patients at risk for NSD while optimizing the use of clinical genetics evaluation and potential genetic testing.

KEYWORDS

congenital heart disease, pulmonary valve dysplasia, rasopathy

1 | INTRODUCTION

Valvar pulmonary stenosis (vPS) with or without pulmonary valve dysplasia accounts for approximately 8-12% of all congenital heart diseases (CHD). Prevalence estimates for vPS in the general population range from about 3-5 per 10 000 live births.[1-4] Valvar pulmonary stenosis is characterized by obstruction due to the fusion of the valve leaflet commissures; echocardiographic features include a normal sized valve annulus, systolic doming of the valve leaflets, and post-stenotic dilation of the proximal main pulmonary artery. Pulmonary valve dysplasia as described has a small valve annulus and thickened valve leaflets, with limited and asymmetrical cusp movement.[5,6] Diagnoses of vPS are commonly made in individuals with Noonan syndrome (NS), a genetic condition characterized by short stature, CHD, and characteristic facial features[7] estimated to affect 1 in 1000 to 1 in 2500 live births.[8] The frequency of vPS in NS is reported to be about 50%-70%.[9-13]

NS exhibits autosomal dominant inheritance with age-related variability in the phenotype.[14] NS displays significant genetic heterogeneity; at least nine genes are known to cause NS, with *PTPN11* being the most common, although at least 17 genes in the RAS-MAPK signaling pathway have been associated with related RASopathies and Noonan spectrum disorders (NSD) which share a considerable phenotypic overlap.[15-28] Sharland et al reported an average age at diagnosis of 9 years and indicated that diagnosing patients at an earlier age would be beneficial.[8] A more recent study reported an average age at diagnosis of 7 years.[29] Importantly, 60% of the cases are sporadic with no family history of disease,[12] highlighting the need to systematically ascertain *de novo* cases as early as possible. In addition to vPS, hypertrophic cardiomyopathy (HCM) is another common cardiac finding among individuals with NSD, which occurs in approximately 20%-30% of the patients and can present in infancy or as age increases.[8,9] The clinical presentation and extracardiac features associated with NS can be highly variable, and some features may be subtle and easily overlooked.

Dysmorphic facial features tend to be most striking in newborns and most subtle in adults. Infants may display feeding difficulties (75%), failure to thrive, and developmental delay.[8,12] Delayed puberty is common, and males with a history of cryptorchidism (77%) can have fertility issues.[8,30] Skeletal abnormalities most commonly include pectus deformities (70%). Short stature is observed in 50%-70% of people with NS.[8,31] Hematological abnormalities (30%-65%) commonly include coagulation defects.[8,32] The prematurity rate in NS has been described as up to 53% compared to a prematurity rate of 9.6% worldwide.[9,33,34] Congenital dysplasia, hypoplasia, or aplasia of the lymphatic channels can lead to lymphedema (49%), which may be progressive in nature and potentially life-threatening.[10,35-37] Renal abnormalities (10%) may occur, though typically minor. Hearing loss (40%)[8] can be conductive or sensorineural, mild to profound, and may lead to speech delay.[38-40] Guidelines for care have been established to optimize the complex management of individuals with NS.[11,41,42]

Because vPS comprises a significant portion of CHD in the general population, it is important to ascertain which patients with vPS are most likely to have a syndromic diagnosis and thus require evaluation by genetics. Pulmonary valve dysplasia has been noted to occur more frequently (25%-35%)[43-45] in individuals with NS.[46,47] Thus, previous studies have suggested that vPS with a dysplastic PV is an informative finding in patients with NS since it is typically identified less frequently in patients with non-syndromic vPS.[46-49]

Currently, there are no guidelines for patients with vPS regarding referral for the evaluation of NSD. Many of the associated medical concerns such as cardiac defects, hearing loss, developmental delays, feeding difficulties, and failure to thrive present in infancy or childhood have significant implications.[42,50,51] Appropriate genetics referrals in this population would optimize patient care by reducing the number of patients who remain undiagnosed until later in life, by which time opportunities for early intervention for such concerns have been lost. Studies suggest that there are higher risks associated with cardiac interventions for patients with NSD as well as higher rates of re-intervention.[10,52] Thus, being able to accurately ascertain individuals at-risk for NSD may be important prior to intervention. Furthermore, guidelines for referral would optimize genetics resource utilization including clinical evaluation and testing. Therefore, we sought to investigate the prevalence of NSD in a pediatric population with vPS and identify the most distinguishing cardiac and extracardiac phenotypic characteristics between those with and without NSD.

