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Genotypic and Phenotypic Characterization of Noonan Syndrome: New Data and Review of the Literature

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Noonan syndrome (NS) is an autosomal dominant disorder, characterized by short stature, minor facial anomalies, and congenital heart defects. In approximately 50% of cases the condition is caused by missense mutations in the *PTPN11* gene on chromosome 12, resulting in a gain of function of the protein SHP-2. In this study, PTPN11 mutation analysis was performed in 170 NS patients. In 76 (45%) of them a mutation was identified. We report on the distribution of these mutations, as well as on genotype–phenotype relationships. The benefit of the NS scoring system developed by van der Burgt et al. [(1994); Am J Med Genet 53:187–191] is shown, among physicians who consequently based their diagnosis on the NS scoring system the percentage mutation positive subjects was 54%, whereas this percentage was only 39% among physicians who made less use of the scoring system. In two patients with some uncommon manifestations mutations were found in the C-SH2 domain, a region in which defects are not often identified in NS. A trend was observed in patients carrying the 922A → G change (Asn308Asp) receiving normal education. In one patient with NS and mild juvenile myelomonocytic leukemia (JMML) the mutation 218C → T (Thr73Ile) was found. This confirms previous findings indicating that individuals with NS with specific mutations in PTPN11 are at risk of developing JMML.

KEY WORDS: Noonan syndrome; PTPN11; juvenile myelomonocytic leukemia (JMML); C-SH2 domain

INTRODUCTION

Noonan syndrome (NS, OMIM 163950) is an autosomal dominant condition with a variable phenotype comprising short stature, congenital heart defects, and minor facial anomalies [Noonan, 1968]. The main facial findings of NS are hypertelorism with down-slanting palpebral fissures, ptosis, and low-set posteriorly angulated ears with a thickened helix. The cardiovascular defects most commonly associated with this condition are pulmonary stenosis and hypertrophic cardiomyopathy. Other manifestations are webbed neck, chest deformity, mild mental retardation, cryptorchidism, feeding difficulties, bleeding diathesis, and lymphatic dysplasias. The incidence of NS is estimated to be between 1:1,000 and 1:2,500 live births [Nora et al., 1974].

Tartaglia et al. [2001] have shown that missense mutations in the *PTPN11* gene on chromosome 12 (12q24) account for approximately 50% of NS cases. PTPN11 encodes the non-receptor protein tyrosine phosphatase (PTP) SHP-2. This enzyme is involved in a wide variety of intracellular signal cascades down-stream to receptors for growth factors, cytokines, and hormones and is required in several developmental processes [Neel et al., 2003; Tartaglia et al., 2004]. SHP-2 is composed of two src homology 2 domains, N-SH2 and C-SH2, at the amino terminus, a single central phosphatase domain (PTP) and a carboxy-terminal tail (Fig. 1a).

The N-SH2 and PTP interdomain interaction controls the switching of the SHP-2 protein between its active and inactive state [Hof et al., 1998]. The majority of mutations associated with NS affect such interaction destabilizing the catalytically inactive conformation of the protein. This results in a gain of function of SHP-2 [Tartaglia et al., 2001; Fragale et al., 2004].

Since PTPN11 was identified as a major NS gene, several reports described PTPN11 mutations in relation to clinical manifestations in NS-patients [Tartaglia et al., 2002; Musante et al., 2003; Sznajer et al., 2003; Zenker et al., 2004]. In every survey pulmonic stenosis was more prevalent in NS patients with a mutation in PTPN11, whereas hypertrophic cardiomyopathy, was more frequently diagnosed in mutation-negative cases. All authors documented a clustering of mutations within the N-SH2 domain (exon 3) and PTP-domain (exons 7, 8, and 13), with the 922A → G substitution (Asn308Asp) being the most common mutation (Fig. 1b).

