



Patient Report

Paris-Trousseau-type macrothrombocytopenia without 11q deletion

Zuhre Kaya,¹ Ulker Kocak,¹ Ferda Perçin,² Shinji Kunishima,³ Meryem Albayrak,¹ Turkiz Gursel,¹ and Candan Ozogul⁴¹Pediatric Hematology Unit, Department of Pediatrics, and ²Departments of ²Clinical Genetics and ⁴Histology, Medical School of Gazi University, Ankara, Turkey and ³Department of Hemostasis and Thrombosis, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan**Key words** hereditary, macrothrombocytopenia.

Hereditary macrothrombocytopenias (MTCP) are a heterogeneous group of rare disorders, characterized by giant platelets, thrombocytopenia and bleeding diathesis of variable degree. They are distinguished from each other by the mode of inheritance, specific clinical and laboratory abnormalities and underlying genetic defect. MTCP can occur as an isolated abnormality or be accompanied by additional hematological abnormalities, congenital malformations or Alport-like features such as nephritis, deafness and cataract.^{1,2} Giant platelet granules in association with dysmegakaryopoiesis are a characteristic feature of Paris-Trousseau (PT) or 11q23 deletion syndrome. We here describe a family with PT-type MTCP without 11q deletion.

Case report

An 11-year-old-girl was found to have thrombocytopenia during preoperative evaluation for ear tube insertion. A detailed history indicated frequent epistaxis since infancy, delayed speaking and learning difficulty at school. She had short stature (5th percentile) and an atypically narrow, elongated face with prominent nose bridge and thin upper lip (Fig. 1). Ophthalmologic examination was normal. Audiogram showed bilateral sensorineural deafness. Neurocognitive assessment indicated mild mental retardation on Stanford–Binet Intelligence Scale, impairment in both receptive and expressive language ability, decrease in attention span and visual perception. On complete blood count the platelet count was low, ranging between 10 and 30 × 10⁹/L on repeated measurements, as were elevated mean platelet volume and giant platelets in peripheral blood smear. No Döhle-like inclusion bodies could be identified in leukocytes. Red cell morphology was normal. Template bleeding time was 15 min (normal <7 min). Platelet aggregation in response to adenosine diphosphate, collagen, epinephrine and ristocetin in standard and low doses were normal. Coagulation measurements including prothrombin time, activated partial thromboplastin time, and the levels of plasma fibrinogen, von Willebrand factor antigen,

ristocetin cofactor activity, and factor VIII activity were normal. Bone marrow examination showed increase in micromegakaryocytes. Urine analysis and blood chemistry including kidney and liver function tests and lipid profile were normal. Family investigation indicated that two of her male siblings also had MTCP (Table 1). One of the siblings with MTCP also had delayed speaking and atypical face similar to the proband but no hearing loss, while the other sibling had only MTCP. Physical and laboratory examinations in the parents and two female siblings were normal. There was no family history of renal failure and/or progressive hearing loss. Renal ultrasonography and echocardiogram were normal in the proband, her siblings, and parents. Hearing tests were also normal except for the proband. Examination of platelets under electron microscopy showed that some platelets contained giant α -granules in the proband (Fig. 2). According to genotyping of intragenetic polymorphic sites of the *MYH9* gene in all of the family members, the affected children had no common *MYH9* haplotype (Fig. 3). Thus the MTCP phenotype in the family was not linked to the *MYH9* locus. Paternity was confirmed by examination of MCT118 and YNZ22 loci.

Chromosome analysis of peripheral leukocytes using the high-resolution binding technique showed a normal karyotype for all family members and fluorescence *in situ* hybridization of chromosome 22 at band q11.2, using the DiGeorge and velocardiofacial syndrome commercial TUPLE1 and N25 dual color locus specific probes (Cytocell, Cambridge, UK), demonstrated two hybridization signals without evidence of a microdeletion. We also carried out electronic microarray analysis (Nanogen, San Diego, CA, USA) to detect the nine common mutations (35delG, R184P, M34T, L90P, 167delT, 235delC, IVS1+1G>A, delE120, Q124X) for the *Connexin 26* gene (*GJB2*) and mitochondrial A1555G mutation in all of the family members. Although the father had no mutation, we detected the A1555G mutation in all three children. Although the mother had no mutation for *GJB2* gene, we could not obtain a definite result for mitochondrial mutation because of some technical restrictions. To test the partial deletion on chromosome 11q22.3–11q24.3 regions including the *FLII* gene locus, four microsatellite DNA markers (D11S1302, D11S411, D11S925, D11S912) residing between 100 and 131 Mb intervals were used. Site-specific polymerase chain reaction, 6–7% denaturing gel electrophoresis and silver