2 | METHODS

An IRB-approved retrospective medical record review of patients born between 09/01/2012 and 04/23/2016 who had diagnoses of vPS and had at least one echocardiogram performed at Cincinnati Children's Hospital Medical Center (CCHMC) was completed. Data obtained from the chart review were recorded and stored in a REDCap database. For the purposes of this study, individuals with complex congenital heart malformations which may have vPS as an associated finding, or which might influence the structure or function of the PV, were excluded. Individuals with other types of pulmonary stenosis (subvalvar, supravalvar, peripheral pulmonic) in the absence of vPS were excluded.

2.1 | Genetic diagnoses

NSD diagnosis was recorded if the patient had either a clinical diagnosis by genetics evaluation or a diagnosis confirmed by molecular testing. Genetics evaluation was defined as an evaluation by a clinical geneticist with a documented physical exam. Evaluations and notes pertaining to sessions run by genetic counselors alone were not recorded as genetics evaluations, as they did not include a physical exam. Genetic testing including multigene NSD panels (ranging from 11 to 16 genes) and *PTPN11* single gene sequencing completed among 4 clinical genetic testing laboratories over a 4-year period was reviewed. For the purposes of this study, NSD included cardio-facio-cutaneous syndrome (CFC), Costello syndrome, NS with multiple lentigines (NSML), and neurofibromatosis type 1 (NF1) in addition to

NS due to the overlap of similar clinical features, including vPS, associated primarily with NS. Studies have demonstrated that individuals with NF1 may exhibit a distinctly Noonan-like phenotype.[53,54] The non-NSD group included those with normal NSD genetic test results and those who had not undergone genetic testing. Individuals with a diagnosis of a genetic condition outside of NSD were included in the cohort but excluded from data analysis comparisons.

2.2 | Cardiac characteristics

Diagnoses of vPS indicated by ICD-9 or ICD-10 codes were confirmed through positive identification of vPS in at least one echocardiogram report from CCHMC. Echocardiogram reports and cardiology progress notes were used to extract information regarding the diagnosis of vPS and additional diagnoses of cardiac disease and cardiology interventions. Congenital heart defects beyond vPS, including HCM, aortic stenosis (AS), atrial septal defects (ASD), ventricular septal defects (VSD), patent foramen ovale (PFO), and patent ductus arteriosus (PDA) were collected.

2.3 | Extracardiac clinical characteristics

Data pertaining to the clinical characteristics associated with NS were extracted from the medical record. The framework for choosing appropriate clinical characteristics was based on the van der Burgt criteria.[55] Diagnoses of clinical conditions were recorded if the patient had seen a specialist in the field pertaining to that diagnosis. Ophthalmological abnormalities included in data collection were ptosis, strabismus, amblyopia, nystagmus, myopia, hyperopia, astigmatism, and presbyopia. Neurological abnormalities included seizures, craniosynostosis, hydrocephalus, and Arnold Chiari malformation. Neurological and ophthalmological abnormalities were collapsed into two categories, respectively, during data analysis. Short stature, prematurity, and developmental delay were recorded when noted in the problem list.

2.4 | Statistical analyses

To address whether there were significant differences between the NSD and non-NSD groups, the clinical characteristics associated with NS that were hypothesized to be significant were selected. This included pectus carinatum/excavatum, neurological abnormalities, ophthalmological abnormalities, developmental delay, short stature, prematurity, dysplastic PV, and additional cardiac abnormalities (HCM, AS, ASD, VSD, PDA, PFO). The additional cardiac abnormalities were initially analyzed independently for significance between groups. Those that reached statistical significance independently were retained as independent clinical characteristics. Additional cardiac abnormalities that did not reach significance independently were collapsed into a group labeled "additional cardiac abnormalities" and analyzed as a single characteristic. Additionally, we looked at the vPS intervention status, age at initial vPS intervention, initial PV peak gradient, and initial PV annulus diameter to explore the differences between the NSD and non-NSD groups regarding the vPS disease status. Age at initial genetics evaluation was compared to investigate the differences in ages at which the NSD group and the non-NSD group first presented to genetics. A univariate analysis was completed, and the clinical characteristics that subsequently reached a statistical significance of P less than .2 were selected for further analysis. This was performed using a logistic regression model to test for the association between the number of clinical characteristics observed and presence of the NSD diagnosis. To investigate whether significant differences were present between those with a documented genetics evaluation and those without a documented genetics evaluation, we utilized the same clinical characteristics described above, with the exception of age at initial geneticist evaluation.