Some mutations have been linked to clinical characteristics. Tartaglia et al. [2003] identified the 218C → T (Thr73Ile) mutation in four of five NS patients with juvenile myelomonocytic leukemia (JMML), a malignant hematopoietic disorder of early childhood. The mutations 836A → G (Tyr279Cys) in exon 7 and 1403C → T (Thr468Met) in exon 12 appear to be confined to individuals clinically diagnosed with LEOPARD syndrome (LS, multiple lentigines, ECG-abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal gentalia, retardation of growth, and sensorineural deafness, OMIM 151100) [Digilio et al., 2002; Legius et al., 2002]. Finally, Tartaglia et al. [2002]

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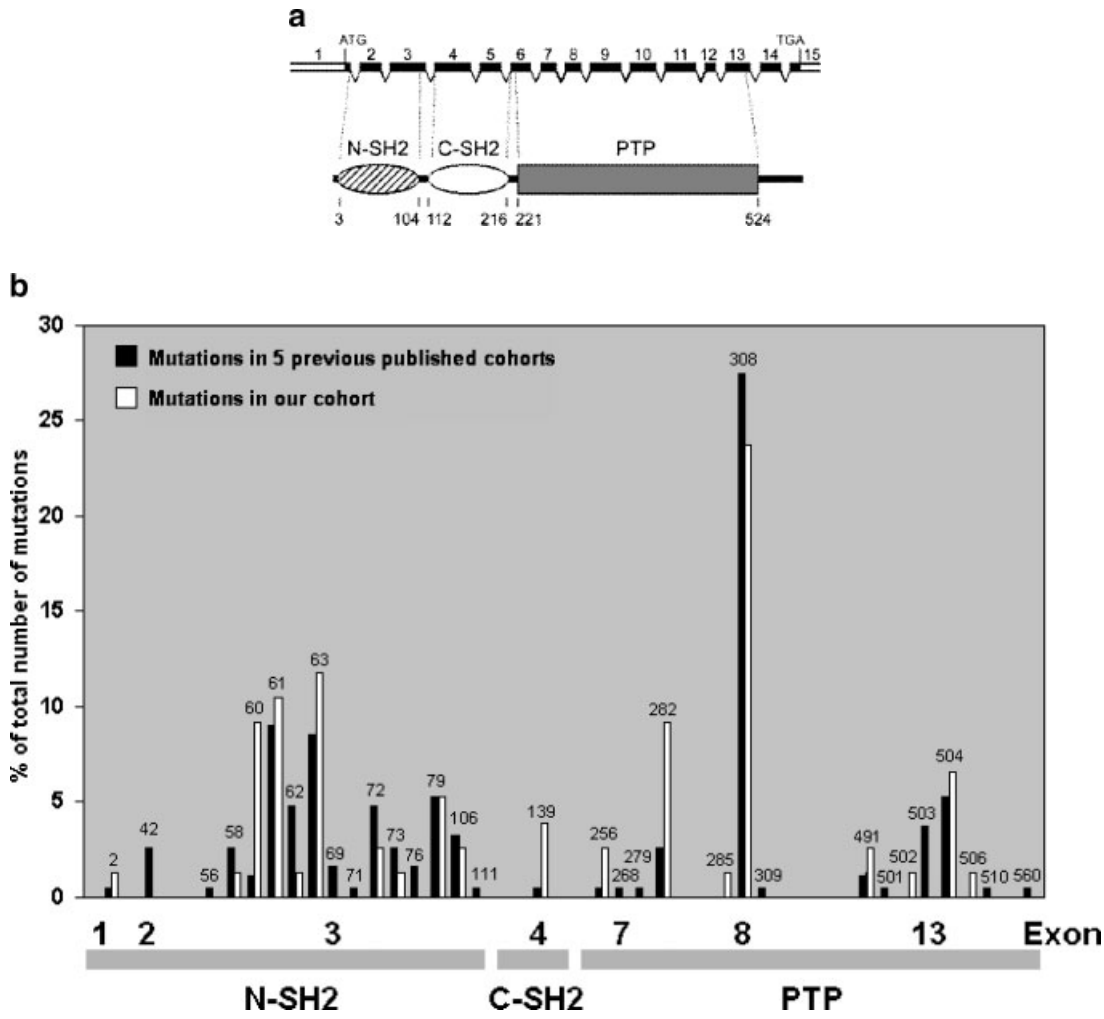

