RASopathies and Hemostatic Abnormalities: Key Role of Platelet Dysfunction

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Abstract

Background: Bleeding anomalies occur in patients affected by Noonan syndrome. No study has been performed in patients with molecularly confirmed RASopathy. We aimed to characterize the frequency and types of bleeding disorders in patients with RASopathies and evaluate any significant association with laboratory findings.

Patients and methods: Forty-nine individuals (*PTPN11*, n=27; *SOS1*, n=7; *RIT1*, n=3; *SPRED1*, n=1; *LZTR1*, N=3; *RAF1*, n=2; *BRAF*, n=4; *MEK1*, n=1; *MEK2*, n=1), and 49 age- and sex-matched controls were enrolled in the study. The “Paediatric Bleeding Questionnaire Scoring Key” was administered to patients and families. Laboratory screening tests including clotting factors dosing, platelet count, prothrombin time, and partial thromboplastin time, were employed both in patients and controls to characterize the bleeding diathesis. A subgroup of 29/49 patients and 29/49 controls was also tested for platelet function.

Results: Regardless the gene involved, pathological bleeding scores were recorded in 14 (28.5%) patients. Among these, 7 were mutated in *PTPN11* (26% of total cases with *PTPN11* mutations), 3 in *SOS1* (43%), 2 in *RIT1* (67%), 1 in *BRAF* (25%), and 1 in *MEK1*. Compared to patients with normal bleeding scores, those with pathologic bleeding score showed higher prevalence of splenomegaly ($p=0.006$), prolonged aPTT ($p = 0.04$), lower levels of coagulation factor V (FV) ($p = 0.001$), factor VII (FVII) ($p = 0.003$), factor X (FX) ($p = 0.0008$) and factor XIII (FXIII) ($p = 0.002$), higher vWAg ($p = 0.04$), and lower platelet sensitivity to Ristocetin ($p = 0.001$), arachidonic acid (AA) ($p = 0.009$) and collagen ($p = 0.01$). The presence of hematomas inversely correlated with FV ($p = 0.002$), FVII ($p = 0.003$), FX ($p = 0.002$) and FXIII ($p = 0.004$) levels, and directly correlated with platelet response to collagen ($p = 0.02$) and AA ($p = 0.01$). The presence of splenomegaly directly correlated with occurrence of hematoma ($p=0.006$), platelet response to Ristocetin ($p = 0.04$) and AA ($p = 0.04$), and inversely correlated with FV levels ($p = 0.03$).

Conclusions: Patients with RASopathies and a bleeding tendency exhibit multiple coagulation-related laboratory abnormalities, including platelet-related disorders. Splenomegaly is frequently detected and might be a suggestive sign for qualitative platelet dysfunction. A comprehensive clinical assessment should be carried out at diagnosis, during the follow-up and before any surgical procedures. Since there is currently no consensus on management of bleeding complications, it is important that physicians closely monitor these patients.

Introduction

RASopathies represent one of the most common family of genetic syndromes affecting development and growth. These disorders are caused by germline mutations in genes encoding components of the Ras/mitogen activated protein kinase (MAPK) pathway, a ubiquitous and highly conserved signal transduction cascade critical to normal development with a key role in cell cycle regulation, differentiation, proliferation, apoptosis, and senescence [1-3]. The proteins involved in these disorders

include multiple members of the RAS family of GTPases (*HRAS*, *NRAS*, *HRAS*, *MRAS*, *RRAS*, *RRAS2* and *RIT1*), core components of the MAPK cascade (*BRAF*, *RAF1*, *MAP2K1*, *MAP2K2* and *MAPK1*), and both positive (*SOS1*, *SOS2*, *SHOC2* and *PPP1CB*) and negative (neurofibromin and *SPRED1*) regulators of Ras function. This signaling cascade controls the function of a large number of downstream effectors, including a variety of nuclear regulatory proteins involved in the control of gene expression and cell cycle progression, and several cytoplasmic effectors controlling diverse cellular processes (e.g., differentiation, metabolism and survival) [1-3].

