SUPPLEMENTARY APPENDIX

Phenotipic features of patients

The clinical and laboratory findings of the 4 families with *NBEAL2* mutations are reported in more detail (Table 1S) as follows.

Family 1. The proband was a male evaluated at the age of 28 years for a spontaneous severe epistaxis. He had a previous history of mild epistaxis and easy bruising.[1,2] Leukocyte count was 3.7 x 10^9/L with normal differential, hemoglobin was 147 g/L and platelet count was 47 x 10^9/L with a mean platelet volume (MPV) of 10.5 fL. Blood smears, prepared without anticoagulant and stained with MGG, showed that most platelets lacked azurophilic granules and therefore had a grayish tonality. Platelet macrocytosis and giant platelets were also evident (Table 1S). The patient also had moderate (28%) reduction of plasma levels of coagulation factor V.[2] At present (age 42 years), the proband has moderate splenomegaly (15.5 cm in diameter), high vitamin B_{12} levels and a slight increase in the bone marrow reticulin network. We included in the analysis both patients' parents, who had no thrombocytopenia or bleeding (Table 2S). The proband's mother, brother and two children had a borderline low level of plasma factor V (41%, 52%, 47% and 43%, respectively).

Family 2. A 5 year-old boy from southern Italy was referred for moderate bleeding diathesis, characterized by easy bruising and frequent epistaxis. His platelet count ranged from 58 to 100 x 10^9/L; MPV was 11.1 fL. Prothrombin time and activated partial thromboplastin time were normal, while his bleeding time was prolonged (11 minutes, normal values ≤7.5 min) (Table 1S). Analysis of the MGG-stained blood smears showed gray platelets, giant platelets, and anisopoikilocytosis. The proband's father, mother and 2 year-old brother had no history of pathological bleeding or thrombocytopenia. Their main clinical features are detailed in Table 2S.

Family 3. The proband was a 72 year-old male (II-1) with personal history of thrombocytopenia that was first identified during routine laboratory examination at the age of 53.[3] His 68 year-old brother (II-2) had a diagnosis of immune thrombocytopenia at the age of 42, which was never treated. Both had a life-long bleeding diathesis characterized by epistaxis, easy bruising and

excess bleeding from minor wounds, after tooth extraction and after surgery. Their parents, who were first cousins, and other family members had normal platelet counts and no bleeding tendency. Platelet counts were 65 x 10^9/L for the proband and 30x10^9/L for his brother. Although MPV was normal, large platelets with gray appearance were evident on blood smears (Table 1S). Platelet aggregation revealed a mild defect in platelet response to ADP, epinephrine, collagen and arachidonic acid in the proband. Both patients developed moderate leukopenia, had elevated serum vitamin B_{12} levels and bone marrow biopsy revealed grade 3 and grade 2 fibrosis (scale 0 to 3), for II-1 and II-2, respectively. Individual II-2 only showed an enlarged spleen. Proband's two daughters and son had no history of abnormal bleeding, thrombocytopenia, or other signs of the disease (Table 2S).

Family 4. The proband (IV-4), who was an 11 years-old boy at diagnosis, and other 6 members (IV-5, III-7, III-8, II-4, II-2, III-1) were previously described.[4] Briefly, the proband had thrombocytopenia, severe α-granule deficiency, giant platelets and bleeding diathesis. Individuals III-1 and III-7 had personal history of epistaxis, platelet macrocytosis and prolonged bleeding time (Table 1S and 2S). In addition, patient III-1 had thrombocytopenia. All the other family members had normal platelet counts and no history of bleeding diathesis. Moreover, individuals III-1, II-2, II-4, III-7 all displayed mild reduction of platelet α-granule content.[4] We also enrolled five additional family members, III-5, IV-1 and IV-3, who had mild reduction of platelet α-granule content, as well as III-4 and IV-2, who had no platelet abnormalities.

The main clinical and laboratory features of the probands of families 5-11 are summarized in Table 3S. On examination of MGG-stained blood smears, all probands showed α-granule deficiency and a variable percentage of gray appearing platelets. Moreover, all of them had macrothrombocytopenia and 4 of them also had giant platelets. In 4 cases thrombocytopenia and the α-granule defect were inherited as an autosomal-dominant trait, while the remaining 3 patients had no familial history of thrombocytopenia and/or abnormal bleeding. Using a validated clinical and laboratory algorithm,[3,5] we could rule out the diagnosis of any known forms of inherited thrombocytopenia. In particular, the laboratory work-up of these patients included the study of *in vitro* platelet function (Born's method),[6] flow cytometry evaluation of platelet surface glycoproteins,[6] the immunofluorescence screening test for *MYH9*-related disease,[7] and the screening for mutations of the 5'UTR of the *ANKRD26* gene.[8] None of these

examinations was consistent with the diagnosis of any known form of congenital thrombocytopenia (Table S3). Moreover, the diagnosis of the X-linked thrombocytopenia associated with mutations of *GATA-1* was also excluded because of the inherited transmission pattern and/or the absence of dyserythropoiesis or imbalanced hemoglobin chain synthesis.