Fig. 1. **a**: The *PTPN11* gene and the protein it codes for: SHP-2. SHP-2 consists of two Src homology domains 2 (N-SH2 + C-SH2) and a protein tyrosine phosphatase (PTP) domain. **b**: Distribution of PTPN11 mutations in five previous published reports [Musante et al., 2003, n = 23; Sarkozy et al., 2003, n = 23; Sznajer et al., 2003, n = 55; Tartaglia et al., 2002, n = 54, Zenker et al., 2004, n = 32] and in the current cohort of 76 Noonan syndrome (NS) patients. The numbers above the columns indicate the location of the amino acid substitutions. The schematic domain structure of SHP-2 is also shown. N-SH2 and C-SH2 indicate respectively the two amino-terminal Src homology two domains, PTP indicates the PTP domain.

noted that in their cohort no subject carrying the common Asn308Asp mutation was enrolled in special education.

In this study the manifestations of 56 NS patients with a proven PTPN11 mutation are analyzed. We performed a genotype–phenotype analysis, reviewed the literature on this subject, and evaluated the NS scoring system developed by van der Burgt et al. [1994]. Special attention was paid to two patients with some uncommon traits, both carrying a 417G → C mutation in the C-SH2 domain and to a patient presenting with mutation 218C → T (Thr73Ile), which is correlated with a high risk of developing JMML.

PATIENTS AND METHODS

Patients

In the laboratory for DNA-diagnostics of the University Medical Center Nijmegen (UMCN) the *PTPN11* gene was screened for mutations in 170 unrelated index patients, who were clinically considered as NS patients. Of these patients 63 (37%) were diagnosed by 3 physicians of the UMCN, who based their diagnosis on the NS scoring system developed by van der Burgt et al. [1994]. The other 107 patients (63%) were diagnosed by referring physicians. From 56 of 76 mutation-positive patients informed consent was obtained for further study. This group included 32 males (57%) and 24 females (43%). We sampled data on minor anomalies, height, heart defects, sternal malformations, webbed neck, cryptorchidism, hematologic findings, feeding difficulties, psychomotor development, and family history.

Mutation Screening

Genomic DNA was isolated from blood by a simple salt-precipitation method [Miller et al., 1988]. Exons 1, 2, 3, 4, 7, 8, 12, 13, and 14 were amplified by PCR using the primers described by Tartaglia et al. [2002] that were adapted for use with M13 sequencing primers. All PCRs except for exon 1 were carried out using AmpliTaq Gold (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) starting with an initial denaturation step (93°C, 7 min) followed by 37 PCR cycles (93°C, 30 sec; 55°C, 1 min; 73°C, 1 min) and a final elongation step (72°C, 5 min). Exon 1 was amplified using the Invitrogen enhancer solution system (Invitrogen, Breda, The Netherlands) starting with 5′ denaturation at 95°C, 36 PCR cycles (95°C, 30 sec; 60°C, 30 sec; 68°C, 1 min) followed

TABLE I. Noonan Syndrome (NS) Scoring System [van der Burgt et al., 1994]

Finding	A = major	B = minor
1. Facial	Typical face	Suggestive face
2. Cardiac	Pulmonary valve stenosis and/or typical ECG	Other defect
3. Height	<p3	<p10
4. Chest wall	Pectus carinatum/excavatum	Broad thorax
5. Family history	First degree relative definite NS	First degree relative suggestive NS
6. Other	All three (males): mental retardation, cryptorchidism, lymphatic dysplasia	One of mental retardation, cryptorchidism, lymphatic dysplasia

Definite NS, 1A plus one of 2A–6A or two of 2B–6B; 1B plus two of 2A–6A or three of 2B–6B.

by a final elongation step (68°C, 7 min). All PCR products were bidirectionally sequenced using M13 primers, except for exon 1 that could only be sequenced with the forward primer. Sequencing was performed using Big Dye Terminator kit version 1.1 followed by analysis on an Applied Biosystems 3730 capillairy sequencer (both from Applied Biosystems). Results were analysed using the PHRED basecaller and mutations were identified using PHRAP/PolyPHRED software and the Consed viewer (all from University of Washington, Seattle).

