ARTICLE

The Spectrum of Cardiac Anomalies in Noonan Syndrome as a Result of Mutations in the *PTPN11* Gene

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ABSTRACT

OBJECTIVE. Noonan syndrome is a clinically homogeneous but genetically heterogeneous condition. Type 1 Noonan syndrome is defined by the presence of a mutation in the *PTPN11* gene, which is found in ~40% of the cases. Phenotype descriptions and cardiac defects from cohorts with Noonan syndrome were delineated in the "pregenomic era." We report the heart defects and links to gene dysfunction in cardiac development in a large cohort of patients with type 1 Noonan syndrome.

METHODS. This was a retrospective, multicenter study based on clinical history, pictures, and medical and cardiologic workup over time. Data were collected by referral geneticists. Mutation screening was performed by direct sequencing of exons 2, 3, 4, 7, 8, 12, and 13 and their intron-exon boundaries, which harbor 98% of identified mutations the *PTPN11* gene.

RESULTS. A *PTPN11* gene mutation was identified in 104 (38.25%) of 274 patients with Noonan syndrome. Heart defect was present in 85%. The most prevalent congenital heart defects were pulmonary valve stenosis (60%), atrial septal defect, ostium secundum type (25%), and stenosis of the peripheral pulmonary arteries (in at least 15%). Pulmonary valve stenosis and atrial septal defect, ostium secundum type, were significantly associated with the identification of a mutation in the *PTPN11* gene. Ventricular septal defect and most left-sided heart defects showed a trend toward overrepresentation in the group without a mutation.

CONCLUSION. We compared our data with previous series and integrated the comprehension of molecular *PTPN11* gene dysfunction in heart development.

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Key Words
Noonan syndrome, cardiac defect, *PTPN11* gene, phenotype, genotype

Abbreviations
NS—Noonan syndrome
CHD— congenital heart defect
PVS—pulmonary valve stenosis
HCM— hypertrophic cardiomyopathy
ASD-OS—atrial septal defect of ostium secundum type
VSD—ventricular septal defect
RAS—rat sarcoma viral oncogene homolog
MAPK—mitogen-activated protein kinase
Egfr— epithelial growth factor receptor
NFAT—nuclear factor of activated T cell
LEOPARD—lentigines, EKG anomalies, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, deafness

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NOONAN SYNDROME (NS) is an autosomal dominant multiple congenital anomalies syndrome that is characterized by minor facial anomalies (hypertelorism, downward slant of the palpebral fissures with ptosis, and low-set posteriorly angulated ears), short stature, congenital heart defects (CHDs), and variable learning disabilities. With an estimated incidence of 1 in 2000,[1] NS turned out to be 1 of the most common genetic condition in pediatrics and the second most common syndromal form of CHD, after trisomy 21.[2] Type 1 NS corresponds to the causally homogeneous subgroup of patients who have NS and are molecularly defined by the presence of a mutation in the *PTPN11* gene. Mutations in *PTPN11* are responsible for ~40% of the cases of NS and for >95% of patients with LEOPARD syndrome (lentigines, EKG anomalies, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, deafness). The penetrance of the mutations of *PTPN11* is 100%, but their expressivity is variable even within families. Very recently, mutations in *KRAS*[3] and *SOS1*[4,5] were shown in, respectively, <5% and 15% of *PTPN11*-negative patients with NS. *PTPN11*, *SOS1*, and *KRAS* are part of the *RAS* signaling pathway. The causative gene(s) remains to be found for roughly half of patients with NS.

A broad spectrum of cardiac phenotypes has been recognized in NS. Pulmonary valve stenosis (PVS), hypertrophic cardiomyopathy (HCM) and atrial septal defect of ostium secundum type (ASD-OS) are the most common defects in NS, but ventricular septal defect (VSD), peripheral pulmonary stenosis, atrioventricular canal, aortic stenosis, mitral regurgitation, aortic coarctation, and coronary anomalies can also be seen in NS. Previous studies of cohorts of patients with NS suggested that the prevalence of specific types of CHD was different in type 1 NS and in NS without *PTPN11* mutation.[6–11] We report the cardiac anomalies in a large cohort of patients with type 1 NS to delineate the spectrum of cardiac defects that are caused by *PTPN11* mutations and better understand the role of *PTPN11* in heart development.