REFERENCES

1. Espanol I, Hernandez A and Pujol-Moix N. The magic of immersion oil: gray platelet syndrome. Haematologica. 1998;83(5):474-75.
2. Pujol-Moix N, Pecci A, Oliver A, Borrell M, Estivill C and Nomdedeu JF. Gray platelet syndrome associated with plasma factor V deficiency in two Spanish families. Blood E-letter. 2011; Response to: Gunay-Aygun M et al. Gray platelet syndrome: natural history of a large patient cohort and locus assignment to chromosome 3p. *Blood [e-Letter]* http://bloodjournal.hematologylibrary.org/letters.
3. Glembotsky AC, Marta RF, Pecci A, De Rocco D, Gnan C, Espasandin YR, et al. International collaboration as a tool for diagnosis of patients with inherited thrombocytopenia in the setting of a developing country. J Thromb Haemost. 2012;
4. De Candia E, Pecci A, Ciabattoni G, De Cristofaro R, Rutella S, Yao-Wu Z, et al. Defective platelet responsiveness to thrombin and protease-activated receptors agonists in a novel case of gray platelet syndrome: correlation between the platelet defect and the alpha-granule content in the patient and four relatives. J Thromb Haemost. 2007;5(3):551-9.
5. Noris P, Pecci A, Di Bari F, Di Stazio MT, Di Pumpo M, Ceresa IF, et al. Application of a diagnostic algorithm for inherited thrombocytopenias to 46 consecutive patients. Haematologica. 2004;89(10):1219-25.
6. Noris P, Perrotta S, Bottega R, Pecci A, Melazzini F, Civaschi E, et al. Clinical and laboratory features of 103 patients from 42 Italian families with inherited thrombocytopenia derived from the monoallelic Ala156Val mutation of GPIbalpha (Bolzano mutation). Haematologica. 2012;97(1):82-8.
7. Savoia A, De Rocco D, Panza E, Bozzi V, Scandellari R, Loffredo G, et al. Heavy chain myosin 9-related disease (MYH9 -RD): neutrophil inclusions of myosin-9 as a pathognomonic sign of the disorder. Thromb Haemost. 2010;103(4):826-32.

8. Noris P, Perrotta S, Seri M, Pecci A, Gnan C, Loffredo G, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood. 2011;117(24):6673-80.

Table 1S. Clinical and laboratory features of 5 patients carrying *NBEAL2* biallelic mutations.

Family (Patient)	Gender/Age at diagnosis of GPS	Previous diagnosis of ITP	Platelet count ($x10^9$/L) [Cell counter - Microscopy]	Mean Platelet Volume (fL)	Bleeding score[b]	Bleeding Time	Splenomegaly / diameter (cm)	Myelofibrosis[e]	Other findings
1 (II-1)[2]	M/28	No	48 - 57	10.5	2	>10 min[c]	Yes / 15.5	Slight	High vitamin B_{12} level Leukopenia Deficiency of coagulation factor V (28%)
2 (II-1)	M/5	No	60 - nd	11.1	2	11 min[d]	Yes /10.3	nd	High Vitamin B_{12} level
3 (II-1) [3]	M/72 (53)[a]	No	56 - 65	8.4	2	nd	No	Yes	High vitamin B_{12} level Leukopenia
3 (II-2)[3]	M/68 (42)[a]	Yes	26 - 30	8.6	2	nd	Yes /15.9	Yes	High vitamin B_{12} level Leukopenia
4 (IV-4)[4]	M/11	nd	85 - nd	13.7	2	>20 min[c]	Yes /13	Yes	High Vitamin B_{12} level

[a]In brackets age at diagnosis of thrombocytopenia.

[b]World Health Organization (WHO) bleeding score. Grade 0: no bleeding; Grade 1: petechiae; Grade 2: mild blood loss (no need for hospital admission or access to emergency); Grade 3: gross blood loss (hospital admission or access to emergency or iron therapy required or red cell transfusions after surgery or delivery); Grade 4: debilitating blood loss (red blood cell transfusion required for spontaneous haemorrhages).

