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Clinical and Hematologic Findings in Noonan Syndrome Patients With *PTPN11* Gene Mutations

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Reports on Noonan syndrome (NS) have documented multiple types of coagulation defects and bleeding diathesis, and a wide range of clinical presentations. Early studies suggested that a large proportion of NS patients have coagulation defects, whereas more recent reports indicate low rates of coagulopathy. The aim of this study was to evaluate phenotypic characteristics, *PTPN11* gene mutations, and hematological and coagulation parameters in 30 clinically diagnosed cases of NS. One of the NS patients had a history of easy bruising; however, his hematological and coagulation tests were normal. None of the other patients had clinical coagulation problems. In the NS group, values for platelet count, activity of factors XI, XII, and protein C were significantly lower than the corresponding means for the control group. However, the results of coagulation tests in the NS group were diagnostically inconclusive and only one patient had clinical signs of coagulopathy. Interestingly, two NS patients had low protein C activity. One of these children had an A1517C mutation and transient myelodysplasia. The other patient had a C1528G mutation in exon 13 that has not been reported previously. Neither of these individuals experienced a thrombotic event or any complication during approximately 3 years of follow-up. For all patients clinically diagnosed with NS, a thorough history of coagulation issues should be taken and first-line coagulation testing should be done to evaluate for bleeding diathesis. However, if these assessments reveal nothing abnormal, complications related to coagulation are unlikely and extensive testing is unnecessary.

Key words: Noonan syndrome; bleeding diathesis; myelodysplasia; protein C; coagulation; factor VIII; factor XI; factor XII; *PTPN11* gene

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INTRODUCTION

Noonan syndrome (NS) (OMIM 163950) is a relatively common genetic disorder that was first described by Noonan and Ehmke [Noonan and Ehmke, 1963; Noonan, 1968]. Most cases are sporadic, but familial cases occur and are transmitted by autosomal-dominant inheritance. The reported prevalence of NS ranges from 1 in 1,000 to 1 in 2,500 [Mendez and Opitz, 1985; Allanson, 2007]. Cases are initially diagnosed by clinical examination and detection of key features and can be confirmed by molecular analysis of related genes. The genes associated with NS (listed according to identification frequency) are as follows: *PTPN11* (approximately 50% of cases), *SOS1* (10–15%), *RAF1* (3–10%), *KRAS* (fewer than 3%), *MEK1* (approximately 3% of cases), and *BRAF* (fewer than 2%) [Ferrero et al., 2008; Sarkozy et al., 2009; Tartaglia et al., 2010].

The main clinical characteristics of NS are short stature, dysmorphic facial features, webbed neck, congenital heart defects (frequently pulmonary valve stenosis [PS] and/or hypertrophic cardiomyopathy [HCM]), hypoplastic external genitalia and

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cryptorchidism in males, sternal deformity, variable degrees of developmental delay, and lymphatic hypoplasia/dysplasia. Phenotypic facial features include hypertelorism with down-slanting palpebral fissures, ptosis, and low-set posteriorly angulated ears with a thickened helix. Mild mental retardation and hearing difficulty are also relatively common [Allanson, 1987, 2007].

The literature on NS notes thrombocytopenia, von Willebrand disease, prolonged bleeding time, and coagulation factor deficiencies (mainly FVIII, FXI, and FXII) as coagulation defects in these patients [de Haan et al., 1988; Witt et al., 1988; Sharland et al., 1992; Singer et al., 1997]. In some cases, combinations of these manifestations have been documented. However, no correlations have been detected between coagulation test results and clinical history of bleeding disorders in NS patients. Furthermore, only a few reports have noted low protein C (PC) activity in NS [Ganesan and Kirkham, 1997; Firth et al., 2005].

The aim of this study was to investigate phenotypic characteristics, *PTPN11* gene mutations, and hematological and coagulation parameters in 30 clinically diagnosed cases of NS, and to compare the hematological findings to those in a group of healthy children.