METHODS

Patients with NS or LEOPARD syndrome were recruited through a multicenter network of clinical geneticists from France, Switzerland, and Belgium for molecular analysis of the *PTPN11* gene. The patients were entered systematically in the study, on the basis of the order by which samples arrived in the laboratory for diagnosis. Checklists were systematically sent to referring geneticists independently and before completion of the DNA screening. Call for supplementary data was not based on the identification (or not) of a mutation. Specific cardiologic data were derived from records of transthoracic echocardiography and, when available, from cardiac catheterization and/or heart surgery. Because the data were mainly retrospective, distinction between PVS with normal valves and pulmonary valve dysplasia was hampered by the impossibility to check retrospectively for the presence of valvular dysplasia in patients for whom only pulmonary stenosis was mentioned. For this reason, we decided to merge the 2 anomalies under the term PVS.

Mutation screening focused on hot-spot locations in the *PTPN11* gene, which covered 98% of identified mutations.[6–9] Exons 2, 3, 4, 7, 8, 12, and 13 of the *PTPN11* gene and their intron-exon boundaries were sequenced on both strands from genomic DNA for each patient on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Mutations were identified using ABI PRISM SeqScape 2.0 (Applied Biosystems) by comparison with the reference sequence for genomic and complementary DNA sequence (GenBank accession Nos. NT_009775.14 and NM_002834).

Statistical analysis for qualitative data and comparison used χ^2 and exact Fisher test. Significance was determined as $P < .05$. Informed consent was obtained for inclusion and DNA screening. The ethics committee of AP-HP Bichat University Hospital (Paris, France) approved the study.

RESULTS

A total of 291 patients with NS or LEOPARD syndrome were initially enrolled in the study. The 19 patients who presented with café-au-lait spots and/or lentigines were found to share a small number of specific mutations. Because their clinical and molecular diagnosis was LEOPARD syndrome, they were studied and published separately.[12] The final cohort of patients with NS comprises 149 boys and 123 girls. The mean age in the cohort is 7 years for the groups both with and without mutation (lower quartile: 2 years; upper quartile: 13 years).

A mutation in the *PTPN11* gene was identified in 104 (38.25%) patients. Table 1 summarizes the cardiologic findings in patients. The c.922A→G (p.N308D) nucleotide substitution represents the most prevalent mutation (18.25%) in our patients. Table 2 presents the spectrum of CHD by functional domain, by exon, and, specifically, by those observed with the p.N308D mutation.

Among patients with a mutation, the apparent rate of heart defect is 85%. Because the selection criteria for offering molecular testing were clinically based and potentially biased toward overrepresentation of CHD-bearing patients, the true penetrance of CHDs in type 1 NS can nevertheless not be established objectively. The most prevalent CHD in type 1 NS are PVS (60%), ASD-OS (25%), and stenosis of the peripheral pulmonary arteries (in at least 15.4%). PVS and ASD-OS are significantly associated with the presence of a mutation ($P < .01$), and coarctation of aorta and HCM are associated with the absence of mutation. VSD and most left-sided heart defects show a trend toward overrepresentation in the group without a mutation. Comparison with previously reported series of genotyped NS is difficult. We summa-

TABLE 1 **Comparison of CHD Frequency Between *PTPN11*-Positive (Type 1 NS) and *PTPN11*-Negative Patients in Our Cohort**

CHD	Patient With an Identified *PTPN11* Mutation ($n = 104$), *n* (%)	Patient Without an Identified *PTPN11* Mutation ($n = 168$), *n* (%)	*P*
PVS	62 (59.60)	53 (31.55)	<.0001
ASD	26 (26.90)	22 (13.10)	<.012
Atrioventricular canal	2 (1.90)	3 (1.80)	NS
HCM	12 (10.60)	38 (22.60)	.022
Coarctation of aorta	1 (0.96)	10 (5.95)	.042
VSD	7 (6.70)	23 (13.70)	.074
Aortic stenosis	2 (1.90)	8 (4.70)	.22
Mitral insufficiency	5 (4.81)	12 (7.10)	.43
Peripheral pulmonary artery stenosis	16 (15.40)	17 (10.10)	.20

NS indicates not significant.