The group with diagnoses of genetic conditions outside of NSD was compared to non-NSD group in terms of clinical characteristics to determine whether or not these groups could be combined. Due to significant differences between these two groups, the individuals with a diagnosis of a genetic condition outside of NSD were included in the cohort but excluded from data analysis comparisons. The results are presented as proportions and percentages for categorical variables and means and standard deviations, or medians and interquartile values (for not normally distributed data), for continuous variables. Chi-square test, Fisher's exact test, t test, Wilcoxon rank-sum test, and ANOVA were used for comparisons among the groups. A significance level was established at alpha less than 0.05. For multiple testing, we used the Bonferroni correction adjusted for correlations among variables employing the SISA website.[56] For a comparison between the NSD and non-NSD groups and between individuals with and without a clinical genetics evaluation, the threshold for significance was 0.005. Both comparisons had 15 variables with a Spearman's correlation coefficient of 0.15. Statistical analysis was performed using JMP Genomics 8.0 and SAS 9.4 software.[57]

3 | RESULTS

A total of 306 patients were reviewed for inclusion in the study. Of the 306 charts reviewed, 102 patients were excluded based on the previously described criteria, leaving a cohort of 204 patients with vPS eligible for the study. Patients were also excluded due to the lack of confirmatory documentation of vPS on cardiology clinic notes and/or echocardiogram studies at CCHMC. The mean age of the cohort was 2.01 years at the time of data collection with a standard deviation of 1.07. The majority (72.5%) of the patients were White, followed by Black/African American (15.2%), and Other/Unknown (12.3%).

3.1 | Genetic testing and diagnoses in vPS population

A documented clinical genetics evaluation was found in 37 of the 204 patients (18.1%), including evaluations made during inpatient hospital visits. Genetic testing for NSD was performed in 20 of the 37 (54.1%) patients with a genetics evaluation. NSD diagnoses were

made in 12 of the 20 (60%) patients, making up 5.9% of the study cohort (Table 1). Compared to a prevalence of 0.04%-0.1% of NS in the general population, the 5.9% prevalence rate found in our study cohort indicates a significantly enriched population ($P < .0001$). *PTPN11* mutations were identified in 7 of the 12 (58.3%) individuals, *NF1* mutations in 2 of the 12 (16.7%), with *KRAS*, *BRAF*, and *RAF1* mutations each identified in one individual (8.3%). All individuals who had genetic testing, either positive or negative, underwent a gene panel testing for NSD, except for one individual in the NSD group who had a single gene test for *PTPN11*. All patients in the non-NSD group with a "negative" molecular test had at least 12 genes included in the analysis. The genes not included on all panels were *RASA2*, *RIT1*, *SOS2*, *SPRED1*, and *NF1*. Four individuals in the non-NSD group with a "negative" molecular test did not have an analysis of *NF1*. The mean age at NSD diagnosis was 9.3 months. Of the 12 patients with diagnosed NSD, 1 (8.3%) had a confirmed *de novo* mutation and 2 (16.7%) had confirmed familial mutations. The remainder of the patients had an unknown mode of inheritance.

There were seven patients with a genetics evaluation who were not tested for NSD, but instead were diagnosed with other unrelated genetic conditions, confirmed through molecular testing. This group included the Cornelia de Lange syndrome, Williams syndrome ($n = 2$), 22q11.2 deletion syndrome, Alagille syndrome, Hurler syndrome, and a patient with multiple chromosomal copy number variants. One patient did not have an evaluation by genetics but was diagnosed through molecular testing to have Pendred syndrome. Overall, 20 of the 204 patients in the vPS cohort had a genetic diagnosis, indicating a total syndromic prevalence of 9.8%. The eight patients with diagnoses of genetic conditions outside of the NSD were excluded from all further statistical analyses.