MATERIALS AND METHODS

We investigated 30 consecutive children (9 girls and 21 boys) who were clinically diagnosed with NS at our hospital between 2003 and 2009. Patient age ranged from 6 months to 17 years (mean ± standard deviation 6.1 ± 5.1 years). Hematological parameters were compared to those of a control group of 30 healthy children (18 girls and 12 boys) who ranged in age from 8 to 12 years (mean ± standard deviation 10.7 ± 0.8 years). Informed consent was obtained from parents of all subjects. The study protocol was approved by the Research and Ethics Committee of Başkent University Faculty of Medicine (Project No. KA 07/46) and was conducted in accordance with the ethical principles described in the Declaration of Helsinki.

Each NS patient was evaluated using a standard protocol that consisted of a detailed physical examination, measurements of growth parameters, and psychometric testing. As well, a clinical geneticist assessed for evidence of dysmorphic findings, including minor anomalies. Photographs of the children were taken to record their morphologic features. Family histories were also recorded. Clinical diagnosis and referral for molecular genetic analysis were done based strictly on the NS scoring system developed by van der Burgt et al. [1994] (Table I).

Blood was collected from patients and controls and was sent to the Laboratory for Molecular Diagnostics at the Center for Human Genetics, University of Leuven, Belgium. These samples were analyzed for mutations of the *PTPN11* gene, specifically exons 2, 3, 4, 7, 8, 12, and 13. These sites were screened at the genomic level using denaturing high-performance liquid chromatography.

In addition, standard blood testing was carried out for all patients and controls in the Hematology Laboratory at Baskent University Ankara Hospital. Venous samples were collected into three separate tubes from a single needle puncture. The assays for bleeding diathesis and their corresponding normal ranges were as follows: thrombocyte count (150,000–400,000/μl), prothrombin time (PT) (11–15 sec), international normalized ratio (INR) (1–1.2), activated partial thromboplastin time (aPTT) (24–40 sec), bleeding time platelet aggregation in response to adenosine diphosphate (BT I) (55–85%) and epinephrine (BT II) (60–90%), PC activity (70–140% in individuals older than 6 months), FVIII activity (85–125%), FXI activity (70–110%), and FXII activity (70–110%).

Complete blood count and thrombocyte count were determined using an automatic hemocytometer that was calibrated daily (Cell-Dyn 3700, Abbott, Santa Clara, CA). PT, aPTT, INR, and activity levels of FVIII, FXI, FXII, and PC were measured using an automatic coagulometer (Diagnostica Stago, Asnires, France). Bleeding times I and II were determined using an aggregometer that was calibrated daily (Apact 4004, Ahrensburg, Germany).

Echocardiographic exams (Acuson Sequoia C256, CA) were done in all 30 NS cases. Three patients exhibited no abnormal findings and the remaining 27 had their cardiac diagnoses confirmed with cardiac catheterization. Three of the 27 patients had HCM only. The other 24 underwent surgical intervention to correct heart defects.

The 30 NS patients also underwent genital physical examination and abdominal ultrasonography to assess for urogenital anomalies. All were also evaluated with psychometric tests according to age: Danver tests (adapted for Turkish children) for those younger than 2 years, the Stanford–Binet test for those 2–6 years, and the Wechsler Intelligence Scale for Children-Revised test for those 6–16 years.

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

Findings	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.

Statistical Analysis

The Shapiro–Wilk test was used to assess the normality of each variable's distribution, and Levene's test was used to assess homogeneity of variance in the different groups. For variables that were normally distributed and had homogeneous variance, the Student's *t*-test was used to compare data for pairs of groups and one-way analysis of variance (ANOVA) and the Tukey test were used to compare data for three groups. It was not possible to establish parametric test assumptions for some variables. In these cases, comparisons were made using the Mann–Whitney *U*-test (two groups) or the Kruskal–Wallis test (three groups). Multiple comparisons between pairs of groups were carried out using Dunn's test. Results were expressed as the number of observations (n) and the mean ± standard deviation and median (mean ± SD, M). Categorical data were analyzed using Fisher's exact test and the two-proportional *z* test. All data analyses were performed using the Statistical Package for the Social Sciences (SPSS v. 17.0; SPSS, Inc., Chicago, IL). A *P*-value <0.05 was considered statistically significant.