RESULTS

Frequency and Distribution of Mutations

In 76 (45%) of 170 clinically suspected NS patients sent for PTPN11 analysis a mutation was identified. In total we detected 24 different mutations, all heterozygous missense changes.

Of all mutations 48.7% were located in the N-SH2 domain, a same percentage affected the PTP domain, while 2.6% of lesions were within the C-SH2 domain (Fig. 1b). The mutation most often detected in our survey was the 922A → G change (Asn308Asp) in exon 8 (21%).

In patients diagnosed by the physicians involved in this study, who based their diagnosis on the NS scoring system (Table I), the percentage of mutation-positive subjects was 54% (34 out of 63). In the patient group diagnosed by referring physicians this percentage was 39% (42 out of 107).

Of the 56 mutation-positive NS patients included in our clinical study, 39 subjects were unrelated sporadic patients and 17 subjects were index patients of independent families. In all 17 families the mutation co-segregates with the syndrome. Among the families segregating the disorder, transmission of the mutation was maternal in 13 families and paternal in 4 families.

Genotype–Phenotype Relationships

In Table II the distribution of several characteristics of NS is summarized, grouped by the exon in which a mutation was detected. The most frequent traits in our study group are a typical face (86%) and cryptorchidism in males (84%). Valvular pulmonary stenosis was the most common congenital heart defect, present in 68% of patients. Hypertrophic obstructive cardiomyopathy was diagnosed in 7% and 25% of subjects did not have any heart defect at all.

TABLE II. Frequency of Characteristic NS Findings Grouped by the Exon in Which a Mutation Was Detected

	Exon 3 (%)	Exon 4	Exon 7 (%)	Exon 8 (%)	Exon 13 (%)	Total (%)
No. of subjects	26	2	5	15	8	56
Sex: male	15 (58)	0	3 (60)	7 (47)	7 (88)	32 (57)
Familial	10 (38)	0	1 (20)	2 (13)	4 (50)	17 (30)
Short stature						
<p3	20 (77)	2	4 (80)	13 (87)	2 (25)	41 (73)
<p10	4 (15)		1 (20)	2 (13)	3 (38)	10 (18)
>p10	2 (8)			0	3 (38)	5 (9)
Heart defect						
VPS	19 (73)	1	2 (40)	10 (67)	6 (75)	38 (68)
HOCM	2 (8)			1 (7)	1 (13)	4 (7)
ASD	9 (35)		1 (20)	4 (27)	3 (38)	17 (30)
Other	4 (15)			2 (13)	1 (13)	7 (13)
No heart defect	5 (19)	1	2 (40)	4 (27)	1 (13)	14 (25)
Face						
Typical	24 (92)	1	4 (80)	13 (87)	6 (75)	48 (86)
Suggestive	2 (8)	1	1 (20)	1 (7)	2 (25)	7 (13)
Normal				1 (7)		1 (2)
Cryptorchidism	14 (93)		2 (40)	6 (86)	5 (71)	27 (84)
Pectus deformity	14 (54)	1	1 (20)	6 (40)	2 (25)	24 (43)
Webbed neck	5 (19)	1	0	2 (13)	2 (25)	10 (18)
Poor feeding in infancy	16 (62)	1	2 (40)	9 (60)	7 (88)	35 (63)
Bleeding abnormalities						
Easy bruising	16 (62)	2	0	10 (67)	4 (50)	32 (57)
Abnormal blood test	4 (15)	1	0	3 (20)	0	8 (14)
Delayed developmental milestones and/or special education	15 (58)	2	2 (40)	5 (33)	4 (50)	28 (50)

VPS, valvular pulmonary stenosis; HOCM, hypertrophic obstructive cardiomyopathy; ASD, atrial septal defect.

C-SH2 Domain

The 417G → C mutation resulting in a Glu139Asp substitution affecting the C-SH2 domain, was identified in three sporadic patients. One of them was not included in the study, because of lack of complete clinical characterization. A short description of the other two patients has been presented before [Jongmans et al., 2004].