TABLE 2 **Spectrum of CHDs Reported With the 3 Functional Domains of the SHP2 Protein and Within the 7 Screened Exons**

CHD	Exon, *n* (%)									
	N-SH2 Domain			C-SH2 Domain		PTP Domain				N308D ($n = 19$)
	All	2	3	All	4	All	7	8	13	8
All CHD	44	1	43	8	5	52	6	27	19	19
PVS	27 (43.55)	0	27 (43.55)	5 (20.00)	2 (40.00)	46	5 (71.40)	26 (25.00)	15 (79.00)	11 (57.90)
ASD	13 (50.00)	1 (100.00)	12 (49.00)	3 (9.00)	2 (9.00)	10	1 (3.80)	5 (19.00)	4 (23.00)	5 (26.30)
Atrioventricular canal	0	0	0	0	0	0	0	0	0	0
HCM	5 (18.50)	0	3 (30.00)	0	0	7	2 (28.60)	4 (30.00)	1 (7.00)	2 (10.50)
Coarctation of aorta	0	0	0	0	0	1	0	1 (0.96)	0	0
VSD	0	0	0	1 (20.00)	1 (6.00)	7	0	7 (6.70)	0	0
Aortic stenosis	0	0	0	0	0	3	0	2 (1.90)	1 (7.00)	1 (5.20)
Mitral insufficiency	5 (18.50)	0	5 (100.00)	0	0	5	0	5 (4.80)	0	0

The last column summarizes the CHD associated with the single common N308D mutation located on exon 8.

rized the published data on CHDs in type 1 NS in Table 3 and compared the relative incidence of major CHDs in type 1 NS versus the general population based, for example, on prevalence data from Eurocat Database registries from European Western countries and/or Italian and/or Iceland databases (Table 4).

DISCUSSION

PTPN11 is a gene that is located on chromosome band 12q24.1 and encodes the nonreceptor protein tyrosine phosphatase SHP-2. The protein contains a phosphatase domain (PTP domain) and 2 amino-terminal SH2 domains (N-SH2 and C-SH2) that selectively bind to short amino acid motifs that contain a phosphotyrosyl residue. These SH2 domains promote SHP-2 association with cell surface receptors and cell adhesion molecules' and modulate the cells' responses to extracellular signals by regulating the phosphotyrosine content of specific intracellular proteins. More specific, SHP-2 functions as an intracellular enhancer of signal transduction for several growth factors, hormones, and cytokine receptors that are involved in the RAS/MAPK (rat sarcoma viral oncogene homolog/mitogen-activated protein kinase) signaling pathway, although its precise role in the pathway, as inhibitor of RAS inactivation by GAP (guanosine-triphosphatase–activating protein) proteins or as enhancer of the kinase cascade beyond RAS activation, remains elusive.[13]

The N-SH2 domain is a conformational switch: it either binds to and inhibits the PTP domain or binds phosphotyrosine proteins and allows the PTP catalytic domain to bind to specific phosphoproteins residues and hydrolyze them.[8] Almost all reported mutations so far are missense exon changes, except for a unique in-frame 3-bp deletion.[14] Almost all NS mutations are gain-of-function mutations that seem to alter N-SH2–PTP interaction, turning protein into a constitutionally active state.[15–17] Some of them might further affect SH2 domain-phosphopeptide affinity and/or substrate specificity.[18] Mutations cluster in 7 exons (2, 3, 4, 7, 8, 12, and 13). Those exons encode the interacting portions of SH2 and the PTP domains or the hinges segment binding the 3 domains. Exons 3 and 8 harbor 80% of the mutations. A single mutation (c.922A→G-p.N308D-exon 8) is present in ~20% of patients. Contrasting with NS mutations, LEOPARD syndrome mutants are catalytically defective and act as dominant negative mutations.[16,19,20]