3.2 | Clinical characteristics compared between the NSD and non-NSD groups

Seven clinical characteristics were found to be significantly associated with a NSD diagnosis including developmental delay, HCM, short stature, pectus carinatum/excavatum, neurological abnormalities, ophthalmological abnormalities, and AS. Four of these clinical characteristics, including ophthalmological abnormalities, developmental delay, HCM, and short stature, were significantly different after Bonferroni correction ($P < .0001$). Pectus carinatum/excavatum, neurological abnormalities, and AS did not reach statistical significance after multiple adjustment but were significant at a nominal level ($P < .05$). Characteristics that did not reach significance independently included vPS intervention status, dysplastic PV, initial PV annulus diameter, age at initial vPS intervention, initial PV peak gradient, age at initial geneticist evaluation, prematurity, and additional cardiac abnormalities, which were analyzed independently and as a grouped variable (ASD, VSD, PDA, PFO) (Table 2). Secondary analysis showed no significant differences in age at diagnosis for vPS between children with and without NSD (median = 59.0 days, IQR = 40.8-133.3 days and median = 51.5 days, IQR = 8.0-143.8 days, respectively, $P = 0.37$).

Ophthalmological abnormalities were collapsed during data analysis into one category. Of the patients with NSD, ophthalmological abnormalities were found in 7 of the 12 individuals (58.3%), with ptosis in 4 of the 12 (33.3%) and astigmatism in 4 of the 12 (33.3%) most frequently observed. Two or more ophthalmological abnormalities were identified in 5 of the 12 (41.7%) patients with NSD. Likewise, neurological abnormalities were collapsed during data analysis into a single category. Neurological abnormalities were present in 3 of the 12 (25%) individuals with NSD, with the most frequent finding being seizures, found in 2 of the 12 patients (16.7%).

There was a statistically significant association between the number of significant clinical characteristics present in an individual and positive NSD diagnosis ($P < .0001$). A logistic regression analysis showed a 4.8-fold increase in the odds for a positive NSD diagnosis for each significant characteristic present in an individual (OR = 4.8, 95% CI = 2.6-8.6).

The cohort was divided into subgroups based on NSD diagnosis status, history of a genetics evaluation, and history and results of any molecular testing for NSD (Figure 1). Looking at the number of significant clinical characteristics present in the non-NSD group, 153 of the 184 (83.2%) individuals had 0 of the identified significant clinical characteristics. Overall, 31 of the 184 (16.8%) individuals in the non-NSD group displayed one or more of the 7 significant clinical characteristics. A subgroup of the non-NSD cohort included eight patients (4.3%) who had negative genetic testing for NSD. Of these patients, 5 of the 8 (62.5%) had 0 of the significant clinical characteristics, 1 of the 8 (12.5%) had 1 characteristic, 1 of the 8 (12.5%) had 2 characteristics, and 1 of the 8 (12.5%) had 5 of the significant characteristics. None of these patients received a clinical diagnosis of NSD. In total, 22 of the 184 (12.0%) individuals in the non-NSD group had at least one significant clinical characteristic and had never been evaluated by genetics or had genetic testing for NSD.

3.3 | Clinical characteristics of patients with and without a genetics evaluation

Clinical genetics evaluation was completed in 30 of the 196 (15.3%) individuals of the study cohort, excluding patients with a syndromic diagnosis outside of NSD. Of the clinical characteristics compared between the group with a genetics evaluation and the group without a genetics evaluation, 12 clinical characteristics were significantly different (Table 3). Of these 12 clinical characteristics, 7 were significant after Bonferroni correction. These included vPS intervention status ($P = .00081$), developmental delay ($P < .0001$), HCM ($P < .0001$), short stature ($P < .0001$), pectus carinatum/excavatum ($P = .0033$), neurological abnormalities ($P = .0002$), and ophthalmological abnormalities ($P < .0001$). After multiple adjustments, 5 of the 12 clinical characteristics, including VSD, AS, dysplastic PV, prematurity, and initial PV peak gradient, did not reach statistical significance but were significant at a nominal level ($P < .05$). There were no differences in additional cardiac abnormalities, initial PV annulus diameter, and age at initial vPS intervention between