RESULTS

Genetic analysis revealed *PTPN11* mutations in 11 (36.7%) of the 30 NS cases. All but one of the NS cases (Patient 4) were sporadic. The father of Patient 4 exhibited typical phenotypic features of the syndrome but declined molecular genetics testing. All 11 mutations were heterozygous missense mutations and were located in exons 3, 4, 8, and 13. The major clinical features of the NS patients with *PTPN11* mutations are summarized in Table II.

Among the 11 children with *PTPN11* mutations, PS was the most common cardiac defect (5/11; 45%). Two of the girls (Patients 8 and 11) had sensorineural deafness, and Patient 11 had a new *PTPN11* mutation C1528G in exon 13.

When the NS patients were grouped according to the presence/absence of a *PTPN11* mutation, analysis revealed that mild mental retardation and short stature (<3p) were significantly more frequent in the group with mutations. However, there were no statistically significant differences between the mutation-positive and mutation-negative groups with respect to any of the hematology results or presence of moderate mental retardation, PS, chest deformity, genitourinary anomalies (including cryptorchidism), or

TABLE II. Major Clinical Features of the 11 Patients With Noonan Syndrome Who Had *PTPN11* Mutations

Patient no.	Age (years)/sex	*PTPN11* mutation (nucleotide substitution)/exon	CHD	Height	Thorax deformity	Genitourinary anomaly	Mental retardation	Other major clinical features
1	0.6/M	A1517C (p.Q506P)/3	PS, VSD	<3 p	—	Cryptorchidism	Mild	Myelodysplasia, hepato-splenomegaly, low PC activity
2	14/M	A1510G (p.M504V)/13	AS	<3 p	Pectus excavatum	Cryptorchidism	Mild	
3	2.5/M	A236G (p.Q79R)/3	PS, ASD	<3 p	Pectus excavatum	—	Mild	
4	1.5/M	C174G (p.N58K)/3	PS	<3 p	—	Cryptorchidism	Normal	Familial case, easy bruising but normal coagulation tests
5	1/F	A1510G (p.M504V)/13	PS	<3 p	—	—	Normal	
6	11/F	T854C (p.F285S)/8	PS, VSD	<3 p	—	—	Moderate	
7	6/M	A922G (p.N308D)/8	PS	3–10 p	—	Cryptorchidism	Moderate	
8	1/F	G417C (p.E139D)/4	PS	3–10 p	Pectus excavatum	—	Mild	Sensorineural hearing loss
9	11/M	G181A (p.D61N)/3	PS	<3 p	Pectus excavatum	—	Mild	
10	15/M	A922G (p.N308D)/8	PS, ASD	<3 p	Pectus excavatum	Cryptorchidism	Mild	
11[a]	5/F	C1528G (p.Q510E)/13	PS, HCM	<3 p	—	—	Moderate	Sensorineural hearing loss, low PC activity

CHD, congenital heart defect; PS, pulmonary valve stenosis; HCM, hypertrophic cardiomyopathy; ASD, atrial septal defect; VSD, ventricular septal defect; PC, protein C.

[a]Has a new mutation of *PTPN11* gene.

sensorineural hearing loss. Table III summarizes the statistical analysis of clinical features in NS patients with and without PTPN11 mutations.

Findings for Coagulation Parameters

None of the 30 NS patients exhibited thrombocytopenia; however, the NS group had a significantly lower mean platelet count and significantly lower mean activity levels of FXI, FXII, and PC than the control group. The NS group results for PT, aPTT, INR, and BT II were also statistically different from the corresponding control findings, but the absence of clinical problems and the nature/severity of test abnormalities revealed no definitive coagulation-related diagnoses (Table IV). Results for platelet count, PT, aPTT, INR, and PC in the mutation-positive and mutation-negative NS patients were also statistically different. Comparison of mean PC activity in the 19 mutation-negative NS patients and the 30 controls revealed no significant difference.