Correspondence: Ulker Kocak, MD, Gazi University School of Medicine, Department of Pediatrics, Pediatric Hematology Unit, Besevler, 06500, Ankara, Turkey. Email: ulkerkocak@gazi.edu.tr

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Fig. 1 Proband (middle) and one of her siblings (right) with dysmorphic face.

staining were used to genotype the patients. Haplotype analysis clearly showed that the entire region was intact in all family members (Fig. 4).

Discussion

We describe three children from one family (one female and two male) with MTCP and bleeding diathesis. MTCP was associated with giant α -granules in platelets, micromegakaryocytes, mental and growth retardation, facial dysmorphism and sensorineural deafness in the proband, with facial dysmorphism similar to proband in one of her male siblings, whereas it existed as an isolated abnormality in the other one. Absence of parental consanguinity and involvement of both sexes suggested autosomal dominant inheritance.

Inherited thrombocytopenia with giant platelets is the common characteristics of a number of rare disorders including Bernard-Soulier syndrome, *MYH9* disorders, velocardiofacial syndrome, Paris-Trousseau-Jacobsen (PTJ) syndrome, gray platelet syndrome, dyserythropoietic anemia with thrombocytopenia, and X-linked thrombocytopenia with thalassemia. The diagnosis of these disorders can be made on the characteristic cellular and somatic abnormalities and identification of underlying genetic defect.^{1,2}

The diagnosis of Bernard-Soulier syndrome, which is the most frequent form of congenital MTCP syndromes, could be excluded on normal platelet agglutination response to ristocetin. Furthermore, normal von Willebrand antigen and activity in the patients indicated that bleeding diathesis is not due to defects in the von Willebrand factor/glycoprotein Ib axis. Gray platelet

Table 1 Clinical and laboratory features of the family

	Gender	Bleeding Symptoms	Speaking disorder	Hearing test	Cataract	Face appearance	Nephritis	Platelet number $\times 10^9/L$	MPV (fl)	Platelet morphology	Bleeding time (min)
Father		Prolonged bleeding after tooth extraction	Stumble	Normal	Absent	Normal	Absent	236	8	Normal	7
Mother		Childhood epistaxis	Absent	Normal	Absent	Normal	Absent	296	8	20% giant	5
Sibling 1	Female	Absent	Absent	Normal	Absent	Normal	Absent	286	7.8	Normal	7
Sibling 2	Male	Epistaxis	Delayed speaking (age 6)	Normal	Absent	Normal	Absent	13	17.8	Giant	14
Sibling 3 (Proband)	Female	Epistaxis	Delayed speaking (age 7)	Sensorineural hearing loss	Absent	Narrow elongated	Absent	10	15.6	Giant	15
Sibling 4	Male	Epistaxis	Absent	Normal	Absent	Narrow elongated	Absent	14	17	Giant	14
Sibling 5	Female	Absent	Absent	Normal	Absent	Normal	Absent	212	7	Normal	6

MPV, mean platelet volume.

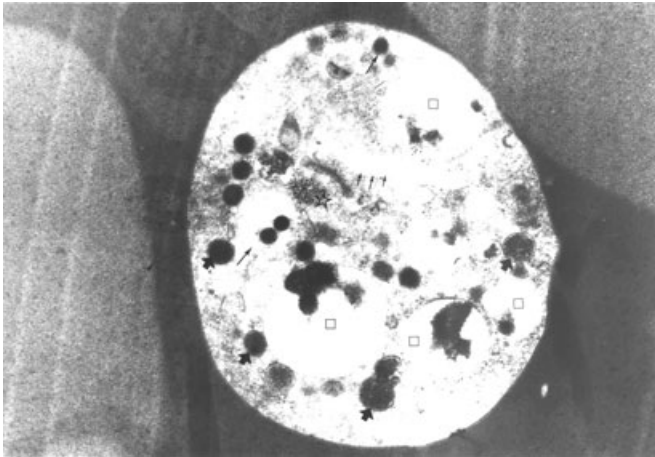


Fig. 2 Minimally enlarged α -granules (thick arrows) of platelets of the proband on electron microscopy (original magnification $\times 21\,000$). Thin arrows, δ -granules.