The RASopathies include Noonan syndrome (NS, OMIM 163950), Noonan syndrome with multiple lentigines (formerly known as LEOPARD syndrome; LS, OMIM 151100) cardiofaciocutaneous syndrome (CFC, OMIM 115150), Costello syndrome (CS, OMIM 218040), Mazzanti syndrome (OMIM 607721 and 617506), neurofibromatosis type 1 (NF1, OMIM 162200), Legius syndrome (OMIM 611431), and other emerging disorders [1-7]. These diseases are characterized by a clinical overlap, with similar craniofacial, cardiac, skeletal, and cutaneous features, variable neurocognitive impairment and behavioural abnormalities, defective postnatal growth, and a variably enhanced risk of cancer. Despite their clinical similarity, each of these disorders has unique phenotypic features that likely result from a differential impact of the specific dysregulation of the involved pathway(s) on development [1-4].

A major clinical complication of NS is the abnormally high risk of bruising and spontaneous bleeding, which has been reported in 50–90% of patients [8]. In NS, bleeding abnormalities are often associated with factor XI (FXI) deficiency and platelet abnormalities. Since a significant number of patients with NS require surgery, their tendency to bleed is a relevant concern. For instance, a duodenal hematoma has been reported as complication of endoscopic biopsy in NS [9] and argues for the early identification of subjects at risk before the procedure among affected individuals to set up the optimal management [10-20].

SHP2, the protein encoded by *PTPN11* (the gene most frequently mutated in NS) is thought to play an important role in platelet function. Indeed, SHP2 participates in multiple intracellular signalling pathways in response to a wide range of growth factors, cytokines and hormones [21]. It has also been implicated in regulating signalling from a variety of platelet and megakaryocyte receptors. In particular, megakaryocyte/platelet (MP)-specific deletion of Shp2 results in macrothrombocytopenia, and platelets become hyper-responsive to anti-CLEC-2 antibody and fibrinogen [22-23]. Of note, at odds with NS patients with activating mutations of this gene and high prevalence of bleeding disorders [2, 3], patients with LS [24], who are heterozygous for a different class of mutations in the same gene, do not have bleeding disorders [2, 3].

Here, we systematically evaluated *ex vivo* a series of coagulation and platelet function variables in a cohort of molecularly characterized RASopathy patients to investigate the prevalence of common haemostatic abnormalities in NS and related disorders and identify possible genotype-phenotype correlations.

Patients And Methods

All the subjects were enrolled at the Clinical Genetic Pediatric Department of the University "Federico II" of Naples (Italy). In total, 49 patients (18 females, 31 males) were included in the study. Their clinical diagnosis (NS, N=43; CFCS, N=2; LS, N=2; NFNS, N=2) had molecularly been confirmed. The mean age at enrollment in the study was 10.8 years (ranging from 0 to 49 years). A sex- and age-matched control group including 49 subjects (18 females, 31 males; mean age 11.2 years, ranging from 1 to 49 years) was also enrolled. Only patients with a molecular diagnosis of RASopathy were considered. The cohort presented the following distribution: *PTPN11* (27, 53.1%), *SOS1* (7, 14.3%), *RIT1* (3, 6.1%), *SPRED1* (1, 2.0%), *LZTR1* (3, 6.1%), *RAF1* (2, 4.1%), *BRAF* (4, 8.2%), *MEK1* (1, 2.0%) and *MEK2* (1, 2.0%). All patients underwent anamnestic recall, clinical and auxological examination, and spontaneous hematomas, ecchymoses and epistaxis were individually evaluated. The "Pediatric Bleeding Questionnaire (PBQ) Scoring Key" [25] was administered to patients and families to assess the patients' tendency to bleed and bruise. PBQ score results above 3 were considered indicative of an enhanced tendency to bleeding or bruising forming. The degree of bleeding diathesis was also evaluated by laboratory screening tests including clotting factors dosing, platelet count, prothrombin time (PT) which measures the integrity of the extrinsic system as well as factors common to both systems and activated partial thromboplastin time (aPTT), which measures the integrity of the intrinsic system and the common component in both patients and controls, performed according to standard methods.