TABLE 1 Characterization of patients with NSD diagnosis

Birth (y)	Age vPS Dx (mo)	Age vPS intervention (mo)	Additional cardiac diagnoses	NSD Dx	Variant classification	Age NSD Dx (mo)	Molecular results (gene, DNA variation, protein variation)	Findings significant in population present in individual
2012	12		Mitral valve prolapse, tricuspid valve prolapse	NS	Pathogenic	17	*RAF1*, c.769T>C, p.Ser257Pro	HCM
2013	2		ASD, PFO	NS, (d)	Pathogenic	14	*PTPN11*, c.172A>G, p.Asn58Asp	Ptosis, hyperopia, astigmatism
2013	2		Dysplastic PV, ASD	CFC	Pathogenic	6	*BRAF*, c.1914T>G, p.Asp638Glu	Ptosis, hyperopia, astigmatism, seizures
2013	5		Dysplastic PV, PFO, subvalvar PS	NSML	Pathogenic	3	*PTPN11*, c.1391G>C, p.Gly464Ala	HCM, pectus carinatum
2013	7	11	Dysplastic PV, VSD, PFO	NS, (f)	Pathogenic	10	*PTPN11*, c.923A>G, p.Asn308Ser	HCM, ptosis, hydrocephalus
2013	1	6	Dysplastic PV, ASD, VSD, PFO	NS	Likely pathogenic	8	*PTPN11*, c.766C>G, p.Gln256Lys	Ptosis
2014	1	2	Dysplastic PV, PFO, supravalvar PS	NF1, (f)	Likely pathogenic	7	*NF1*, c.3251C>G, p.Pro1084Arg	Myopia, astigmatism
2014	2		ASD, VSD, PDA, bilateral SVC, supravalvar PS	NS	Pathogenic	2	*KRAS*, c.173C>T, p.Thr58Ile	HCM, strabismus, nystagmus, hyperopia, seizures, craniosynostosis
2015	3		Dysplastic PV	NF1	Pathogenic	21	*NF1*, c.5440C>T, p.Gln1814Ter	
2015	3	9	Dysplastic PV, ASD, left pulmonary artery stenosis	NS	Pathogenic	14	*PTPN11*, c.182A>G, p.Asp61Gly	Pectus carinatum
2015	<1	4	Dysplastic PV, PFO, trileaflet aortic valve	NS	Pathogenic	6	*PTPN11*, c.923A>G, p.Asn308Ser	AS, myopia, astigmatism
2016	<1		Trileaflet aortic valve	NS	Pathogenic	3	*PTPN11*, c.228G>C, p.Glu76Asp	AS

Abbreviations: AS, aortic stenosis; ASD, atrial septal defect; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PS, pulmonary stenosis; PV pulmonary valve; SVC, superior vena cava; VSD, ventricular septal defect; (f), known familial mutation, (d), confirmed de novo mutation.

TABLE 2 Clinical characteristics compared between NSD group and non-NSD group

Characteristic	NSD [*n* = 12 (6%)]	No NSD [*n* = 184 (94%)]	*P* value
PV dysplasia	8 (67[a])	95 (52)	.38
vPS intervention status	5 (42)	50 (27)	.28
HCM	4 (33)	0	<.0001*
AS	2 (17)	3 (2)	.031
Additional cardiac abnormalities	9 (75)	127 (69)	.76
Prematurity	4 (33)	41 (23)	.48
Developmental delay	8 (67)	17 (9)	<.0001*
Short stature	5 (42)	5 (3)	<.0001*
Pectus carinatum/excavatum	2 (17)	1 (<1)	.01
Neurological abnormalities	3 (25)	8 (4)	.022
Ophthalmological abnormalities	5 (58)	10 (5)	<.0001*
Characteristic	**NSD**	**No NSD**	***P* value**
Initial PV peak gradient (mm Hg)	M = 30.0, SD = 13.6 [12[b]]	M = 28.7, SD = 18.1 [180]	.81
Initial PV annulus diameter (cm)	M = 0.8, SD = 13.6 [10]	M = 0.9, SD = 0.2 [174]	.62
Age at initial vPS intervention (days)	M = 196.4, SD = 103.6 [5]	M = 120.6, SD = 211.8 [50]	.44
Age at initial genetics evaluation (days)	M = 225.3, SD = 180.1 [12]	M = 150.6, SD = 211.7 [18]	.31

Abbreviations: AS, aortic stenosis; HCM, hypertrophic cardiomyopathy; M, mean; PV, pulmonary valve; SD, standard deviation; vPS, valvar pulmonary stenosis.
[a]Numbers in parentheses indicate percentage of individuals within the subgroup.
[b]Numbers in brackets indicate "*n*."
*P value significant after Bonferroni correction.