Patient 1 (Fig. 2) was a 27-year-old woman. She had severe feeding problems in infancy and was treated for pulmonic stenosis. Intellectual development was delayed and she attended special school. As a child she had a triangular face, hooded eyelids, and low-set ears. She is of short stature and she has 2 café-au-lait spots and multiple naevi. At 18 she developed obstructive hydrocephalus, which was caused by a low-grade hypothalamic glioma.

Patient 2 was a 9-year-old girl who was born with a urachal cyst. She had several convulsions shortly after birth for which no cause was found. Her length is below the third centile. She has hypertelorism, down-slanting palpebral fissures, low-set ears, webbed neck, wide nipples; she bruises easily. In the first years of life she developed normally but now she attends a special school. Cardiac evaluation did not show any abnormalities. There are no skin changes except for a small hemangioma.

JMML

We studied one patient with the mutation 218C → T (Thr73Ile) which is correlated with a high risk of developing JMML [Tartaglia et al., 2003]. As presented before the patient had classical NS, and presented after delivery with thrombocytopenia and hepatosplenomegaly. He was diagnosed with JMML, based on morphologic studies of peripheral blood and bone marrow smears. Because of massive splenomegaly a splenectomy was performed at 6 months. After splenectomy his clinical condition improved continuously without cytotoxic therapy. He is now 5 years old and the white blood cell count has normalized [Jongmans et al., 2004].

DISCUSSION

In this study, we analyzed the manifestations of mutation positive NS-patients and evaluated the frequency and distribution of the mutations found in these individuals. The mutation detection rate is 45%, which corresponds with that in most other publications [Tartaglia et al., 2002; Sznajer et al., 2003]. In one report mutations were detected in 60% of patients [Zenker et al., 2004]. The authors state that this relatively high percentage was the result of stringent inclusion criteria. On the other hand they mention a significant bias in their study: most of their propositi (58%) had been recruited from pediatric cardiology departments. This leads to an overrepresentation of patients with heart defects, a strong characteristic of NS.

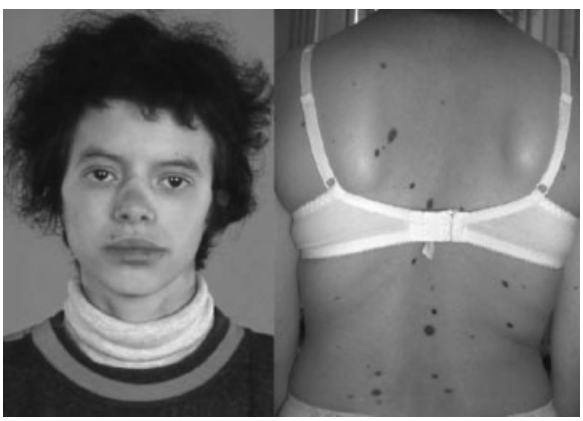

Fig. 2. Patient 1, note the multiple naevi on the back.

The finding that a relatively high percentage of patients undoubtedly fitting the NS condition did not carry a mutation in PTPN11 confirms previous studies indicating that NS is a genetically heterogeneous syndrome. This was already proven by linkage analysis of a NS syndrome-family that did not show linkage to the 12q24 critical region [Jamieson et al., 1994]. Furthermore there is evidence for an autosomal recessive form of NS [van der Burgt and Brunner, 2000].

The distribution of the mutations identified in our study corresponds with that documented by other studies (Fig. 1b). Almost all mutations (97%) affected residues located within or close to the interacting surfaces of the PTP and N-SH2 domains. This is in agreement with the hypothesis that NS is caused by activating mutations that destabilize the intramolecular interaction involving the N-SH2 and PTP domains.

Evaluating the NS Scoring System

van der Burgt et al. [1994] developed a diagnostic method in which six characteristic NS items are scored (Table I). This scoring system was consequently used by the three physicians involved in this study, who accounted for 37% of the patients in our study group. We do not know whether the referring physicians, responsible for the other 63% of patients, used this scoring system, but suppose that they used it on a less frequent basis.