Twenty-nine out of the 49 patients with RASopathy (13 females, 17 males; mean age 11.8 years) and 29 control individuals with no history of bleeding underwent a platelet function test. It consists in a "turbidometric platelet aggregometry", which measures platelet aggregation in platelet-rich plasma (PRP, obtained by centrifugation of 9 volumes of freshly collected whole blood [from the antecubital vein] in plastic tubes containing one volume of 3.8% trisodium citrated and centrifuged for 15 min at 700xg) [10-11]. The method is based on the detection of difference in light transmission by a photometer after adding a platelet agonist to PRP under stirring conditions (1,000 rpm). Samples can be exposed to a wide range of agonists, which can give an insight into different pathways of platelet activation/aggregation; in the current study ADP, arachidonic acid (AA), collagen and ristocetin have been used. Aggregation measurements provide an aggregation index curve describing changes in the intensity of the light transmission of the PRP samples vs a comparator sample of platelet-poor plasma (PPP) obtained from the citrated blood centrifuged at 2,000xg for 15 min after removing the supernatant PRP.

Statistical analysis

Each numerical variable was expressed as mean $\pm$ SD. Statistical analyses were performed using SPSS package. Differences in the clinical and biochemical parameters between patients and controls as well as between patients with pathological bleeding score and patients with normal bleeding score were analysed using the t-test for paired data. Genotype-phenotype correlations were performed taking into account the individual gene involved and type of mutation. Fisher exact test was used in these comparisons to assess the statistical significance of deviations. To analyse possible correlations

between the severity of features and specific gene involved, Spearman's correlation study and association study were performed. A p-value <0.05 was considered to be significant in all comparisons.

Results

All patients underwent anamnestic recall, clinical examination, including auxological parameters, evaluation of hematomas, ecchymoses and epistaxis. Auxological parameters were normal for specific growth chart in 47/49 patients. Bleeding was recorded in 18/49 (36.7%) patients; hematomas and or ecchymoses were recorded in 13/49 (26.5%) patients, while epistaxis and bleeding of oral bleedings were reported in 3/49 (6.12%) and 11/49 (22.4%) patients, respectively. Menorrhagia was recorded in 3/8 (37%) females.

Pathologic bleeding score (PBQ ≥ 3) was recorded in 14/49 (28.5%) patients. Significant scores included: PBQ of 3 in 11 patients; PBQ of 4, 5 and 7 in single patients each.

The degree of bleeding diathesis was also evaluated by laboratory screening tests (clotting factors dosing, platelet count, PT and aPTT assessment) in patients and compared to age- and sex-matched control group. Patological laboratory tests included: prolonged PT (35 patients), aPTT (16 patients); PT/aPTT (13 patients). One subject presented thrombocytopenia (platelet count below 100/mm³). 17/49 (32.6%) patients had splenomegaly. Being reported in 12/49 (24.4%) patients, factor VII (FVII) partial deficiency, isolated or in combination with the deficiency of other vitamin K-dependent factors, was the most frequent coagulation factors abnormality. The optical aggregometry test was pathologic in 19/29 patients, demonstrating platelet dysfunction in 65% of RASopathy patients. Platelet sensitivity to ADP (3.14 ± 1.14 vs 1.84 ± 0.46 ; $p < 0.0001$) and collagene (0.59 ± 0.23 vs 0.42 ± 0.13 ; $p = 0.0015$) was significantly lower in patients than in controls, whereas platelet response to AA ($p = 0.3$) and ristocetin ($p = 0.2$) was similar in the two groups. Patients with pathologic bleeding score showed higher prevalence of splenomegaly ($p = 0.006$).