Two of the subjects with NS (Patients 1 and 11) had low PC activity (42% and 49%, respectively; normal range 70–140% for individuals older than 6 months). The results of this test were in normal limits in the parents of these patients. Only two of the NS children had clinical hematologic problems. One patient (Patient 1) had transient myelodysplasia, low PC activity, and hepatosplenomegaly, and the other (Patient 4) had a history of easy bruising, though coagulation tests were normal.

As noted, 24 of the 30 NS patients underwent corrective heart surgery. None of these individuals had a clinical bleeding problem in the operative or postoperative periods.

DISCUSSION

van der Burgt et al. [1994] developed a diagnostic method that involves scoring of six characteristic NS features (Table I). As mentioned previously, we selected our patients for *PTPN11* gene analysis according to these criteria. The frequency of *PTPN11* mutation detection in our study was 36.7%. In other investigations of larger numbers of patients who were diagnosed using the NS scoring system, this rate ranged from 40% to 60% [Tartaglia et al., 2002; Yoshida et al., 2004; Zenker et al., 2004; Jongmans et al., 2005]. The authors stated that this relatively high percentage was the result of stringent inclusion criteria. Documented prevalences of *PTPN11* mutations differ for familial and sporadic NS cases, with lower mutation frequency in the latter. For example, Musante et al. [2002] found mutation prevalences of 45% in familial cases (5 of 11 patients) and 26% in sporadic cases (18 of 68 patients). The corresponding findings in other work by Tartaglia et al. [2002] were 59% and 37%. In our study, all but 1 of the 30 NS cases was sporadic. Previous researchers have noted that it seems at least to be

TABLE III. Comparisons of Clinical Features With the Noonan Syndrome Patients Grouped According to Presence/Absence of *PTPN11* Mutation

	Mutation (+), n (%)	Mutation (−), n (%)	*P*-value
No. of patients	11 (37)	19 (63)	0.032
Age in years [mean ± SD, median]	6.4 ± 5.4 [6.0]	5.9 ± 4.9 [4.0]	0.948
Sex ratio (M:F)	7:4	14:5	0.687
Short stature			
<3p	9/11 (82)	7/19 (37)	<0.01
3–10p	2/11 (18)	3/19 (16)	0.867
Cardiac defects	11/11 (100)	16/19 (84)	0.059
PS	5/11 (45)	4/19 (21)	0.168
AS	1/11 (9)	3/19 (15)	0.578
PS plus VSD	2/11 (18)	1/19 (5)	0.309
PS plus ASD	2/11 (18)	2/19 (11)	0.573
PS plus HCM	1/11 (9)	2/19 (11)	0.898
HCM	0/11 (0)	3/19 (16)	0.059
CoA	0/11 (0)	1/19 (5)	0.304
Chest deformity	5/11 (45)	6/19 (32)	0.451
Mental retardation			
Mild	6/11 (55)	3/19 (16)	<0.05
Moderate	3/11 (27)	5/19 (26)	0.955
Hearing loss	2/11 (18)	0/19 (0)	0.118
Genital abnormality	5/11 (45)	5/19 (26)	0.290
Cryptorchidism (males)	5/7 (71)	5/14 (36)	0.094
Other	0/11 (0)	0/19 (0)	1.000
Hematological abnormalities	2/11 (18)	0/19 (0)	0.118
Bleeding diathesis	1/11 (9)	0/19 (0)	0.294
Myelodysplasia	1/11 (9)	0/19 (0)	0.294
JMML	0/11 (0)	0/19 (0)	1.000

PS, pulmonary valve stenosis; HCM, hypertrophic cardiomyopathy; ASD, atrial septal defect; VSD, ventricular septal defect; CoA, coarctation of the aorta; JMML, juvenile myelomonocytic leukemia.

