

Paris-Trousseau Syndrome Platelets in a Child With Jacobsen's Syndrome

L. Krishnamurti,^{1*} J.P. Neglia,¹ R. Nagarajan,¹ S.A. Berry,² J. Lohr,³ B. Hirsch,⁴ and J.G. White^{1,4}

¹Department of Pediatrics, Division of Hematology/Oncology/Blood and Marrow Transplantation, University of Minnesota Medical School, Minneapolis, Minnesota

²Department of Pediatrics, Division of Genetics and Metabolism, University of Minnesota Medical School, Minneapolis, Minnesota

³Department of Pediatrics, Division of Cardiology, University of Minnesota Medical School, Minneapolis, Minnesota

⁴Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota

The thrombocytopenia in an infant with clinical features of Jacobsen's syndrome characterized by multiple congenital anomalies, cardiac defects, psychomotor retardation, and deletion of chromosome 11 at 11q23.3 has been evaluated. Study of his platelets in the electron microscope revealed giant alpha granules in his cells identical in appearance to those reported in the family with Paris-Trousseau syndrome. As a result, the Paris-Trousseau syndrome appears to be a variant of the Jacobsen syndrome, and the thrombocytopenia observed in all cases of chromosome 11q23.3 deletion due to dysmegakaryopoieses. Giant alpha granules are frequently observed in normal platelets during long-term storage and may form in Jacobsen and Paris-Trousseau platelets during prolonged residence in the bone marrow. *Am. J. Hematol.* 66:295–299, 2001.

© 2001 Wiley-Liss, Inc.

Key words: thrombocytopenia; blood platelet disorders; chromosomes, human, pair 11; chromosome deletion; growth disorders; psychomotor disorders

INTRODUCTION

A novel genetic thrombocytopenia with platelet inclusion bodies, dysmegakaryopoiesis, mild congenital anomalies, and mental retardation associated with chromosome 11 deletion at 11q23 was recently reported [1–3]. The platelet inclusion bodies were found to be giant α granules present in 15% of the cells in peripheral blood. This condition had not been described previously, and as a result the authors termed it the Paris-Trousseau Syndrome.

The Jacobsen syndrome is also associated with deletion of chromosome 11 at q23.3 [4–10]. Typical anomalies include trigonocephaly, facial dysmorphism, cardiac defects, syndactyly, and psychomotor retardation, although none of these features is invariably present [4]. Approximately 47% of the patients with Jacobsen syndrome were found to be thrombocytopenic [1], but investigation of their platelets by electron microscopy has not been reported.

The present investigation has evaluated the fine structure of peripheral blood platelets from a child with char-

acteristic features of the Jacobsen syndrome. Results indicate that the child's platelets contain inclusions identical to those observed in Paris-Trousseau syndrome platelets.

CASE REPORT

A male baby was born at 35 weeks gestation to a primigravida. The infant was small for gestational age, weighing 1,880 g with an occipitofrontal circumference of 32.5 cm. He had a two-vessel umbilical cord, and the Apgar scores were 2 and 5 at 1 and 5 min, respectively. Severe respiratory distress syndrome ensued which required surfactant therapy and mechanical ventilation for 13 days. Examination at birth revealed multiple congen-

*Correspondence to: Lakshmanan Krishnamurti, M.D., Box 484 UMHC, 420 Delaware St. SE, Minneapolis, MN 55455. E-mail: krish010@tc.umn.edu

Received for publication 31 March 2000; Accepted 4 October 2000

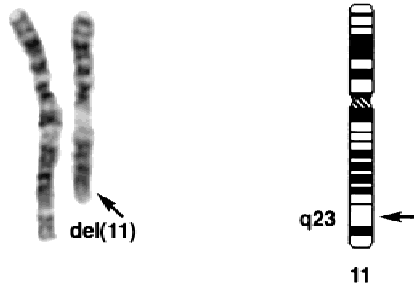


Fig. 1. Cytogenetic analysis of lymphocyte chromosomes from the patient described in this report. The distal long arm of chromosome 11 has a deletion extending from 11q23.3 to the 11q terminus.