Patients with pathologic BQ score showed lower levels of Factor V (FV) (74.9 ± 17 vs 93 ± 12 , $p = 0.001$), FVII (57.8 ± 14 vs 73.7 ± 14 , $p = 0.003$), factor X (FX) (66.7 ± 13 vs 83.6 ± 12 , $p = 0.0008$), factor XIII (FXIII) (58.4 ± 21 vs 86.6 ± 20 , $p = 0.002$), higher vWAg (85 ± 45 vs 60 ± 15 , $p = 0.04$), and longer aPTT (38 ± 4.2 vs 33 ± 3.7 , $p = 0.04$).

Platelet sensitivity to Ristocetin (1.3 ± 0.3 vs 1.1 ± 0.1 ; $p = 0.001$), to AA (0.8 ± 0.6 vs 0.4 ± 0.8 ; $p = 0.009$), and to collagene (1.2 ± 1.1 vs 0.4 ± 0.18 ; $p = 0.01$) was significantly lower in patients with pathologic PBQ score (**Table 1**).

Correlation analysis showed that *PTPN11* patients with NS have higher prevalence of Factor XII (FXII) deficiency ($r = 0.56$, $p = 0.03$). The presence of hematomas inversely correlated with FV ($r = -0.5$, $p = 0.002$), fFVII ($r = -0.4$, $p = 0.003$), FX ($r = -0.56$, $p = 0.002$), and FXIII ($r = -0.56$, $p = 0.004$), and directly correlated with platelet response to collagen ($r = 0.42$, $p = 0.02$) and platelet response to AA ($r = 0.44$, $p = 0.01$). The presence of splenomegaly correlated with the presence of hematoma ($r = 0.387$, $p = 0.006$),

platelet response to Ristocetin ($r = 0.36$, $p = 0.04$), and platelet response to AA ($r = 0.35$, $p = 0.04$) and inversely correlated with factor V levels ($r = -0.35$, $p = 0.03$).

Discussion

RASopathies can be associated with an increased risk of bleeding and bruising, and a variety of bleeding abnormalities have been described in these disorders, but their causes still remain unclear.

The aim of the current study was to identify the frequency and type of bleeding disorders in patients with RASopathies, and evaluate possible links with associated laboratory findings and genotype. Pathological bleeding score was recorded in almost half of the patients, regardless of the gene involved. No significant genotype-phenotype correlation was identified. The role of the small number of patients cannot be excluded. The mutational spectrum of patients with bleeding disorders was largely overlapping with that of the NS general population, including mutations in *PTPN11*, *SOS1*, and *RIT1* genes. Similar to unselected NS cohorts, *PTPN11* mutations were responsible for half of the cases. Another 30% of patients had mutations in *SOS1* and *RIT1*, whereas *BRAF* and *MAP2K1* mutations were identified in single families. None of the patients with bleeding disorders harbored mutations in *RAF1* and *LZTR1*, two genes frequently mutated in NS. Considering that bleeding disorders have previously been described in patients with mutations in both these genes [9, 26, 27], the absence of these complications among the tested patients with *LZTR1* and *RAF1* mutations is likely related to the limited size of the study cohort.

Possible genotype-phenotype correlations with coagulation defects in the context of RASopathies were initially hypothesized for *PTPN11* and *SOS1*. A study performed on 27 NS patients identified a *PTPN11* mutation in 21 and bleeding defects in 9, and therefore a correlation between *PTPN11* mutations and bleeding defects in the context of NS was hypothesized. To date, more than 20 genes responsible for RASopathies have been described and correlations with bleeding disorders cannot be excluded. However, despite the limited number of cases described in the studies reported so far, the present results together with those previously published support the absence of significant correlations with bleeding defects in RASopathies.