In these two groups there is a clear difference in percentage of mutation positive patients. In the group diagnosed by the physicians using the NS scoring system, this percentage is 54%, in the referred group 40%. This underlines the benefit of the scoring system. Furthermore, all 56 mutation-positive patients matched the criteria of the NS scoring system.

Despite these results we cannot exclude the possibility of missing mutations in rare atypical cases that test positive for PTPN11 mutations when the scoring system is strictly used. However, seen the above described difference in mutation positive percentages in our study population it seems at least to be cost-effective to use the scoring system.

C-SH2 Domain

Both patients with the 417G → C change (Glu139Asp, C-SH2 domain) presented with classical NS manifestations, but exhibited some remarkable findings as well. One patient had been diagnosed with a hypothalamic glioma. As far as we know this is the first report documenting the association of a PTPN11 mutation with a solid tumor in patients with NS. The multiple naevi in this patient are remarkable as well. Although pigmentation abnormalities can be present in NS (café-au-lait spots 10%, pigmented naevi 25% [van der Burgt, 2000]), this patient had an extreme number of naevi.

The clinical findings of only one other sporadic patient with a mutation in residue 417 were described by Musante et al. [2003] in a patient with short stature and pulmonary stenosis, hepatosplenomegaly, and leukocytosis of unknown origin since the neonatal period.

Finally a 417G → T change (Glu139Asp) was identified in an English father and his two children [Tartaglia et al., 2002]. They show typical Noonan facies and are of normal intelligence. The father had a normal echocardiography and ECG findings, the son had slightly left ventricular thickening but no pulmonary stenosis and the daughter had a murmur as an infant but later a normal echocardiogram. It is of interest that both children had profound bilateral sensorineural deafness, but as it was not present in their father it may suggest an alternative genetic cause of deafness. No defect in the *GJB2*/

CX26 gene was identified. On the subject of hearing loss in NS very few reports have been published. Sharland et al. [1992] concluded that nerve deafness is unusual (3%) in NS. In their study (n = 151) hearing loss was reported in 40%, but in most of these individuals this was due to serous otitis media.

The spectrum of clinical manifestations observed in the above mentioned patients is a good example of the phenotypic variability seen in NS. The clinical spectrum of many more patients with a mutation in the C-SH2 domain must be studied to determine if there is difference in clinical presentation compared to NS patients with a mutation in the PTP or N-SH2 domain.

The effect of mutations in C-SH2 on the function of SHP-2 is not known, although it is likely that they will be gain-of-function mutations as well. It was shown that biphosphopeptides that bind both the C-SH2 and N-SH2 domain stimulate the activity of SHP-2 much more potently and at much smaller phosphopeptide concentrations, than occupancy of only one of both domains by a single phosphorylated peptide [Pluskey et al., 1995]. Thus, the C-SH2 domain does not have a direct role in activation of SHP-2, but increases the concentration of ligand in the proximity of N-SH2 by binding one site of a biphosphorylated ligand [Hof et al., 1998].

LS

In one 2-year-old patient with typical face, CHD (pulmonary stenosis, atrial septal defect (ASD), and hypertrophic cardiomyopathy), webbed neck, cryptorchidism, and delayed psychomotor development we detected a 1517A → C substitution (Gln506Pro, PTP domain). By binding Ala72 the glutamine residue at position 506 is directly involved in the hydrogen bonding network that stabilizes the interface between the N-SH2 domain and the PTP active sites [Hof et al., 1998]. Mutations in Ala72 have been reported in NS by other groups. This is only the third time a mutation is detected in its binding partner in the PTP domain. Loh et al. [2004] documented this amino acid change in a NS patient diagnosed with JMML. Interestingly, Conti et al. [2003] identified this mutation in a single LS patient.

Since lentigines develop later in life, especially in young patients, such as ours, the definitive diagnosis LS is difficult to make. Although it looks like the GLN506Pro mutation can give rise both to NS and LS, it is too early for final conclusions as the number of patients with this mutation is small, and the patients were young.

