Brief report

The mutational spectrum of *PTPN11* in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease

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Germ line *PTPN11* mutations cause 50% of cases of Noonan syndrome (NS). Somatic mutations in *PTPN11* occur in 35% of patients with de novo, nonsyndromic juvenile myelomonocytic leukemia (JMML). Myeloproliferative disorders (MPDs), either transient or more fulminant forms, can also occur in infants with NS (NS/MPD). We identified *PTPN11* mutations in blood or bone marrow specimens from 77 newly reported patients with JMML (n = 69) or NS/MPD (n = 8). Together with previous reports, we compared the spectrum of *PTPN11* mutations in 3 groups: (1) patients with JMML (n = 107); (2) patients with NS/MPD (n = 19); and (3) patients with NS (n = 243). Glu76 was the most commonly affected residue in JMML (n = 45), with the Glu76Lys alteration (n = 29) being most frequent. Eight of 19 patients with NS/MPD carried the Thr73Ile substitution. These data suggest that there is a genotype/phenotype correlation in the spectrum of *PTPN11* mutations found in patients with JMML, NS/MPD, and NS. This supports the need to characterize the spectrum of hematologic abnormalities in individuals with NS and to better define the impact of the *PTPN11* lesion on the disease course in patients with NS/MPD and JMML. (Blood. 2005;106:2183-2185)

Introduction

The *PTPN11* proto-oncogene encodes Src-homology tyrosine phosphatase 2 (SHP-2), a protein tyrosine phosphatase with a role in signal transduction and hematopoiesis.[1,2] Somatic *PTPN11* mutations exist in 35% of juvenile myelomonocytic leukemia (JMML) specimens and are less frequent in other leukemias.[3-6] SHP-2 relays signals from activated growth factor receptors to Ras. *PTPN11*, *KRAS2, NRAS,* and *NF1* mutations are found in mutually exclusive subsets of patients with JMML.[3,4] These data support the hypothesis that hyperactive Ras signaling plays a central role in JMML.

Germ-line *PTPN11* mutations cause approximately 50% of cases of Noonan syndrome (NS),[7,8] a congenital disorder characterized by facial anomalies, short stature, and heart defects.[9] Whereas NS is frequently inherited as an autosomal dominant condition, almost half of the constitutional *PTPN11* mutations found in NS arise sporadically. Germ-line *PTPN11* mutations are also found in patients with multiple lentigene syndrome (LS), a rare developmental disorder clinically related to NS.[9] Infants with NS are predisposed to developing a myeloproliferative disorder (NS/MPD), which may regress without treatment or follow an aggressive clinical course similar to JMML.[10-14] By contrast, cases of JMML that arise in patients without NS have a poor prognosis without hematopoietic stem cell transplantation.[15-18] Recent studies show that children with JMML have improved outcomes when they are treated aggressively early in the course of disease.[18] Therefore, differentiating JMML from NS/MPD and identifying patients with NS/MPD who will require aggressive treatment are important clinical questions. We identify *PTPN11* mutations in 77 newly reported patients with JMML and NS/MPD, and compare the mutational spectrum in JMML, NS/MPD, and NS/LS to determine if genotype-phenotype correlations exist that may help guide diagnosis and clinical management.

Study design

Tissue samples (bone marrow, peripheral blood, and, rarely, buccal swab and skin fibroblasts) from patients with JMML and NS/MPD were collected under Institutional Review Board–approved protocols at each institution and with informed consent. DNA was extracted and analyzed for mutations in Freiburg, Germany; New York, New York; Rome, Italy; Nagoya, Japan; and San Francisco, CA. Since the data accumulated thus far have

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Table 1. *PTPN11* mutations in 77 newly reported children with JMML or NS/MPD

Cohort, no. cases	Nucleotide substitution	Amino acid substitution
JMML, N = 69		
1	155C > G*	Thr52Ser
1	178G > C	Gly60Arg
9	179G > T	Gly60Val
6	181G > T	Asp61Tyr
6	182A > T	Asp61Val
1	182A > G	Asp61Gly
5	214G > A	Ala72Thr
4	215C > T	Ala72Val
20	226G > A	Glu76Lys
6	227A > G	Glu76Gly
1	226-227GA > AT*	Glu76Met
5	1508G > C	Gly503Ala
4	1508G > T	Gly503Val
NS/MPD, N = 8		
2	182A > G	Asp61Gly
1	215C > G	Ala72Gly
2	218C > T	Thr73Ile
1	1492C > T	Arg498Trp
1	1504T > G*	Ser502Ala
1	1517A > C	Gly506Pro

*Novel mutation.

demonstrated that *PTPN11* mutations in myeloproliferative disorders occur in exons 3 (~90%) or 13 (~10%),[3,4] these were the only exons screened in many of the European and American cases as previously described.[3,4,8] Mutations in the JMML cases from Nagoya were detected by analyzing exons 3, 8, and 13 employing standard cloning and sequencing techniques.