syndrome was excluded on normal platelet stainability and incompatible ultrastructural abnormalities.¹ None of the patients exhibited the red cell abnormalities seen in dyserythropoietic anemia with thrombocytopenia and X-linked thrombocytopenia with thalassemia.^{3,4}

Sensorineural deafness in association with MTCP is a strong indication for Fechtner or Epstein syndromes. These two disorders, together with May-Hegglin anomaly and Sebastian syndromes, are now called *MYH9* disorders because they are linked to mutations in the *MYH9* gene, which encodes non-muscle myosin heavy chain IIA.^{5,6} Döhle-like leukocyte inclusions, nephritis, sensorineural hearing loss and cataract are seen in different combinations in families with Fechtner and Epstein syndromes. Patients may develop nephritis or hearing loss later in life and can be designated as having May-Hegglin anomaly or Sebastian syndrome in childhood. Furthermore, mild involvement of kidney or ear may remain undetected. Therefore, the clinical spectrum of *MYH9* disorders may vary from a mild

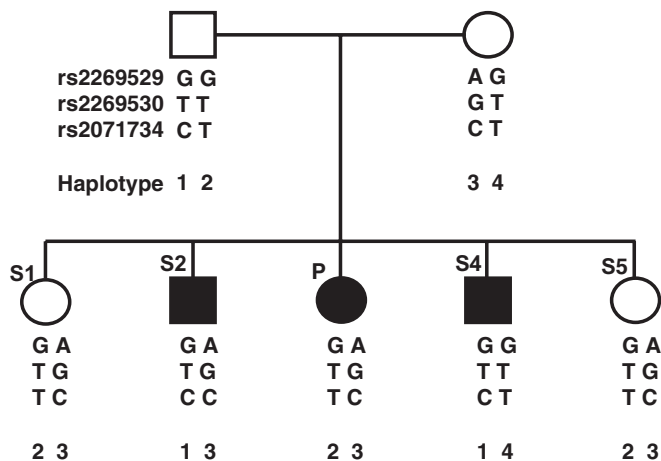


Fig. 3 Genotyping of intragenetic polymorphic sites of the *MYH9* gene in all the family members. (■, ●), affected individuals.

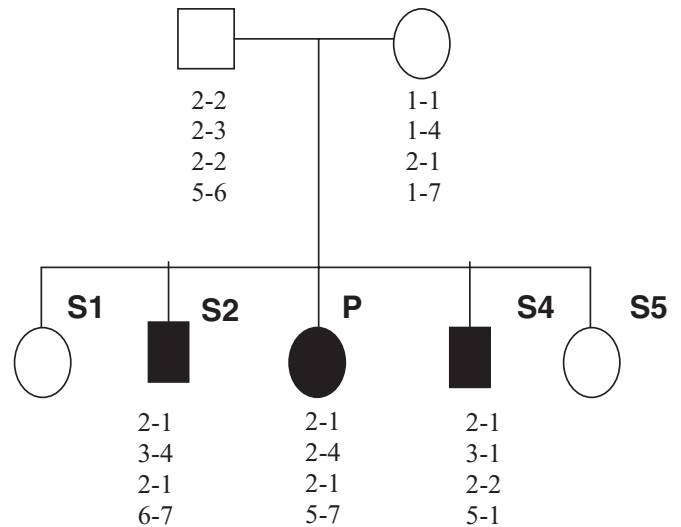


Fig. 4 Haplotypes of four microsatellite markers on chromosome 11q22.3-11q24.3 region. (■, ●), affected individuals. Order of DNA markers: D11S1302, 100.62 Mb; D11S4111, 108.59 Mb; D11S925, 118.47 Mb; D1S912, 131.26 Mb.

asymptomatic MTCP to progressive renal failure and/or hearing loss in association with thrombocytopenia. Severe bleeding tendency, which was reflected by markedly prolonged bleeding time (>15 min) in the present patient, is rarely encountered in *MYH9* disorders. Moreover, MTCP phenotype in the present family was not linked to the *MYH9* locus.