ital anomalies. These included low-set ears, contractures of fingers of the left hand, overlapping toes bilaterally, spooned out toenails, bilateral colobomas, perimembranous VSD, and coarctation of aorta. There was no enlargement of the liver or spleen. Hemolytic disease of the newborn occurred on the first day of life due to ABO incompatibility between the mother and baby (mother's blood group was O, and baby's blood group was A). At birth the bilirubin was 4.8 mg/dl, Hgb 15.7 g/dl. At 4 hr of life, bilirubin was 5.9 mg/dl, Hgb 11.3 gm/dl, and direct antiglobulin test was positive. Double volume exchange transfusion was performed, and subsequently the hyperbilirubinemia resolved. The infant was persistently thrombocytopenic with platelet counts in the 30,000–50,000 range if unsupported by transfusions. Hemoglobin remained stable in the normal range for age after the neonatal period. White blood count was in the normal range for age with a normal differential count. Screening tests for coagulation were within the normal range. Peripheral blood smear examination revealed thrombocytopenia with normal red cell and white blood cell morphology. No abnormality in platelet morphology was detectable on light microscopy. Bone marrow examination was not performed because it was not required for diagnosis or treatment of the patient. Cytogenetic analysis of peripheral blood lymphocytes revealed a deletion of the distal long arm of one chromosome 11, extending from 11q23.3 to the 11q terminus (Fig. 1). Fluorescence in situ hybridization further showed that the mixed-lineage leukemia (MLL) locus (mapped to band 11q23) was proximal to the breakpoint and thus was not included in the deleted segment (data not shown). Since parental blood chromosome studies revealed normal karyotypes, this deletion in chromosome 11 apparently arose *de novo*.

The infant received several transfusions in the neonatal period. Over the next 6 months his platelet count remained stable at 50,000–60,000 without any transfusions. He did not have excessive bruising or hemorrhage. He successfully underwent repair of coarctation of the aorta at 6 months of life. He received platelet transfu-

sions perioperatively and did not have any bleeding problems during or after surgery.

Platelet Studies

Blood from the child was obtained after a 2-week interval during which he had not been transfused. The sample was mixed immediately with citrate/citric acid anticoagulant (9:1), sedimented at 200g at room temperature for 20 min. Platelet-rich plasma (PRP) was transferred to 5-ml tubes and placed in a 37°C water bath for 30 min.

Fixation was accomplished by combining 1-ml samples with an equal volume of 0.1% glutaraldehyde in White's saline [a 10% solution of a 1:1 mixture of (1) 2.4 mmol/l NaCl, 0.1 mmol/l KCL, 46 mmol/l MgSO₄, and 64 mmol/L Ca(NO₃)₂·4H₂O and (2) 0.13 mol/l NaHCO₃, 8.4 mmol/l NaH₂PO₄, and 0.1 g/l of phenol red, pH 7.4] [11,12]. After 15 min the samples were centrifuged to pellets and the supernatant fixative removed and replaced with 3% glutaraldehyde in the same buffer. The samples resuspended in the second aldehyde fixative were maintained at 4°C for 30 min and then sedimented to pellets. The supernatant was removed and replaced with 1% osmic acid in distilled water containing 1.5% potassium ferrocyanide for 1 hr at 4°C. All samples were dehydrated in a graded series of alcohols and embedded in Epon 812. Thin sections cut from the plastic blocks on an ultramicrotome were examined unstained or after staining with uranyl acetate and lead citrate to enhance the contrast. All examinations were made in a Phillips 301 electron microscope.

RESULTS

Thin sections of platelets from the patient with Jacobson's syndrome revealed cells of normal size and discoid configuration supported by circumferential coils of microtubules. Alpha granules, dense bodies, and mitochondria were normal in size, number, and distribution in most of the cells. However, giant alpha granules were present in about 15% of his platelets (Figs. 2, 3, and 4A–D). Contact sites between membranes of alpha granules resulting in fusion were evident in many examples. The appearance is identical to the giant alpha granules in Paris-Trousseau platelets [1,2] and in normal cells during long-term storage *in vitro* [13].

DISCUSSION

Paris-Trousseau syndrome was reported in a mother and child with a relatively mild phenotype, chronic thrombocytopenia, and deletion of 11q23.3 [1–3]. Both patients had prolonged bleeding time, and the mother had hemorrhagic manifestations associated with pregnancy. Platelet survival was within normal limits. Platelet ag-