Results and discussion

Results of the *PTPN11* mutational screening performed on the 77 newly reported patients (JMML = 69, NS/MPD = 8) are listed in Table 1. Two mutations, 155C > T (Thr52Ser) and 226-227GA > AT (Glu76Met), had not been previously documented in JMML or other malignancies.[3-6,19,20] Figure 1 shows an updated compendium of *PTPN11* mutations previously documented in JMML or NS/MPD combined with the current cohort of 77 newly reported patients. The series includes 107 JMML cases, 19 patients with NS/MPD, and 243 patients with NS or LS. While other exons are commonly mutated in the germ line of patients with NS and LS, only cases with exon 3 and 13 mutations are listed in Figure 1.

All defects with the exception of 2 result in an amino acid substitution. While there is overlap with respect to the substitutions seen in patients with NS/MPD and NS (Figure 1; Asp61Gly, Tyr62Asp, Ala72Gly, Thr73Ile, Ser502Thr, and Gly503Arg) or LS (Arg498Trp, Gln506Pro), only the Asp61Gly mutation is shared among patients with JMML, NS/MPD, and NS. Remarkably, 45 (42%) of the mutations found in JMML cases alter codon 76. Mutations affecting Glu76 are rare in individuals with NS (< 3% of cases), but are conservative (Glu76Asp) when they occur.

We identified a 214G > A (Ala72Thr) mutation in the bone marrow specimen of a 5-month-old girl with JMML for whom umbilical cord was collected at birth. Remarkably, the cord blood contained the same *PTPN11* mutation, which was absent in the child's buccal cells and parental DNA. Despite the assumption that JMML arises in utero, this is, to our knowledge, one of the first demonstrations that a somatically acquired JMML-associated *PTPN11* mutation occurred before birth.

In this newly reported cohort of 77 patients (Table 1), 6 missense changes, including 1 novel mutation affecting exon 13, 1504T > G (Ser502Ala), were identified in 8 NS/MPD specimens. We had buccal swab DNA (n = 2) and skin fibroblast DNA (n = 1) available for analysis from 3 patients and confirmed the presence of the *PTPN11* mutation in each case, supporting the germ-line origin of the lesion. Together with previous data, the Thr73Ile substitution was identified in 8 out of 19 NS/MPD cases (Figure 1), representing the most common mutation in patients with NS/MPD. This mutation is uncommon in NS patients without MPD, and has not been observed among patients with JMML.

Analysis of DNA from the unaffected parents of 3 children with NS/MPD with different *PTPN11* mutations (Asp61Gly, Ser502Ala, and Gly506Pro) demonstrated the sporadic origin of each lesion. Consistent with these results, all previous children with NS/MPD carrying a mutated *PTPN11* gene have been documented to be sporadic cases.[3,14] These findings suggest that NS/MPD is associated with mutations that might be associated with decreased fertility or have more severe consequences on fetal survival. This is consistent with the observation that mutations associated with NS/MPD have only rarely been identified in patients with familial NS cases, and that mutants detected in families transmitting NS are unlikely to be associated with NS/MPD.[7,21-25]

We identified 1 patient with JMML carrying a somatic 182A > G (Asp61Gly) mutation previously associated with NS.[7] The mutation was found in hematopoietic cells but was absent in skin fibroblasts. The same mutation was found in 2 patients with NS/MPD. In one of the latter patients the germ-line nature of this lesion was confirmed by analyzing buccal cells. To our knowledge, the 182A > G (Asp61Gly) mutation is the only *PTPN11* mutation associated with JMML, NS/MPD, and NS.