Association of MTCP with mental and growth retardation and facial dysmorphism in the proband is suggestive of a contiguous gene syndrome. Velocardiofacial syndrome is a contiguous gene syndrome, caused by hemizygous deletion of chromosome 22q11.2. The most prominent features include cleft palate, cardiac abnormalities, learning disabilities and facial dysmorphism characterized by prominent tubular nose, narrow palpebral fissures and slightly retruded mandible. Patients with this disorder are obligate carriers of Bernard-Soulier syndrome and have mild thrombocytopenia with large platelets.⁷ Analysis of the 22q11 region using locus-specific DNA probes did not indicate deletion in the present patient or her siblings. In addition, none had the major clinical signs of velocardiofacial syndrome.

Paris-Trousseau syndrome is a rare disorder characterized by mild bleeding tendency, MTCP with giant platelet α -granules, and a subpopulation of megakaryocytes with dyspoietic changes in bone marrow.⁸ It is caused by 11q23.3-q24.1 deletion, which contains the *FLII* gene, a transcription factor responsible for megakaryocyte maturation.⁹⁻¹⁴ PT syndrome is a variant of Jacobsen syndrome in which 11q23.2-qter deletion causes mental and growth retardation, trigonocephaly, facial dysmorphism and malformations of heart, kidney, intestinal system and lower extremities.¹⁵⁻²⁰ The clinical phenotype of previously reported patients with PTJ syndrome varies with the location of the breakpoint. Although giant α -granules in the platelets and bone marrow dysmegakaryopoiesis in the proband suggested PT syndrome, haplotype analysis clearly showed that the entire region

Table 2 Summary of published cases of 11q deletion disorder, including the present study

	Previous cases ^{8–20} with 11q deletion (n)	Present subjects (n)
No. subjects	128	3
Sex (M/F)	41/87	2/1
Clinical findings		
Dysmorphic face	53/54	2/3
Short stature	38/55	1/3
Mental/Psychomotor retardation	24/31	2/3
Hearing loss	4/9	1/3
Eye abnormality	26/37	0/3
Congenital heart defects	59/111	0/3
Renal abnormalities	8/107	0/3
Skeletal abnormalities	5/33	0/3
Endocrine abnormality	4/23	0/3
Intestinal abnormality	14/102	0/3
Hematologic findings		
Thrombocytopenia/pancytopenia	74/86	3/3
Bone marrow examination		
Dysmegakaryopoiesis	15/23	3/3
Micromegakaryocytes	15/23	3/3
Ultrastructural studies		
Giant α -granules in platelets	15/23	3/3
Cytogenetics		
11q deletion	128/128	0/3

between 11q23.3 and 11q24.3 was intact in all of the family members. In addition, neither the proband nor her siblings had typical craniofacial features or congenital abnormalities of this syndrome (Table 2), but we could not exclude the possibility of a point mutation in the *FLII* gene leading to impairment in the synthesis or function of FLII protein.

Sensorineural deafness was the most prominent finding in the proband. It rarely occurs in PTJ syndrome, probably due to the loss of *TECTA* gene locus at 11q23.3.¹³ Analyses of common genetic mutations causing hereditary deafness indicated the presence of mitochondrial *A1555G* mutation in all of the affected siblings, one with and two without hearing loss. This mutation accounts for nearly 5% of syndromic or non-syndromic hearing loss and has a variable expressivity among affected individuals.²¹ Its presence in the proband is probably a coincidence. To our knowledge, coexistence of deafness-associated *A1555G* mutation and hereditary MTCP syndromes has not been described previously.

There have been few reports of inherited thrombocytopenia and dysmegakaryopoiesis without 11q deletion. Olson *et al.* reported three related children with chronic thrombocytopenia, bone marrow micromegakaryocytes and normal karyotype in a consanguineous family.²² Tamary *et al.* described a family with an autosomal recessive thrombocytopenia associated with severe bleeding tendency, dysmegakaryopoiesis, neutropenia and mild dyserythropoietic anemia.²³ Platelet ultrastructure was normal and no mutations in transcription factor genes (*FLII*, *FOG-1*, *VNF-E2*, and *Gfi-1b*) affecting common myeloid progenitors were found in this family. MTCP in the present family differed

from these cases by mode of inheritance (probably autosomal dominant), very mild bleeding tendency and absence of dyserythropoiesis. We therefore believe that it may represent a new entity different from other inherited giant platelet disorders.

In a subset of patients with congenital thrombocytopenia who do not demonstrate characteristic clinical and laboratory features, bone marrow examination may be useful to detect dyspoietic changes in megakaryocytes. Further studies of genes regulating megakaryocyte maturation may clarify the causes of defective megakaryocyte maturation in patients without 11q deletion.

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