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A *PTPN11* allele encoding a catalytically impaired SHP2 protein in a patient with a Noonan syndrome phenotype

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Abstract

The RASopathies are a relatively common group of phenotypically similar and genetically related autosomal dominant genetic syndromes caused by missense mutations affecting genes participating in the RAS/mitogen-activated protein kinase (MAPK) pathway that include Noonan syndrome (NS) and Noonan syndrome with multiple lentigines (NSML, formerly LEOPARD syndrome). NS and NSML can be difficult to differentiate during infancy, but the presence of multiple lentigines, café au lait spots, and specific cardiac defects facilitate the diagnosis. Furthermore, individual *PTPN11* missense mutations are highly specific to each syndrome and engender opposite biochemical alterations on the function of SHP-2, the protein product of that gene. Here, we report on a 5-year-old male with two *de novo* *PTPN11* mutations in *cis*, c.1471C>T (p.Pro491Ser) and c.1492C>T (p.Arg498Trp), which are associated with NS and NSML, respectively. This boy's phenotype is intermediate between NS and NSML with facial dysmorphism, short stature, mild global developmental delay, pulmonic stenosis and deafness but absence of café au lait spots or lentigines. The double-mutant SHP-2 was found to be catalytically impaired. This raises the question of whether clinical differences between NS and NSML can be ascribed solely to the relative SHP-2 catalytic activity.

Keywords

double mutation; LEOPARD; Noonan; *PTPN11*

INTRODUCTION

The RASopathies are a group of phenotypically and genetically related autosomal dominant genetic syndromes caused by mutations in genes encoding proteins participating in signaling through the RAS/mitogen-activated protein kinase (MAPK) pathway and include Noonan syndrome (NS), Noonan syndrome with multiple lentigines (NSML, formerly, LEOPARD syndrome), Costello syndrome, and cardiofaciocutaneous syndrome [Rauen, 2013; Tartaglia and Gelb, 2010]. The RASopathies are relatively common, affecting roughly 1:1000 live births, with NS being the most common. Mutations in *PTPN11*, which encodes the protein tyrosine phosphatase SHP-2, account for 50% of NS and 90% of NSML [Sarkozy et al., 2008; Tartaglia et al., 2011].

NS and NSML share overlapping features including similar facial dysmorphism, hypertrophic cardiomyopathy (HCM) and pulmonic stenosis (PS), and can be challenging to differentiate clinically in infancy [Digilio et al., 2011]. Nonetheless, the two disorders can often be distinguished because patients with NSML, but very few with NS, manifest multiple lentigines, which can be congenital but emerge by age 5 [Digilio et al., 2011; Sarkozy et al., 2008]. In addition, café-au-lait spots are present in ~75% of infants with NSML but are unusual in NS (~10%) [Digilio et al., 2006]. A minority of patients with NSML has sensorineural deafness (10-25%), a finding that is extremely rare in NS. Conversely, 80-90% of individuals with NSML have HCM, which only 20% of those with NS have [Sarkozy et al., 2004; Shaw et al., 2007].

In contrast to the clinical overlap at young age, the individual *PTPN11* missense mutations are highly specific to NS and NSML respectively [Tartaglia et al., 2006]. Moreover, the biochemical alterations engendered by the amino acid substitutions associated with NS and NSML differentiate them quite well. *PTPN11* mutations causing NS increase SHP-2's phosphatase activity, either constitutively or in response to phosphotyrosyl activation, and increase RAS/MAPK signaling [Fragale et al., 2004]. In contrast, *PTPN11* mutations causing NSML strongly reduce SHP-2's activity [Hanna et al., 2006; Tartaglia et al., 2006] and have been suggested to result in dominant-negative effects to signaling through the RAS/MAPK pathway [Kontaridis et al., 2006]. What remains less clear is how such dramatically dissimilar biochemical effects produce such remarkably similar phenotypes.

Here, we report on a patient with two *de novo* *PTPN11* mutations in *cis* affecting exon 13, c.1471C>T and c.1492C>T (p.Pro491Ser and p.Arg498Trp), which are associated with NS and NSML respectively. Biochemically, the double-mutant SHP-2 shows clear loss of phosphatase activity; however, the child's phenotype is intermediate between NS and NSML.