TABLE IV. Comparison of Coagulation Parameters in the Noonan Syndrome Patients With and Without *PTPN11* Mutation, and in the Healthy Controls (Mean ± SD, M)

Parameter	*PTPN11* (+) (n: 11)	*PTPN11* (−) (n: 19)	Controls (n: 30)	*P*-value
PLT	251272.7 ± 78097.4, 245000	278842.1 ± 64989.9, 264000	333550 ± 103314.2,* 326500	<0.05
PT	14.45 ± 0.64,** 14.4	13.68 ± 0.91, 13.7	13.38 ± 0.46, 13.3	<0.01
INR	1.144 ± 0.059,** 1.14	1.084 ± 0.082, 1.07	1.050 ± 0.042, 1.04	<0.01
aPTT	32.75 ± 2.19,* 33.1	31.88 ± 3.95,* 31.9	30.88 ± 1.49,* 30.8	<0.05
BT I	73.26 ± 19.9, 76.7	76.84 ± 15.9, 78.9	76.20 ± 7.57, 75.5	0.467
BT II	70.25 ± 19.9, 78.3	71.01 ± 22.1, 75	64.83 ± 8.69,* 60	<0.05
FVIII	97.09 ± 31.2, 92	91.78 ± 27.0, 86	90.26 ± 12.6, 85.5	0.950
FXI	81.54 ± 18.3, 79	84.42 ± 17.7, 80	107.1 ± 37.1,* 98.5	<0.05
FXII	85.90 ± 18.8, 91	94.31 ± 16.4, 91	114.1 ± 19.7,*** 115.5	<0.001
PC activity	64.18 ± 13.3,* 64	75.73 ± 10.9, 75	75.56 ± 11.6, 73	<0.05

PLT, platelet count; PT, prothrombin time; INR, international normalized ratio; aPPT, activated partial thromboplastin time; BT I (ADP), platelet aggregation in response to adenosine diphosphate; BT II (EPI), platelet aggregation in response to epinephrine; PC, protein C; FVIII, FXI, FXII, activity of factors VIII, XI, and XII, respectively.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

cost-effective to use the scoring system, and have underlined the benefit of this system, recommending it for all cases that are suggestive of NS [Jongmans et al., 2005].

Our study has some limitations. The number of patients was too small to establish definite genotype–phenotype correlations. Also, although in future we plan to investigate other genes known to be responsible for or involved in NS, including *SOS1*, *RAS*, and *KRAS*, these were not investigated in this study.

Table III shows the distribution of several major clinical features of NS in our patients with and without *PTPN11* mutations. Some of these findings were concordant with previous studies, whereas other phenotypic characteristics were not. Comparison of our mutation-positive and mutation-negative groups revealed no significant differences for rates of PS, pectus deformities, genitourinary anomalies including cryptorchidism, hearing loss, moderate mental retardation, and hematological abnormalities. However, short stature (<3p) and mild mental retardation were more frequent in the NS patients with *PTPN11* mutations. Tartaglia et al. [2002] found that, among individuals with NS, those with a mutation in *PTPN11* more often have PS, whereas those with no such mutation more often have cardiomyopathy. The most commonly reported mutation in NS is A922G in exon 8 [Musante et al., 2002; Tartaglia et al., 2002; Jongmans et al., 2005]. Two of the 30 patients in our study had this mutation. Previous reports have stated that NS patients with this mutation have normal intelligence [Tartaglia et al., 2002; Jongmans et al., 2005]. Contrary to this, our psychometric tests revealed mild mental retardation in these children. These two children were from families in a rural area of Turkey with low socioeconomic status. Our findings also conflict with prior reports in other ways as well. We observed no differences between mutation-positive and mutation-negative NS patients with respect to hematological abnormalities, whereas previous researchers have found that such abnormalities are more frequent in those with *PTPN11* mutation [Yoshida et al., 2004]. Further, others have not linked this genetic defect with short stature [Tartaglia et al., 2002; Yoshida et al., 2004].