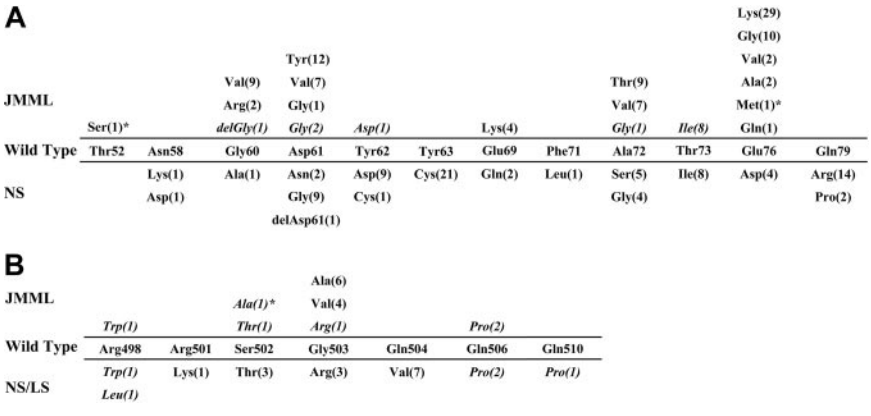

Figure 1. PTPN11 mutations in JMML, NS/MPD, and NS/LS. The middle sections of both panels show wild-type SHP2 amino acid residue at each position. (A) Residues located within the N-SH2 domain encoded by exon 3. (B) Residues located within the portion of the catalytic domain encoded by exon 13. Amino acid substitutions documented in JMML and NS/MPD (italics), and in NS and LS (italics) are shown above and below the wild-type SHP2 sequence, respectively. Del indicates a deletion of this amino acid. Digits in parentheses indicate the numbers of individuals with JMML, NS/MPD, or NS carrying a specific mutation. Novel mutations are identified by asterisks. Whereas virtually all mutations in JMML and NS/MPD are located within these confined regions, mutations associated with NS alone alter other residues of SHP2 in approximately 50% of the cases.[9] Our data, updated to January 2005, includes 107 cases with JMML, 19 with NS/MPD, 181 with NS, and 42 with LS.

The finding of Asp61Gly in JMML and NS/MPD is intriguing as this substitution was used to generate a mouse knock-in model of NS.[26] When homozygous, the Asp61Gly mutant is embryonic lethal, whereas heterozygotes have decreased viability. Of note, while the JMML-like picture in infants with NS usually develops at or shortly after birth, *Ptpn11*$^{Asp61Gly/+}$ mice show signs of a mild myeloproliferative disorder and splenomegaly by 5 months of age.

Similar to what is observed in NS, the vast majority of mutations identified in JMML and NS/MPD alter residues located at the interface between the N-terminal Src homology 2 (N-SH2) and catalytic (protein tyrosine phosphatase [PTP]) domains.[27] They are predicted to promote SHP-2 gain of function by impairing the switch between the active and inactive conformation of the protein, favoring a shift in the equilibrium toward the former. One exception is the Thr52Ser substitution, which affects the N-SH2 phosphotyrosyl-binding site.[27] It has been hypothesized that the genotype-phenotype relationships observed in patients with somatic and germ line mutations of *PTPN11* are due to distinct gain-of-function effects on the protein product SHP-2.[3] Consistent with this view, somatically acquired JMML-associated *PTPN11* mutations are predicted to have a strong gain-of-function effect that might otherwise affect embryonic/fetal development if transmitted in the germ line, explaining why these mutations are not seen in NS. In contrast, germ-line mutations identified in NS are predicted to have weaker hematologic effects. It is also possible that the rare germ-line *PTPN11* lesions observed in NS/MPD can exhibit intermediate effects. Indeed, in vitro and in vivo experiments on primary hematopoietic cells and cell lines show that somatic mutants confer more pronounced effects on cell growth than common mutants only found in NS,[28-30] and exhibit an increased phosphatase activity basally.[3]

Our data raise a number of new questions. First, are somatic *PTPN11* mutations sufficient to initiate MPD and, if not, what are the cooperating molecular lesions? Second, do specific *PTPN11* mutations have different consequences depending on whether they involve the entire hematopoietic compartment or arise as clonal events? Third, do some patients with NS and *PTPN11* mutations develop transient myeloproliferation, which is unrecognized? Finally, does the nature of a *PTPN11* mutation (germ-line versus somatic) and the specific amino acid substitution detected provide information that can be used to guide decisions regarding the need to initiate aggressive treatment in infants presenting with JMML? These questions will be answered through ongoing collaborations between laboratory researchers and clinical investigators who are evaluating and treating children with these disorders.

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