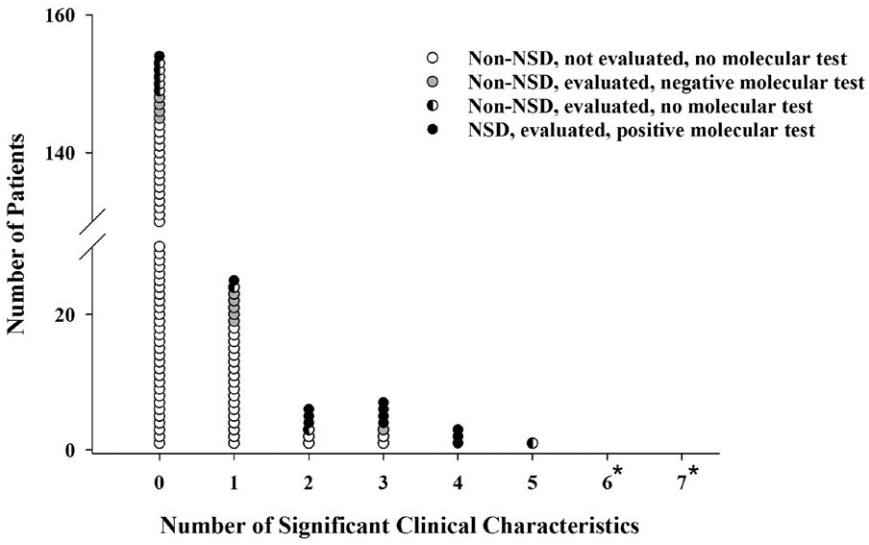

FIGURE 1 Number of significant clinical characteristics present in cohort subgroups based on NSD diagnosis status, history of a genetics evaluation, and history and results of molecular testing for NSD. Asterisks (*) indicate that no individuals met this criterion

individuals with and without a genetics evaluation. Secondary analysis showed no significant differences in age at diagnosis for vPS between children with and without a previous genetics evaluation (median = 51.0 days, IQR = 3.8-80.0 days and median = 56.0 days, IQR = 11.8-151 days, respectively, *P* = 0.16).

4 | DISCUSSION

The prevalence of NS among individuals presenting with vPS is unclear, with limited data to guide clinicians, including cardiologists, on indication for further genetic evaluation. To our knowledge, this is the first description of the cardiac and extracardiac features of NSD in a cohort of patients with vPS. The results of our study indicate the prevalence of NSD in our institution-based population of individuals diagnosed with vPS is ~6%, with a total syndromic prevalence of ~10%. Patients with vPS and NSD were significantly more likely to have HCM, AS, short stature, developmental delay, pectus carinatum/excavatum, ophthalmological abnormalities, and neurological abnormalities compared to individuals with apparently nonsyndromic vPS.

The majority (83.2%) of the non-NSD group had 0 of the 7 significant clinical characteristics present. One or more of the 7 significant

TABLE 3 Clinical characteristics compared between group with a clinical genetics evaluation and group without a clinical genetics evaluation

Characteristic	Evaluation [*n* = 30 (15%)]	No Evaluation [*n* = 166 (85%)]	*P* value
PV dysplasia	21 (70[a])	82 (49)	0.038
vPS intervention status	16 (53)	39 (23)	0.00081*
HCM	4 (13)	0	0.0005*
AS	4 (13)	1 (<1)	0.002*
VSD	5 (17)	10 (6)	0.044
Additional cardiac abnormalities	25 (83)	111 (67)	0.055
Prematurity	12 (40)	33 (20)	0.016
Developmental delay	11 (37)	14 (8)	<0.0001*
Short stature	8 (27)	2 (1)	<0.0001*
Pectus carinatum/excavatum	3 (10)	0	0.0033*
Neurological abnormalities	6 (20)	5 (3)	0.0002*
Ophthalmological abnormalities	11 (37)	6 (4)	<0.0001*
Characteristic	**Evaluation**	**No Evaluation**	***P* value**
Initial PV peak gradient (mm Hg)	M = 36.1, SD = 19.6 [30[b]]	M = 27.4, SD = 17.2 [162]	0.030
Initial PV annulus diameter (cm)	M = 0.8, SD = 0.1 [26]	M = 0.9, SD = 0.2 [158]	0.059
Age at initial vPS intervention (days)	M = 100.0, SD = 108.5 [16]	M = 138.8, SD = 233.6 [39]	0.40

Abbreviations: AS, aortic stenosis; HCM, hypertrophic cardiomyopathy; M, mean; PV, pulmonary valve; SD, standard deviation; vPS, valvar pulmonary stenosis; VSD, ventricular septal defect.

[a]Numbers in parentheses indicate percentage of individuals within the subgroup.

[b]Numbers in brackets indicate "*n*."