According to the literature, approximately two-thirds of individuals with NS have a history of abnormal bleeding or mild-to-severe bruising, and one-third have coagulation defects [Witt et al., 1988; Sharland et al., 1992; Allanson, 2007]. Factor deficiencies in patients with NS are generally stable, but there may be clinical amelioration with age [Allanson, 2007]. It has been reported that severe hemorrhage occurs in 3% of NS cases [van der Burgt, 2007]. However, the exact prevalence of coagulopathy and the mechanism(s) that underlie the association between coagulopathy and NS are not fully understood.

According to reviews published between 1985 and 1988, the estimated prevalence of bleeding disorders in NS was between 20% and 33% [Allanson et al., 1985; Mendez and Opitz, 1985; Witt et al., 1988]. Later studies showed a high frequency (56–67%) of coagulation defects in this patient group [Sharland et al., 1992; Massarano et al., 1996; Singer et al., 1997]. Sharland et al. [1992] did detailed coagulation studies on 72 individuals with NS. They found that 47 of these patients (65%) had history of abnormal bruising or bleeding, 29 (40%) had prolonged aPTT, and 36 (50%) had partial deficiencies of FVIII, FXI, and FXII. The same study reported combined FXI and FXII deficiency in one patient, combined FXI and FVIII deficiency in another patient, and combined FVIII, FXI, and FXII deficiencies in a third patient. Massarano et al. [1996] investigated 18 patients with NS and found increased bruising or bleeding in 12 (67%). They detected prolonged aPTT in 10 cases (56%) linked with low activity levels of clotting factors, particularly FXI and FXII. Bleeding times were normal, though one patient had marginal thrombocytopenia. The authors found that coagulation results were not correlated with bruising history, and they concluded that such findings may not predict bleeding risk in NS cases.

The findings of Massarano et al. concur with those of Sharland et al. [1992], whereas more recent work done after the year 2000 has noted much lower frequencies of bleeding problems between 1% and16% [Musante et al., 2002; Yoshida et al., 2004; Jongmans et al., 2005]. Furthermore, some case reports have documented NS patients with important bleeding problems, and even the rare

abnormality of FXIII deficiency [Sgouros et al., 2004; Tofil et al., 2005; Staudt et al., 2005]. To date, it has been widely held that NS patients are at risk for abnormal bleeding and should be tested for bleeding abnormalities [Singer et al., 1997; Bertola et al., 2003].

Musante et al. [2002] studied genotype and phenotype in 96 NS patients with *PTPN11* mutations. Only one (1.0%) of their subjects was diagnosed with mild von Willebrand syndrome, and this individual experienced bleeding complications after surgery for cryptorchidism but not after tonsillectomy. Two other patients had moderately prolonged aPTT. Another subject reported easy bruising and longer bleeding after having received a cut, though this patient's coagulation test results were normal. Similarly, in our study only one patient (Patient 4) and his father experienced easy bruising. This patient's coagulation tests were normal. As mentioned, the father declined further hematologic tests and molecular genetics analysis. The results of our study are in accord with those of Musante et al. [2002].

Yoshida et al. [2004] investigated 45 patients with NS and found a 6.7% prevalence of bleeding diathesis. Eighteen (40%) of their 45 subjects had *PTPN11* mutations, and the prevalence of bleeding problems in this group was 16%. The authors detected hematological abnormalities only in mutation-positive individuals. In these cases, bleeding diathesis was due to thrombocytopenia, platelet dysfunction, and FXII deficiency. Jongmans et al. [2005] studied 56 NS patients who had *PTPN11* mutations. They noted easy bruising in 32 cases (57%) and abnormal coagulation tests in 8 (14%) patients. The report provides no details about coagulation factor activity.