Consistently Tartaglia et al. [2002] included a young patient with the mutation 836A → G (Tyr279Cys) in their NS cohort. This mutation has frequently been identified in LS patients and as far as we know in no other NS patient. At the time of inclusion this patient had the facial appearance of NS with hypertrophic cardiomyopathy, short stature, and cryptorchidism. He developed severe heart failure and received a cardiac transplant after which he was lost to genetic follow up. He has recently been reassessed and was found to have developed multiple lentigenes. He has moderate hearing loss but this is attributed to chronic otitis media and has also been noted to have bleeding problems, e.g., an operation of tonsillectomy had to be abandoned because of hemorrhage. In retrospect the later development of lentigenes together with cardiomyopathy rather than pulmonary stenosis would probably favor a diagnosis of LS.

JMML

NS has been associated with myeloid disorders [Johannes et al., 1995; Bader-Meunier et al., 1997; Choong et al., 1999]. Tartaglia et al. [2003] identified the 218C → T (Thr73Ile) mutation in four out of five NS patients with JMML. They suggested that a subgroup of individuals with NS, with specific mutations in PTPN11, are at risk of developing JMML. Loh et al. [2004] reported two more patients diagnosed with NS and JMML. In one again the 218C → T (Thr73Ile) mutation was found. In our patients the mutation 218C → T was detected in one subject diagnosed with JMML as well. This supports the hypothesis that the 218C → T mutation is associated with a high risk of developing JMML.

The patient in our cohort has now reached the age of 5 years and his white blood cell counts are completely normal. This corresponds with the clinical course in three out of four NS/JMML patients reported by Bader-Meunier et al. [1997]. In all three cases the hematologic disease improved spontaneously, which is not in agreement with the prognosis of JMML in non-NS patients.

The pathogenesis of JMML is still poorly understood. Deregulation of the RAS signal transduction pathway is thought to play a key role, because in approximately 80% of JMML patients a mutation deregulating RAS function, due to NRAS or KRAS2 oncogenic lesions, loss-of-function mutations in NF1, or gain-of-function defects in PTPN11 can be identified [Tartaglia et al., 2004]. The relatively high prevalence of myeloproliferative features in young children with NS can now be explained with the important role of SHP-2 in the control of myeloid precursor cell proliferation and development, as well as with its modulatory function on RAS signaling.

Thr73 is directly involved in the bond between PTP and N-SH2, like most residues mutated in NS-patients. It is not clear why specifically the Thr73Ile-change causes a predisposition to JMML. Furthermore, it is not clear why the prognosis in NS patients with JMML is better than in non-NS patients with JMML. Tartaglia et al. [2003] hypothesized a model in which distinct gain-of-function thresholds for SHP-2 activity are required to induce cell-, tissue-, or developmental-specific phenotypes, each depending on the transduction network context involved in the phenotype. In such a model, SHP-2 mutants associated with NS would have milder gain-of-function effects, which are sufficient to perturb heart, craniofacial and skeletal development but inadequate to deregulate myeloid proliferation. The PTPN11 mutations observed in isolated JMML would be expected to produce proteins with the highest gains in function. Consistently, these molecular lesions have been observed prevalently as somatic defects, suggesting that they are associated with embryonic lethality. Further research on these subjects is necessary.

922A → G and Normal Education

The 922A → G (Asn308Asp) mutation in exon 8, by Tartaglia et al. [2002] associated with normal intelligence, was present in 13 patients of our clinical study group (23%). This confirms the reputation of this nucleotide as a "mutation hot spot" in NS. Among these 13 patients only 3 attended special school. Except for this suspected correlation with normal education, the phenotype observed in subjects with a mutation in this position was not different from the phenotype in subjects with other mutations.

CONCLUSIONS

The NS scoring system has proven its benefit for clinical use. We recommend its use in cases suggestive of NS.

We confirm the previously observed correlation in patients with the mutation 922A → G (Asn308Asp) receiving normal education.

Mutations in the C-SH2 domain are rare and seem to cause classical NS, with other remarkable findings as well. Further research on this subject is necessary.

Mutations in PTPN11 are correlated with a predisposition to JMML, the mutation 218C → T (Thr73Ile) seems to play a role in this. The correlation between NS and JMML can be explained by the involvement of SHP-2 in RAS-signaling.

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