Among the laboratory findings described in RASopathy patients with bleeding defects, FXI deficiency and platelet abnormalities are most frequently described [10–20]. Patients with NS seem to have an imbalance in fibrinolytic components favouring fibrinolysis. This may be an important contributor to the bleeding [28]. Acquired cases of von Willebrand syndrome have been described and the destruction of the von Willebrand factor could explain bleeding in some NS patients with pulmonary valve stenosis [17]. A number of studies identified no correlation between coagulation study results and bleeding risk in NS [10–29]. In regard of laboratory findings, our RASopathy patients with bleeding disorders showed lower levels of FV, FX, FXIII coagulation factors, higher vWAg, longer aPTT, and lower platelet sensitivity to Ristocetin, AA and collagen. Moreover, they presented a higher prevalence of splenomegaly. Of note, the presence of hematomas was inversely correlated with FV, FVII, FX and FXIII levels, whereas it was directly correlated with platelet response to collagen and platelet response to AA. Other significant correlations

highlighted in this study was the observation that splenomegaly was directly correlated with the presence of hematomas, and to platelet response to Ristocetin and AA, but inversely correlated with FV levels.

Although there is currently no consensus on the best strategy for bleeding disorder diagnosis in NS patients, it has been suggested that, at time of diagnosis of NS, a complete blood cell count (CBC) with differential and PT/aPTT should be performed. Then, in consultation with hematologist, FIX, FXI, and FXII levels, von Willebrand factor, and platelet aggregation should be investigated [30].

Recently, a significant reduction in platelet aggregation, induced by low concentrations of GPVI and CLEC-2 agonists, and a decrease in thrombus growth on a collagen surface under arterial shear stress were demonstrated in a NS mouse model carrying a gain-of-function mutation of SHP2. Conversely, LS-associated SHP2 loss-of-function results in increased platelet activation [31]. This study suggests the presence of thrombopathies linked to platelet signaling defects in NS.

Conclusions

Our findings underline that the combination of platelet dysfunction and coagulation factor deficiency is at the basis of haemostatic abnormalities in patients affected by RASopathies. Splenomegaly, frequently detected in these patients, might be a suggestive sign for platelet dysfunction.

Present and previous studies show that patients with RASopathies can experience multiple bleeding disorders, including platelet-related disorders. Therefore, comprehensive clinical evaluations should be carried out both at diagnosis and in follow-up, in particular if patients are symptomatic and before any surgical procedures. Since there is no current consensus on management of bleeding complications, it is important that physicians closely monitor these patients [9–11]. Further studies are needed to better evaluate these features and set up a specific follow-up.

Abbreviations

AA: arachidonic acid.

CFCS: cardio-facio-cutaneous syndrome,

LS: Leopard syndrome

MAPK: mitogen activated protein kinase

NS: Noonan syndrome,

NF1: neurofibromatosis type 1,

PBQ: Pediatric Bleeding Questionnaire

PT: Prothrombin Time

aPTT: Partial Thromboplastin Time

Declarations

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Authors' contributions

Di Candia Francesca, Marchetti Valeria: designed and directed the study and wrote the manuscript

Cirillo Ferdinando, Di Minno Alessandro, Magliacane Gerardo, Boccia Giovanni performed platelet studies.

Rosano Carmen, Pagano Stefano, Siano Maria. Anna, Falco Mariateresa, Assunto Antonia were involved in patients' follow-up.

Pinna Valentina, De Luca Alessandro, Tartaglia Marco performed molecular analysis

Di Minno G, Daniela Melis and Pietro Strisciuglio: encouraged the study progress and gave substantial cultural contribution.

All authors discussed the results and contributed to the final manuscript.

Availability of data and materials

Data are available by request

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the responsible Institutional Committee on Human Experimentation and with the Helsinki Declaration of 1975 (revised in 2000).

Consent for publication

Informed consent was written by the parents of our patient to participate in this study.

Competing interests

The authors declare that they have no competing interests.