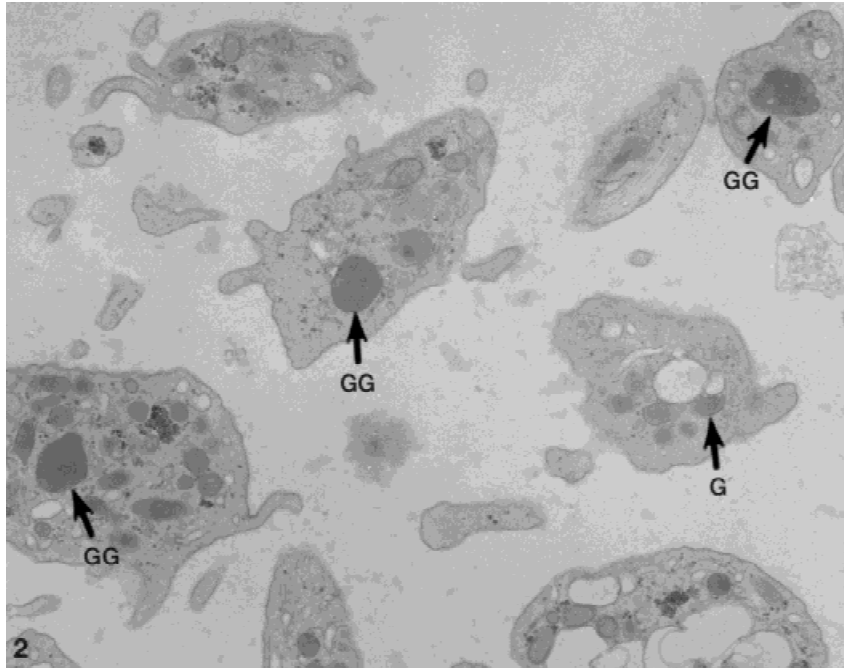


Fig. 2. Thin section of platelets from the patient with Jacobsen's syndrome. Most of the alpha granules (G) in the several platelets are of normal size. However, three platelets in the plane of section contain giant alpha granules (GG). Original magnification $\times 12,000$.

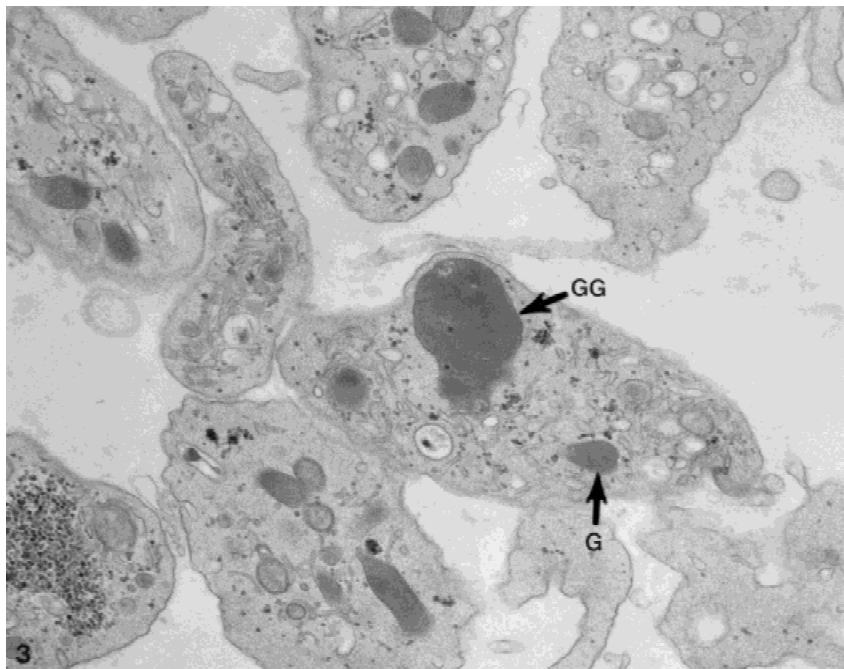


Fig. 3. Thin section of another platelet sample taken from the patient with Jacobsen's syndrome. Some of the giant granules (GG) are massive in size compared to normal alpha granules (G). Original magnification $\times 21,000$.

gregation studies indicated normal platelet function. Bone marrow examination revealed a marked increase in the number of megakaryocytes and the presence of many micromegakaryocytes with dystrophic maturation. Micromegakaryocytes are usually found in myelodysplastic syndromes and myeloproliferative disorders [14]. The findings were suggestive of a dysmegakaryocytopoietic state. Immunocytochemical and ultrastructural studies demonstrated that platelet inclusions were alpha granules that had arisen by a process of fusion [1].

Hematologic abnormalities are common in patients with Jacobsen's syndrome [4–10] who share the genetic defect of 11q23 deletion observed in patients with Paris-Trousseau syndrome [1–3]. Thrombocytopenia is twice as common as neutropenia or anemia and has been reported in 47% of the cases. Despite the frequent occurrence of thrombocytopenia, Jacobsen's syndrome platelets have apparently not been studied previously by transmission electron microscopy.