The spectrum of CHDs has been widely described in

TABLE 3 **Combined Data on CHDs in Type 1 NS From 6 Cohorts**

Parameter	Patients With Type 1 NS							NS Patients Without *PTPN11* Mutation					
	Tartaglia et al[6] (2002)	Sarkozy et al[7] (2003)	Musante et al[8] (2003)	Zenker et al[9] (2004)	Yoshida et al[10] (2004)	Jongmans et al[11] (2005)	This Study	Cumulated (Incidence, %)	Tartaglia et al[6] (2002)	Sarkozy et al[7] (2003)	Musante et al[8] (2003)	Zenker et al[9] (2004)	Yoshida et al[10] (2004)	Jongmans et al[11] (2005)	This Study
No. of cases	54	23	32	34	18	76	104	341 (100.00)	65	58	NM	23	94	27	168
No. of patients with cardiac defect	42	20	28	ND	15	62	86	253 (74.20)	58	54	NM	ND	ND	13	114
PVS	36	13	21	30	10	38	62	209 (82.60)	30	17	NM	12	NM	6	53
ASD	6	2	4	6	10	17	26	73 (28.85)	11	4	NM	4	NM	4	22
Atrioventricular canal	NM	2	NM	NM	NM	NM	2	NP	NM	7	NM	NM	NM	NM	3
HCM	3	3	0	3	NM	4	12	NP	17	4	NM	6	NM	NM	36
Coarctation of aorta	NM	0	NM	NM	NM	NM	1	NP	NM	4	NM	NM	NM	NM	10
VSD	NM	0	3	5	NM	NM	7	NP	NM	3	NM	3	NM	NM	23
Aortic stenosis	NM	NM	NM	NM	NM	NM	2	NP	NM	NM	NM	NM	NM	NM	8
Mitral insufficiency	NM	1	NM	NM	NM	NM	5	NP	NM	2	NM	NM	NM	NM	12
Pulmonary branches stenosis	NM	NM	NM	NM	NM	NM	16	NP	NM	NM	NM	NM	NM	NM	17

NM indicates not mentioned; NP, not possible (missing data).

NS before the era of *PTPN11* gene identification: PVS, ASD-OS, VSD, Ebstein anomaly, and coarctation of aorta were the most common heart defects encountered in patients with NS, as well as HCM.[2,21–24] Among 151 patients who had NS and were aged 1 week to 45 years, Sharland et al[25] reported PVS in 63%, HCM in 17%, and septal defects in 6%. Heart was normal in 10%. In a series of 184 patients, Digilio et al[24] underlined that coarctation of aorta was present in 8.7%. In a cohort of 157 children, Marino et al[2] emphasized high prevalence of atrioventricular canal (15.4%), whereas PVS represented only 38.9%, HCM 9.5%, coarctation of aorta 8.8%, ASD-OS 8%, mitral valve anomaly 5.8%, tetralogy of Fallot 4.4%, VSD 4.4%, patent ductus arteriosus 2.2%, and pulmonary atresia 1.4%.

Since the discovery of *PTPN11* involvement in type 1 NS, 6 small cohorts of patients have been reported from Europe and Asia.[6–11] The spectrum of CHD in type 1 NS cannot be drawn easily from these cohorts (Table 3). In the genotype/phenotype cohort study by Tartaglia et al,[6] only PVS, ASD-OS, and HCM were considered. PVS was present in 70.6% of the group with mutation and in 46.2% of the group without mutation ($P < .01$), whereas HCM was more prevalent in patients without a mutation (26% vs 5.9%; $P < 0{,}01$). The other CHDs were not reported and a "*PTPN11*-negative" group was not described. In a study of 96 patients with NS, Musante et al[8] found a mutation rate of 29%. PVS was present in 60%; no HCM was identified among *PTPN11*-positive patients. There was no information on cardiac findings in the group without mutation, and only PVS, septal defects (of all types), and HCM were reported. The cardiac defects in the *PTPN11*-positive group were limited to the presence (or absence) of PVS, ASD-OS, VSD, and HCM. Sarkozy et al[7] reported 73 patients with NS or LS and identified a mutation in 37% of the cases. Among patients with a mutation, PVS, HCM, partial AVC, and ASD-OS were the most common anomalies. No coarctation of aorta, tetralogy of Fallot, or VSD was observed. A trend for ASD-OS to be correlated with exon 3 mutations was suggested. All mutations in the N-SH2 domain were associated with the presence of a heart defect, whereas in 70%, the mutations occurred in the PTP domain. Zenker et al[9] reported 57 children with NS. The mutation rate was 60%. PVS was noted in 88% and HCM was noted in 3% of *PTPN11*-positive patients. The cardiac findings in the group without mutation were not given. Among 45 cases, Yoshida et al[10] detected PVS in 55% of patients with mutation. As in Tartaglia's and Musante's studies, only PVS, HCM, or ASD-OS was recorded. In the most recent study,[11] only PVS, HCM, ASD-OS were described separately.