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References

1. Tajan M, Paccoud R, Branka S, Edouard T, Yart A. The RASopathy family: consequences of germline activation of the RAS/MAPK pathway. *Endocr Rev.* 2018;39(5):676–700.
2. Tartaglia M, Gelb BD, Zenker M. Noonan syndrome and clinically related disorders. *Best Pract Res Clin Endocrinol Metab.* 2011;25(1):161–79.
3. Aoki Y, Niihori T, Inoue S, Matsubara Y. Recent advances in RASopathies. *J Hum Genet.* 2016;61:33–9.
4. Rauen KA. The RASopathies. *Annu Rev Genomics Hum Genet.* 2013;14:355–69.
5. Tartaglia M, Gelb BD. Disorders of dysregulated signal traffic through the RAS-MAPK pathway: phenotypic spectrum and molecular mechanisms. *Ann N Y Acad Sci.* 2010;1214:99–121.
6. Capri Y, Flex E, Krumbach OHF, Carpentieri G, Cecchetti S, Lißewski C, et al. Activating mutations of RRAS2 are a rare cause of Noonan syndrome. *Am J Hum Genet.* 2019;104(6):1223–32.
7. Motta M, Pannone L, Pantaleoni F, Bocchinfuso G, Radio FC, Cecchetti S, et al. Enhanced MAPK1 Function Causes a Neurodevelopmental Disorder within the RASopathy Clinical Spectrum. *Am J Hum Genet.* 2020;3(3):499–513. 107(.
8. Nugent DJ, Romano AA, Sabharwal S, Cooper DL. Evaluation of bleeding disorders in patients with Noonan syndrome: a systematic review. *J Blood Med.* 2018;9:185–92.
9. Sgouros SN, Karamanolis G, Papadopoulou E, Papageorgiou G, Stefanides G, Nastos H, et al. Post biopsy intramural hematoma of the duodenum in an adult with Noonan's syndrome *J Gastroenterol Hepatol.* 2004 Oct;19(10):1217–9.
10. Artoni A, Selicorni A, Passamonti SM, Lecchi A, Bucciarelli P, Cerutti M, et al. Hemostatic abnormalities in Noonan syndrome. *Pediatrics.* 2014;133(5):e1299–304.
11. Sharland M, Patton MA, Talbot S, Chitolie A, Bevan DH. Coagulation-factor deficiencies and abnormal bleeding in Noonan's syndrome. *Lancet.* 1992;339(8784):19–21.
12. Briggs BJ, Dickerman JD. Bleeding disorders in Noonan syndrome. *Pediatr Blood Cancer.* 2012;58(2):167–72.
13. Kitchens CS, Alexander JA. Partial deficiency of coagulation factor XI as a newly recognized feature of Noonan syndrome. *J Pediatr.* 1983;102(2):224–7.
14. Bertola DR, Carneiro JD, D'Amico EA, Kim CA, Albano LM, Sugayama SM, et al. Hematological findings in Noonan syndrome. *Rev Hosp Clin Fac Med Sao Paulo.* 2003;58(1):5–8.
15. De Haan M, vd Kamp JJ, Briët E, Dubbeldam J. Noonan syndrome: partial factor XI deficiency. *Am J Med Genet.* 1988;29(2):277–82.
16. Massarano AA, Wood A, Tait RC, Stevens R, Super M. Noonan syndrome: coagulation and clinical aspects. *Acta Paediatr.* 1996;85(10):1181–5.
17. Wiegand G, Hofbeck M, Zenker M, Budde U, Rauch R. Bleeding diathesis in Noonan syndrome: is acquired von Willebrand syndrome the clue? *Thromb Res.* 2012;130(5):e251-4.
18. Briggs B, Savla D, Ramchandrar N, Dimmock D, Le D, Thornburg CD. The Evaluation of Hematologic Screening and Perioperative Management in Patients with Noonan Syndrome: A Retrospective Chart

Review *J Pediatr*. 2020;220:154-8.e6.