MATERIALS AND METHODS/RESULTS

Patient Report

This 5-year old Han Chinese male was born to unrelated healthy parents. He was first evaluated by a clinical geneticist at age 2 years 4 months and found to have relative macrocephaly (head circumference 48 cm, 25th centile) with weight and height both below

3rd centile (10.1 kg, 50th centile for age 1 year 2 months and 82 cm, 50th centile for age 1 year 6 months), sensorineural deafness, mild global developmental delay, mild pectus deformity, and bilateral cryptorchidism. This patient also showed mild dysmorphic facial features including hypertelorism (inner canthal, outer canthal, and interpupillary distances all >97th centile), mild ptosis and downslanting palpebral fissures, low-set and posteriorly angulated ears (Fig 1). A thorough dermatologic evaluation confirmed the absence of café au lait spots and lentigines. This patient was re-evaluated at six-month intervals until age 5, but lentigines were never observed. Echocardiography had first been performed at age 2 months demonstrating valvular stenosis with gradient of 40 to 50 mmHg across the lesion. Repeat echocardiography, performed at similar intervals, failed to show progression of the PS or the development of HCM. At the most recent evaluation at age 5, the patient continued to manifest relative macrosomia with persistence of height and weight below 3rd centile. He had developmental delays, achieving 3.5- to 4-year milestones, and was receiving supportive services at school. Overall, this patient had facial dysmorphism and PS, consistent with NSML or NS, and sensorineural deafness, which discriminates towards NSML, but was missing key dermatologic findings that weakened the clinical diagnosis away from NSML.

Molecular Genetics

DNA sequence analysis was performed for *PTPN11*, the most commonly mutated gene for both NSML and NS, and revealed two missense mutations affecting exon 13, c.1471C>T and c.1492C>T, which predicted the p.P491S and p.R498W substitutions, respectively. The p.P491S mutation has previously been associated with NS and the p.R498W allele causes NSML [Lee et al., 2011; Sarkozy et al., 2004]. Analysis of *PTPN11* exon 13 in the parents showed normal sequences. Identity testing using single nucleotide polymorphisms (Sequenom iPLEX Pro Sample ID Panel) ruled out non-paternity (probability of paternity > 99.99991%). Thus, we concluded that the proband had two *de novo* *PTPN11* mutations.

To determine if the two *PTPN11* mutations had arisen in *cis* or *trans*, the proband's *PTPN11* exon 13 was PCR amplified and subcloned (pCR2.1®-TOPO® TA vector, Life Technologies). The inserts of 12 independent clones were sequenced, of which, five showed reference sequence and seven showed both mutations. No clone with only one mutation was observed. Thus, we concluded that the two *de novo* *PTPN11* mutations arose *in cis*.

To assess the effects of the P491S and R498W substitutions on catalytic function, these mutants, the double mutant and the wild-type enzyme were expressed in bacteria, purified and their phosphatase activities were determined *in vitro* basally and following stimulation with the protein tyrosine phosphatase non-receptor type substrate 1 (PTPNS1) bisphosphotyrosyl-containing activation motif (BTAM peptide) [Martinelli et al., 2008]. We then compared the phosphatase activities of the double mutant to wild-type SHP-2 as well as both of the single mutants. As shown in Figure 2, the basal phosphatase activity of the P491S/R498W double mutant protein was barely detectable, similar to the NSML-associated R498W single mutant protein and different from the wild-type and NS-associated P491S single mutant SHP-2 proteins. After BTAM stimulation, the activities of all SHP-2 proteins tested increased significantly. As expected, the NS-associated P491S SHP-2 showed

substantially greater phosphatase activity compared to wild-type protein, while the NSML-associated R498W mutant was catalytically impaired, being characterized by a weak response to stimulation. The P491S/R498W double mutant SHP-2 also had reduced phosphatase activity, comparable to the NSML-associated R498W protein. Based on this finding, we concluded that the P491S/R498W double mutant SHP-2 behaved biochemically like an NSML mutant.

DISCUSSION

In summary, we report on a patient who presented with clinical findings consistent with NS but with sensorineural deafness, an unusual finding. Although the diagnosis of NSML was considered, neither lentigines nor café-au-lait spots were observed. The use of DNA testing, which can often definitively differentiate NS from NSML, was not decisive as the child was found to harbor two *PTPN11* mutations—one associated with NS and the other with NSML. Biochemical characterization of the doubly mutant SHP-2 protein resulting from this boy's *in cis* mutations was most consistent with the diminished phosphatase activity associated with NSML.

Voron et al. [1976] proposed clinical diagnostic criteria for NSML, such that the presence of multiple lentigines plus two of the other cardinal features of NSML were sufficient for the diagnosis. In the absence of lentigines, it was proposed that patients must have three of the other cardinal features of NSML and have an affected first-degree relative. For our proband, the absence of lentigines would prevent a diagnosis of NSML using the first set of criteria and the fact that his *PTPN11* mutations were *de novo* obviates the possibility of applying the second set of criteria. In the current era where DNA testing is available for patients with RASopathy phenotypes, lentigines are still observed in all but the exceptional person harboring NSML-associated *PTPN11* mutations (*n.b.*, to our knowledge, there is no published patient of NSML without lentigines for an individual < 5 years, although there is one allusion to a personal observation of such a patient) [Sarkozy et al., 2008]. Consistent with these considerations, the occurrence of HCM with lentigines and/or café-au-lait spots had been documented in all the subjects reported to carry a heterozygous *PTPN11* missense change predicting the p.Arg498Trp amino acid substitution (Table I). For our subject, the lack of lentigines by age 5 as well the absence of café-au-lait spots or HCM make the NSML diagnosis difficult to sustain.