Preselection criteria may explain differences between our study and the previous ones. Tartaglia et al[6] included patients with clinical phenotype "evocative" of NS and PVS or HCM and/or pectus deformity or electrocardio-

TABLE 4 **Cardiac Anomalies Identified in Our Patients With Type 1 NS (in %)**

Cardiac Anomaly	Prevalence (per 10 000), Atlanta, GA, 1995–1997[37]	Italian Multicentric Study 1992–1993	Iceland Study 1990–1999	Type 1 NS, %	Relative Risk	Eurocat Prevalence Database 1999–2003 (per 10 000 births)
HCM	NM	NM	NM	10.58	NA	NM
Heterotaxias and L-TGA	1.60	3.70	NM	0.00	0.40	3.13
Outflow tract defects, total						
Tetralogy of Fallot	4.70	3.30	3.00	0.00	0.25	3.08
D-transposition of the great arteries	2.40	2.00	1.90	0.00	0.22	6.21
Double outlet right ventricle	2.20	2.00	NM	0.00	0.47	NM
Truncus arteriosus	0.60	NM	NM	0.00	1.25	1.01
Atrioventricular septal defect	NM	5.40	1.40	1.93	5.37	3.63
With Down syndrome	2.40	NM	NM	NA	NA	NM
Without Down syndrome	1.00	NM	NM	1.93	1.93	NM
Total anomalous pulmonary venous return	0.60	0.80	NM	0.00	NA	NM
Ebstein anomaly	0.60	0.50	NM	0.00	NA	NM
Right obstructive defects						
Tricuspid atresia	0.30	0.50	NM	0.00	NA	NM
Pulmonary atresia, intact septum	0.60	0.60	NM	0.00	NA	NM
Pulmonic stenosis, atresia	5.95	7.30	6.50	58.65	28.20	NM
Peripheral pulmonary stenosis	7.00	NM	NM	16.35	23.30	
Left obstructive defects						
Hypoplastic left heart	2.10	1.80	0.70	0.00	NA	NM
Coarctation of aorta	3.50	2.40	3.80	0.96	2.90	3.20
Aortic arch atresia or hypoplasia	0.60	0.70	0.90	0.00	NA	NM
Aortic valve stenosis	0.80	2.20	1.50	2.88	16.00	NM
Mitral insufficiency	NM	1.20	1.50	4.81	35.60	NM
Septal defects						Malformation of cardiac septa: 41.36
VSD	24.98	39.00	45.70	6.73	1.60	NM
ASD	10.00	7.50	12.20	26.92	2.40	NM
Isolated hypertrophy	NM	NM	NM	6.73	NA	NM
Patent ductus arteriosus	8.10	3.80	11.50	0.00	NA	NM
Other major heart defects	9.70	NM	NM	0.00	NA	NM
Total	90.20	NM	NM	NM	NA	NA

The data are listed with the available data on prevalence of cardiac defect in the general population from 3 large studies. Heart defects are grouped following Van Praagh's system.[36] Eurocat Database Prevalence period spanning from 1999 to 2003: cardiac defects encompass anomalies of cardiac chambers and connections, atrioventricular septal defect, transposition of great vessels, tetralogy of Fallot, malformations of valves, malformations of the great arteries and veins, malformations of cardiac septa, coarctation of aorta, common arterial truncus (www.eurocat.ulster.ac.uk). L-TGA indicates L transposition of the great arteries; NA, not applicable; NM, not mentioned.

gram anomaly, whereas Zenker et al[9] used a combination of 2 of 3 criteria: typical heart defect (PVS or HCM), typical craniofacial morphology, and pterygium colli. The last suggested that his higher mutation rate was attributable to more stringent and rigorous inclusion criteria. These criteria may bias to selecting patients with the 2 most prevalent defects. No data have been published on the incidence of peripheral pulmonary stenosis in NS type 1. Physiologically, transient peripheral pulmonary stenosis is encountered in normal newborns (during transition from fetal to postnatal). Its incidence in our series is unlikely to reflect a physiologic transient state.

Comparison of patients with and without mutation is presented in Table 1. PVS was identified in 59.6% among mutation carriers (62 of 104) and 31.5% in the noncarrier group (53 of 168), ASD-OS respectively in 25% (26 of 104) and 13% (22 of 168), and stenosis of the peripheral pulmonary arteries in 15.4% (16 of 104) and 10.1% (17 of 168). Left-sided involvement was associated with absence of an identified *PTPN11* mutation: mitral insufficiency in 4.8% (5 of 104) vs 7.1% (12 of 168), aortic stenosis in 1.9% (2 of 104) vs 4.7% (8 of 168), and coarctation of aorta in 0.96% (1 of 104) vs 5.95% (10 of 168; $P < .01$). Finally, presence of VSD occurred in 6.75% (7 of 104) vs 13.7% (23 of 168; $P < .001$).