19. Sharland M, Burch M, McKenna WM, Paton MA. A clinical study of Noonan syndrome. *Arch Dis Child*. 1992;67(2):178–83.
20. Zenker M, Buheitel G, Rauch R, Koenig R, Bosse K, Kress W, et al. Genotype-phenotype correlations in Noonan syndrome. *J Pediatr*. 2004;144(3):368–74.
21. Tajan M, de Rocca Serra A, Valet P, Edouard T, Yart A. SHP2 sails from physiology to pathology. *Eur J Med Genet*. 2015;58(10):509–25.
22. Senis YA. Protein-tyrosine phosphatases: a new frontier in platelet signal transduction. *J Thromb Haemost*. 2013;11(10):1800–13.
23. Mazharian A, Mori J, Wang YJ, Heising S, Neel BG, Watson SPet. al. Megakaryocyte-specific deletion of the protein-tyrosine phosphatases Shp1 and Shp2 causes abnormal megakaryocyte development, platelet production, and function. *Blood*. 2013;121(20):4205–20.
24. Kontaridis MI, Swanson KD, David FS, Barford D, Neel BG. PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. *J Biol Chem*. 2006 Mar 10;281(10):6785–92.
25. Elbatarny M, Mollah S, Grabell J, Bae S, Deforest M, Tuttle A, et al. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. *Haemophilia*. 2014;20(6):831–5.
26. Gamba G, Marabotto F, Losa L, et al. Co-agulation factor deficiencies and abnormal bleeding in Noonan's syndrome. *Horm Res Paediatr*. 2011;76(Suppl 2):321.
27. Aoki Y, Niihori T, Inoue S, Matsubara Y. Recent advances in RASopathies. *J Hum Genet*. 2016;61(1):33–9.
28. Mehta P, Parker RI. Imbalance of plasminogen activator inhibitor type-1 (PAI-1) and tissue plasminogen activator (t-PA) activity in patients with Noonan syndrome. *J Pediatr Hematol Oncol*. 2010 Oct;32(7):532–6.
29. Troiano M, Gottlieb S, Rey R, et al. Noonan Syndrome: assessment of bleeding disorders. *Horm Res Paediatr*. 2011;76(Suppl 2):21.
30. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet*. 2013;381(9863):333–42.
31. Bellio M, Garcia C, Edouard T, Voisin S, Neel BG, Cabou C, et al. Catalytic dysregulation of SHP2 leading to Noonan syndromes affects platelet signaling and functions. *Blood*. 2019 Dec 19;134(25):2304–17.

Tables

Table 1

Patient	Gene	Bleeding score*	Clotting Factor (% of normal)				Platelet response to**		
			V	VII	X	XIII	ADP	Collagen	Ristocetin
1	SOS1	7	67	48	64	47	2.8	0.8	1.1
2	SOS1	4	83,2	61	87	94,4	2.8	0.4	1.1
3	SOS1	3	55	81	76	73			
4	PTPN11	3	83	47.7	57	42	4	4	1.5
5	PTPN11	3	78.6	52	60	46	4	0.8	1.1
6	PTPN11	5	54	46	55		6	2	1.5
7	PTPN11	3	75	57	88	71	5	2	2
8	BRAF	3	85	58.5	74	29	1.2	0.4	1.1
9	MAP2K1	3	78	72			2	2	1.1
10	PTPN11	3	81	36	78	74	4	0.8	1.1
11	PTPN11	3	58	60.2	47.4		6	0.8	1.5
12	RIT1	3	50.2	46	50	50	5	0.8	1.5
13	RIT1	3	55	44	50.5		5	1.2	1.2
14	PTPN11	3	117	86	62		4	0.4	1.1

*These data are expression of 3 evaluations per patient

** values referred to platelet aggregation are expressed as AC50 or the minimum threshold at which an inducing agent is capable, in a time of three minutes, to give an aggregation response that reaches at least 50%. The response to platelet aggregation is the average of two repetitions.

Platelet aggregation has been evaluated using instrument by Chrono-Log Corporation mod. 700 and Mascia Brunelli

Italia - Milano for reagents including ADP, Collagen e Ristocetin