Interestingly, two of our patients had low PC activity (Patient 1: 42% and Patient 11: 49%). The respective *PTPN11* gene mutations in these cases were A1517C in exon 3 and C1528G in exon 13. Assessment of Patient 1 revealed transient myelodysplasia in the peripheral blood and bone marrow, as well as hepatosplenomegaly of unknown etiology with normal liver function test results (Fig. 1A,B). Patient 11 had normal abdominal ultrasonography findings and normal liver function test results. Neither of these patients has experienced a thrombotic event or any complications during approximately 3 years of follow-up. The blood and bone marrow myelodysplasia afflicting Patient 1 resolved within 2 years.

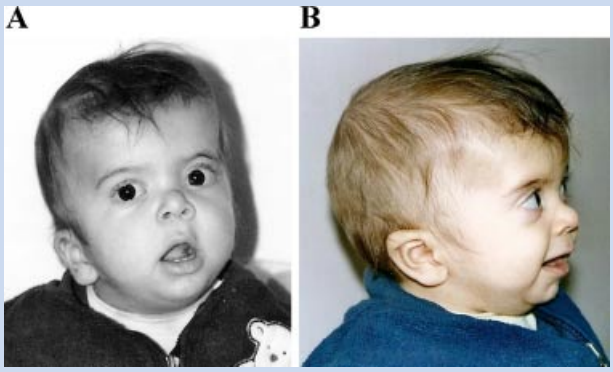

FIG. 1. A,B: Facial dysmorphic features of one of the subjects with Noonan syndrome (Patient 1). Note the low-set ear and thick helix, micrognathia, down-slanted palpebral fissures, and depressed nasal root. This patient also had transient myelodysplasia in the peripheral blood and bone marrow, hepatosplenomegaly, and low protein C activity. Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com

Recently, a similar case to our Patient 1 was reported by Musante et al. [2002]. This child had myelodysplasia and hepatosplenomegaly, and genetic testing revealed a G417C mutation. Hepatosplenomegaly unrelated to cardiac failure is also a frequent finding in NS (26–51% of cases) and myelodysplastic disorders are often associated with hepatosplenomegaly [van der Burgt, 2007]. Numerous authors have found NS to be associated with myeloproliferative disorders [Johannes et al., 1995; Bader-Meunier et al., 1997; Choong et al., 1999; Silvio et al., 2002]. Studies have shown that the C218T mutation in patients with NS is associated with high risk of developing juvenile myelomonocytic leukemia. This hematologic disease most often resolves spontaneously in patients with NS [Tartaglia et al., 2003; Jongmans et al., 2005]. Different from previous reports, the *PTPN11* gene mutation in our patient with myelodysplasia was A1517C. Although this is only one case, it suggests that an A1517C mutation may be another mutation related to transient myelodysplasia.

We were only able to find a single report that discusses a link between PC and NS. Ganesan and Kirkham [1997] documented activated PC resistance in a patient who had Moyamoya disease and NS. The patient was also heterozygous for the factor V Leiden mutation. The authors considered this a coincidental finding. PC deficiency is caused by mutations in the *PROC* gene (OMIM 612283) located at 2q13-q14, and this condition occurs via autosomal-dominant inheritance. The protein is cleaved to its activated form (known as activated PC) on endothelial cells by the thrombin–thrombomodulin complex, and then acts as a serine protease to degrade the activated forms of coagulation FV and FVIII. PC is synthesized in the liver and then released into the bloodstream. It is possible that other unexplained disorders may lead to deficient production of PC in patients with NS.

Previous investigations have clearly revealed that there is an important link between NS and coagulation problems, though our study and other recent reports indicate that the frequency of such associations is low. Patients with NS often require major surgical procedures such as open-heart surgery. If a bleeding disorder is present but not diagnosed, such operations may have serious or life-threatening implications. Every patient with NS should be screened for bleeding diathesis. A thorough history including bleeding or bruising abnormality, and first-line coagulation tests including complete blood count, platelet count, PT, aPTT, and BT should be performed. However, we suggest that, if these initial tests are normal, complications related to coagulation are unlikely and more extensive testing is unnecessary.

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