*P value significant after Bonferroni correction.

clinical characteristics was present in 22 individuals in the non-NSD group (12.0%) without molecular testing or a genetics evaluation, indicating that these patients should be referred for genetics evaluation, as it is possible that some could have an undiagnosed NSD. This is especially true among those with more than one of the clinical significant characteristics which included 4 of the 22 (18.2%) individuals, as a logistic regression analysis found a 4.8-fold increase in the odds of a positive NSD diagnosis for each additional significant clinical characteristic present. Considering the variability in NSD phenotype and that many features may not manifest until later in life, it is worth noting that some individuals in the non-NSD group may have NSD even without associated significant features. This suggests that our presented prevalence of 5.9% could be much higher, and also supports the need for better guidelines to determine which patients with vPS are referred for a genetics evaluation.

PV characteristics would be an ideal method to distinguish the risk for NSD among individuals with vPS since all individuals undergo detailed cardiac evaluation and imaging. Prior research has suggested that a dysplastic valve in addition to vPS is an informative finding in patients with NS since it is typically identified less frequently in patients with nonsyndromic vPS.[43,44,48,49] In our NSD group, 8 of the 12 (67%) had a dysplastic PV. This is higher than the rates of PV dysplasia found in past studies, such as Ishizawa et al (35%), Burch et al (27%), and Bertola et al (24%). This increased rate of valve dysplasia could be due to the accrual of patients from a single institution, which may classify PV dysplasia differently than other institutions. Currently, there is no widely accepted definition of PV "dysplasia," essentially making it a qualitative diagnosis that is noted in clinical echocardiogram reports. Past research has suggested that the PV diameter can be used as a measure of PV dysplasia.[44] The average initial PV annulus dimensions in our study were not significantly different when comparing individuals with and without NSD. Importantly, the presence of a dysplastic PV was ultimately not significantly different between those with and without a diagnosis of NSD in this study. Other PV characteristics compared between the NSD and non-NSD groups were also not statistically different, including vPS intervention status, age of initial vPS intervention, initial PV peak gradient, and initial PV annulus diameter. Additional cardiac abnormalities (ASD, VSD, PDA, PFO) were not significant.

While variables associated specifically with the vPS phenotype were not identified as significant, co-occurring cardiac diagnoses including AS and HCM were significantly more common in the NSD group compared to the non-NSD group. In fact, nearly all individuals in the NSD group had at least one co-occurring cardiac diagnosis in addition to vPS, suggesting that additional cardiac diagnoses are perhaps the rule instead of the exception in NSD (Table 1). AS has a known association with NSD, and the current management guidelines recommend assessing adults for new or previously missed aortic disease including AS.[42,58-60] HCM is a common cardiac finding among individuals with NSD.[8,9] HCM was present significantly more often in patients with NSD, and was identified in 4 of the 12 (33.3%) individuals, while none of the non-NSD patients were diagnosed

with HCM, which suggests that vPS and HCM could be reliable indicators of NSD. Research indicates that patients with NSD and HCM have increased early mortality rates compared to patients with nonsyndromic HCM.[50] Furthermore, late survival, defined as survival to 15 years of age, has been demonstrated to be significantly worse for individuals with both NSD and HCM compared to those with nonsyndromic HCM.[51] It is worth noting that even in individuals with NSD and vPS without HCM, postintervention outcomes for vPS appear less successful compared with outcomes for nonsyndromic vPS. Research shows an increased prevalence of reinterventions and procedure-related complications.[10,52] This further highlights the importance of early consideration of a possible NSD diagnosis in an individual with vPS with or without HCM, as well as the need for ongoing cardiac evaluation. Overall, the results indicate that co-occurring cardiac diagnoses in addition to vPS, especially HCM and AS, are important to consider when evaluating a patient for a possible NSD.

Among individuals with vPS in our cohort who were referred for a clinical genetics evaluation, 12 clinical characteristics were found to be significant, including 6 cardiac characteristics (HCM, AS, VSD, dysplastic PV, vPS intervention status, and initial PV peak gradient). Patients with a clinical genetics evaluation more often had a dysplastic PV, at least one intervention for vPS, or a higher PV peak gradient compared to patients without a clinical genetics evaluation. Patients with co-occurring diagnoses of either HCM, AS, or VSD were more likely to be evaluated, although other cardiac abnormalities (ASD, PFO, PDA) were not significant. This suggests that these characteristics could influence the pattern of genetics referrals for patients with vPS at CCHMC.