Genotype-phenotype correlations within type 1 NS have been briefly addressed. In our cohort, nucleotide substitutions occurred in 44 (42.30%) in the N-SHP domain (exons 2 and 3), 5 (4.8%) in the C-SHP domain, 3 (2.9%) at the N-SHP/C-SHP hinge region, and 52 (50%) in the PTP domain. Exon 2 mutations were identified in <1%. Exon 3 mutations occurred in half of the cases; PVS was associated in 46% (29 of 62) of the cases. Exons 4 and 7 mutations were observed in 4.8% and 5.8%, respectively. Exon 8 mutations occurred in 26%. The N308D substitution has an incidence of 18.26% (19 of 104). PVS is the more common cardiac defect associated with exon 8 mutations, accounting for 58% (11 of

19) of CHD. Mutations in exon 13 account for 18.30%, 55% (10 of 19) being PVS. In Table 4, we compared the frequencies of CHD in patients with NS with their frequencies in the general population, based on registries of congenital anomalies, and computed relative risks (when possible). This relative risk varies widely, from 0.22 for D-transposition of the great arteries to 35.6 for mitral insufficiency.

The spectrum of CHD that is observed with *PTPN11* mutations points to a crucial role for this gene in the normal pattern of right cardiac development and anatomy. Left-sided heart malformations are more prevalent in *PTPN11*-negative patients.

The function of *PTPN11* gene during heart embryogenesis remains to be elucidated. *Ptpn11*, the mouse ortholog of the human *PTPN11*, is required during early mouse development for gastrulation. A mouse that carries the D61G mutation has been produced and recapitulates NS.[26] In a mouse model, Chen et al[27] found that normal epithelial growth factor receptor (Egfr) signaling pathway necessitates interaction of Egfr with Shp2. The Egfr pathway is necessary to permit semilunar valve development. Despite their involvement in NS, expression of PTPN11 does not seem to occur in atrioventricular valves.[18,27] Mice that bear homozygous *Egfr* mutations show electrocardiographic anomalies, aortic stenosis, and regurgitation. In the chick embryo, the hyperproliferative effect of an expressing Shp2 bearing a type 1 NS mutation Q79R on mesenchymal cells in valve primordia was shown to be mediated by extracellular signal–regulated kinase 1/2 activation through the RAS/MAPK pathway. The effect was similarly obtained when transfecting the gene for a constitutively active MEK-1, a downstream molecule that regulates ERK (extracellular signal–regulated kinase) phosphorylation, the mutations of which result in cardiofaciocutaneous syndrome.[28] Finally, *PTPN11* may interfere with calcium/calcineurin/nuclear factor of activated T cell (NFAT) signaling. Calcineurin is a calmodulin-dependent, calcium-activated serine/threonine-specific protein phosphatase. On activation by Ca^{2+}, calcineurin dephosphorylates the NFAT transcription factor, leading to its nuclear translocation and activation. In mice, NFAT activation represses *VEGF* (vascular endothelial growth factor) expression in the myocardium underlying the site of prospective valve formation, which is essential for endocardial cells to transform into mesenchymal cells and, later, for direct valvular elongation and refinement.[29] The calcium/calcineurin/NFAT signal transduction pathway has been implicated in a variety of developmental processes, including in cardiomyocyte maturation, heart valve formation, vascular development,[30,31] and cardiomyocyte hypertrophy.[32] It has been shown that gain-of-function mutants of SHP-2 enhanced fibroblast growth factor-2–mediated Ca^{2+} oscillations in fibroblasts, spontaneous Ca^{2+} oscillations in cardiomyocytes, and reduced nuclear translocation and transcriptional activity of NFAT, pointing to a possible link between the calcium/calcineurin/NFAT pathway and *PTPN11*.[33] It is interesting that *PTPN11* is not involved in nonsyndromic atrioventricular septal defects and coarctation of the aorta[34] or in nonsyndromic HCM.[35]

CONCLUSIONS

Our study of CHDs in type 1 NS illustrates the wide spectrum of developmental anomalies that result from dysregulation of the RAS/MAPK signaling pathway and the difficulty of correlating molecular anomalies with the occurrence of a type of CHD.

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