[c,d]Bleeding time evaluated by the following methods: Ivy (c) or Symplate II (d).

[e]As evaluated by bone marrow biopsy.

Table 2S. Clinical and laboratory features of 13 subjects carrying *NBEAL2* monoallelic mutations.

Family	Familial relationship with the proband [a]	Gender/ Age (yrs)	Platelet count ($x10^9$/L) [Cell counter]	MPV (fL)	Bleeding score [b]	Bleeding Time	Splenomegaly	PB signs of myelofibrosis [d]	Other findings
1[2]	Father (I-1)	M/58	320	9.9	0	Normal [c]	No	No	None
1[2]	Mother (I-2)	F/56	259	10.2	0	Normal [c]	No	No	Borderline level of coagulation factor V (48%)
2	Father	M/37	207	8.0	0	n.d.	No	No	None
2	Mother	F/32	183	11.0	0	Normal [d]	No	No	None
2	Brother	M/2	192	9.0	0	Normal [d]	No	No	None
3[3]	Daughter	F/33	320	7.0	0	n.d.	No	No	None
3[3]	Daughter	F/38	210	7.2	0	n.d.	No	No	None
3[3]	Son	M/39	212	7.9	0	n.d.	No	No	None

4^4	Father (III-7)	M/40	170	9.7	2	11min (v.n.< 9) [c]	No	No	Recurrent epistaxis, easy bruising
4^4	Mother (III-8)	F/38	237	7.3	0	Normal [c]	No	No	None
4^4	Cousin (IV-3)	M/20	312	7.3	0	Normal [c]	No	No	None
4^4	Uncle (III-5)	M/38	227	7.5	0	Normal [c]	No	No	None
4^4	Grandmother (II-4)	F/69	180	7.3	0	Normal [c]	No	No	None

[a] In brackets the identification of patient in the pedigree (shown in Figures 1 and 2)
[b] World Health Organization (WHO) bleeding score (please see note to Table S1)
[c,d] Bleeding time evaluated by the following methods: Ivy (c) or Symplate II (d).
[e] Presence of anisopoikilocytosis with dacryocytes and/or immature erythroid or granulocytic cells at evaluation of PB smears.

Abbreviations: PB: peripheral blood. n.d. = not determined.

Table 3S. Main clinical and laboratory features of 7 unrelated probands with macrothrombocytopenia, α-granule deficiency, and gray platelets but without any *NBEAL2* mutations.

Family	Gender/Age at diagnosis of GPS or thrombocytopenia	Inheritance pattern	Platelet count ($x10^9$/L) [Cell counter - Microscopy]	MPV (fL)	Bleeding diathesis [a]	*In vitro* platelet function [b]	Platelet surface GP expression [c]	Other findings
5[2]	M/3	Sporadic	20-55	8.0	2	n.d.	normal	Deficiency of coagulation factor V (59%); normal vitamin B12 level; 65% decrease in platelet PF4 and β-thromboglobulin by ELISA assay
6	M/44	AD	26-38	13.8	3	normal	normal	None
7	F/25	AD	104-110	13.9	0	normal	normal	Mild neutropenia, normal vitamin B12 level
8	M/20	AD	67-55	10.4	0	normal	Mild reduction of GPIa-IIa	None
9	F/5	AD	37-58	12.5	4	Reduced after 5 μM ADP and 4 μg/mL collagen	normal	Interventricular membranous defect, mild von Willebrand disease type 1
10[3]	F/11	Sporadic	65-42	10.6	3	n.d.	normal	Mitral valve dysplasia, annular pancreas, psychomotor retardation Mild reduction of δ-granules by TEM
11	F/58	Sporadic	61-nd	14.5	1	normal	normal	Normal vitamin B_{12} level Normal δ-granule content by TEM

[a] World Health Organization (WHO) bleeding score (see note to Table 1S).
[b] Platelet aggregometry (Born's method) on platelet rich plasma after stimulation with ADP (5 and 20 μM), collagen (4 and 20 μg/mL), and ristocetin (0.5 and 1.5 mg/mL) (reference 15).
[c] Flow cytometry for GPIb-IX-V, GPIIb-IIIa, and GPIa-IIa (reference 15).
Abbreviations: MPV = mean platelet volume; GP = glycoprotein; n.d.: not determined. TEM = transmission electron microscopy on platelet preparation.