Data from this study suggest that both cardiac and extracardiac features are informative in distinguishing NSD and non-NSD vPS. Ascertainment of infants with vPS without a family history of a NSD is complicated by the age-related variability of the phenotype of a NSD as many features may not be obvious at the time a child is initially diagnosed with vPS. In some cases, diagnosis of a child may lead to diagnoses in apparently asymptomatic parents. Even cardiac characteristics such as HCM and AS may be present in infancy or may develop later in life. For this reason, cardiologists and other providers must pay careful attention to the significant findings for which they are able to assess on an ongoing basis in patients with a history of vPS. Significant findings that may be appreciated upon physical examination include ocular findings of ptosis, pectus deformity, and short stature. Depending on the scope of the examination, care providers may appreciate developmental delay or note a history of neurological features such as seizures.

4.1 | Study limitations

An analysis of our subgroup of individuals with NSD is limited by sample size and the age of our cohort. Because this study included only individuals from infancy through age 4, mild conditions that can present later in life, such as a learning disability, may not have been identified. Additionally, variables such as short stature, developmental delay, and prematurity were noted when documented in the problem list. Incorrect or missed documentation of these variables may have influenced the results. Because only individuals with features prompting a referral to genetics would have been tested for NSD, this study is subject to ascertainment bias. It is possible that we missed individuals with undiagnosed NSD in our cohort of patients with vPS. Approximately 12% of the non-NSD individuals had at least one significant characteristic; some of these individuals may in fact have an underlying NSD. Furthermore, molecular testing in our cohort was not uniform for all individuals and we cannot rule out that a pathogenic mutation was missed due to the omission of genes on some of the NSD panels. The findings from this study would support the inclusion of *NF1* on NSD panels, especially among individuals with vPS. A subgroup of our cohort with genetic diagnoses outside of NSD was not included in our main statistical comparisons due to significant statistical differences between this group and the non-NSD group, which could be a potential limitation. This study is limited by its retrospective nature and accrual of cases from a single tertiary care center. Future studies should include prospective research for patients diagnosed with vPS, which may include universal genotyping for NSD. This could be useful in identifying more accurate extracardiac and cardiac predictors of NSD, especially with a standardized approach to characterize the PV morphology.

4.2 | Clinical implications and recommendations

These data suggest that at least 6% of the patients with vPS have an underlying diagnosis of NSD. However, referring all patients with vPS for genetics evaluation would not be a feasible or efficient use of resources; therefore, it is important to identify a subset of patients with vPS who would benefit from a clinical genetics evaluation and potential genetic testing. An early diagnosis of NSD is critical due to both the implications regarding surgical intervention and complications for cardiac disease, as well as the baseline recommendations for management, which include ongoing cardiac evaluation, regular growth monitoring, developmental assessment, neuropsychological assessment, renal ultrasound, coagulation screening, and ophthalmological evaluation.[42]

Research has found that for patients with NSD, cardiac conditions were the most common reason for initial presentation to medical care, excluding admission to the neonatal intensive care unit. Furthermore, the next most common reasons for presentation included developmental delay, facial dysmorphism, and short stature.[9] Coupled with the results of the present study, it is clear that cardiologists could play a major role in early evaluation and diagnosis of these patients, as the presence of cardiac characteristics including HCM and AS, and extracardiac characteristics including developmental delay, short stature, and pectus deformities in addition to vPS warrants the consideration of the presence of a NSD.

Based on the findings presented in this study, we conclude that it would be reasonable to consider referring patients with vPS and one additional significant clinical characteristic (including HCM, AS, developmental delay, short stature, pectus carinatum/excavatum,

ophthalmological abnormalities, or neurological abnormalities) to genetics for the evaluation of NSD, which is further supported by an odds ratio of 4.8 for each additional clinical characteristic present. Considering over 30% of the patients with a NSD and vPS had a diagnosis of HCM, and none of the non-NSD patients were diagnosed with HCM, cardiologists may consider universally referring patients with both vPS and HCM for genetics evaluation for NSD, as this characteristic appears to be an extremely informative finding. Because of the known evolution and variability of NSD phenotype with age, it is crucial that providers reassess patients with vPS for the presence of the aforementioned significant characteristics during follow-up visits, as some characteristics, such as developmental delay and short stature, may be difficult, if not impractical, to assess in newborns. In addition to the consideration of NSD, due to the overall syndromic prevalence of approximately 10% in this vPS cohort, cardiologists should be aware of any extracardiac features in children with vPS and should have a low threshold for referring for genetics evaluation.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Concept/design, data collection, data analysis/interpretation, drafting article, critical revision of article, approval of article: Anderson

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