One limitation of the foregoing is that the phenotypic spectrum of many genetic disorders is expanding as molecular testing is applied more broadly. It is possible that we will discover that some patients with NSML never develop features currently viewed as seminal or do so at older ages.

To date, physicians and scientists studying NS and NSML have struggled to understand how *PTPN11* mutations that engender opposite effects on SHP-2's phosphatase activity can result in such highly similar phenotypes. Mouse models with disease-relevant mutations introduced into the endogenous *Ptpn11* gene have begun to reveal the pathogenesises. For instance, mice modeling *PTPN11*-associated NS typically show substantial hyperactivation of the Mapks, Erk1/2 [Araki et al., 2004]. In contrast, mice modeling *PTPN11*-associated

NSML, which exhibit HCM, show normal Erk1/2 activation in their myocardium but hyperactivation of Akt; pharmacologic blockade of Akt signaling through mTor using rapamycin prevents or reverses the HCM in the NSML mouse [Marin et al., 2011]. Thus, it is becoming apparent that rather different perturbations in signal transduction elicited from specific RASopathy mutations are resulting in comparable organ-level abnormalities.

While one must draw conclusions about the phenotypic effects of this subject's double *PTPN11* missense mutations with caution, as it is only a single patient, this very rare "experiment of nature" does provide some opportunity for novel insight. Since this patient's double mutant SHP-2 has reduced phosphatase activity, one would have predicted that he would have typical NSML features. Since that is not the case, it implies that perturbations in signal transduction cannot be ascribed simply to the level of SHP-2's phosphatase activity. Rather, mutant SHP-2 proteins must interact with other signaling proteins in a complex fashion that could depend on several factors. Recently published work aimed at addressing this longstanding paradox presented a dual role for the catalytically inhibiting NSML-associated mutations that, similar to NS-associated mutations, also weaken the autoinhibitory conformation via the interaction of the PTP and N-SH2 domains and instead lead the N-SH2 domain to preferentially interact with scaffolding proteins and growth factor receptors [Yu et al., 2013]. Analogously, we speculate that the P491S/R498W protein is more likely to remain activated after stimulation with phosphotyrosyl binding to its N-SH2 and C-SH2 domains, similar to the P491S single mutant SHP-2 (and other NS-associated mutants). Even though the 2nd mutation, R498W, reduces the phosphatase activity, the double mutant protein presumably exhibits its low-level activity in a temporally inappropriate fashion, which may perturb signaling in a manner more typical for NS. Alternatively, the double mutant SHP-2 may be interacting with other proteins because it is in the active conformation for longer in a manner that is independent of its phosphatase activity.

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Figure 1.
Mild dysmorphic facial features at 2 years 4 months (A) and 5 years (B and C).

Figure 2.

In vitro phosphatase activity measured by picomoles of p-nitrophenyl phosphate as a substrate for WT and mutant SHP-2 proteins under basal (white bars) and BTAM stimulated (black bars) conditions. All values represent means and standard deviations from triplicate independent experiments normalized to the basal activity of WT SHP-2. The P491S and R498W mutants exhibited phosphatase activity as expected for NS- and NSML-associated mutant proteins respectively; the P491S/R498W double mutant exhibited phosphatase activity similar to NSML-associated mutations with dramatically reduced basal activity and a muted response to phosphotyrosyl stimulation.

Table IClinical features of individuals carrying *PTPN11* mutations at codon 498.

Amino acid substitution	Patient	Age (years)	HCM	Multiple lentigines	Café-au-lait spots	Deafness	Reference
R498W							
	1	2	+	–	+	–	[Sarkozy et al., 2004]
	2 ^a	34	+	+	–	–	[Sarkozy et al., 2004]
	3	<1	+	+	+	–	[Digilio et al., 2006]
	4 ^b	<1	+	NA	NA	NA	[Kratz et al., 2006]
R498L							
	5	13	+	+	+	–	[Sarkozy et al., 2004]
	6 ^c	39	–	+	–	+	[Du-Thanh et al., 2007]
	7	NA	NA	NA	NA	NA	[Hung et al., 2007]
	8	NA	NA	NA	NA	NA	[Lo et al., 2009]

Hypertrophic cardiomyopathy (HCM)

^a Mother of patient 1.^b This newborn died at 2 months. At the age of 11 days, he exhibited a myeloproliferative disorder with excessively proliferating immature erythroid precursors infiltrating nonhematopoietic organs.^c This patient was reported to have atrioventricular block.