The present study has evaluated a child with charac-

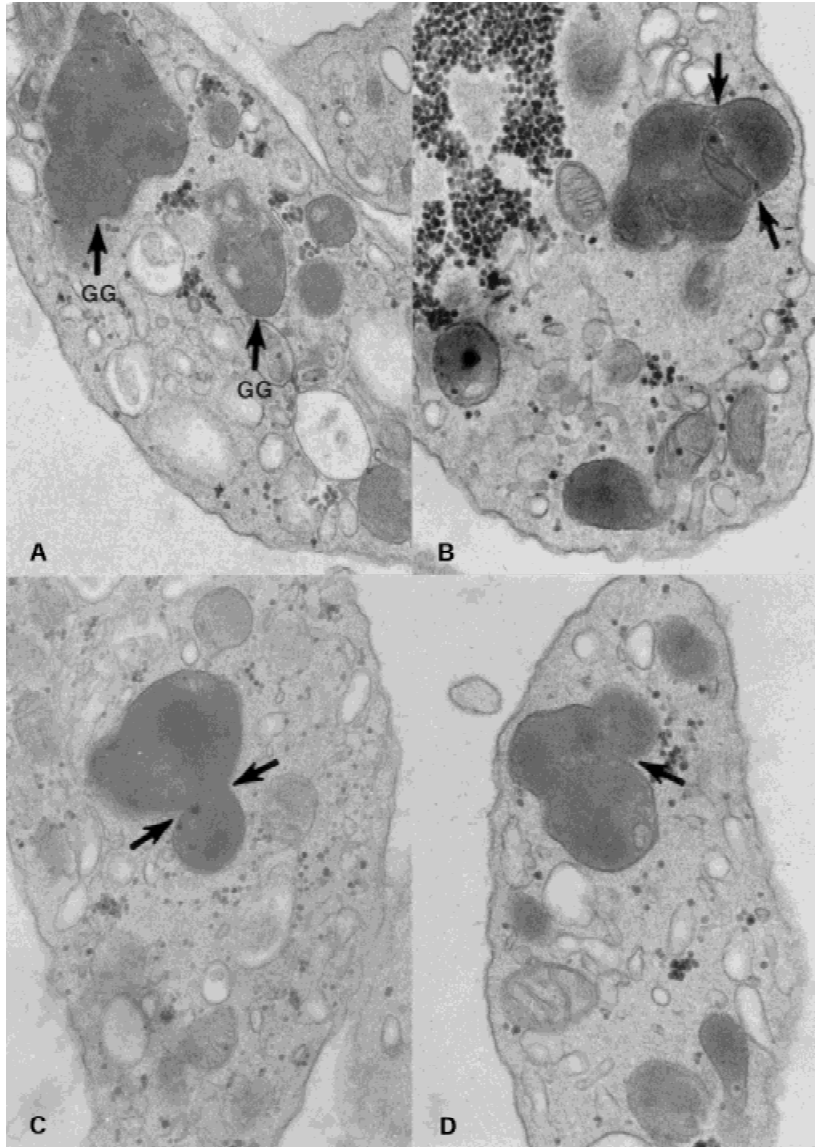


Fig. 4. Thin sections of several platelets from Jacobsen's syndrome patient revealing giant alpha granules (GG). (A) Two giant granules (GG) in the same cell. Arrows in (B–D) indicate possible lines of fusion between smaller alpha granules leading to formation of the giant organelles. Original magnification: $\times 30,000$ (A); $\times 40,000$ (B); $\times 30,000$ (C); $\times 35,000$ (D).

teristic features of the Jacobsen syndrome and deletion of chromosome 11 at 11q23.3. About 15% of his platelets contained giant alpha granules identical to those reported in the Paris-Trousseau syndrome [1]. The results suggest that Paris-Trousseau syndrome is a variant of the Jacobsen syndrome or the same disorder, and all patients with chromosome 11 deletion at 11q23.3 who have thrombocytopenia may have giant alpha granules in their platelets and dysmegakaryopoiesis.

Giant alpha granules are often observed in platelet samples from normal donors [15] and as a result are not considered a sign of pathology. However, giant alpha granules develop in a significant number of platelets exposed to overnight transport or long-term storage [13]. Their appearance is identical to the giant alpha granules of patients with Paris-Trousseau syndrome and, as shown in this study, Jacobsen's syndrome. The dysmegakaryopoiesis in the two syndromes may lead to delayed release

of platelets from bone marrow to peripheral blood, and prolonged presence in bone marrow may favor alpha granule fusion in the same manner as during in vitro storage. Further investigation of thrombocytopenia in Jacobsen's syndrome may reveal molecular mechanisms regulating the release of platelets from bone marrow [3].

REFERENCES

1. Breton-Gorius J, Favier R, Guichard J, Cherif D, Berger R, Debili N, Vainchenker W, Douay L. A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha granules and chromosome 11 deletion at 11q23. *Blood* 1995;85:1805–1814.
2. Favier R, Douay L, Esteva B, Partnoi MF, Gaulard P, Lecompte T, Perot C, Adam M, Lecrubier C, Van den Akker J, Lasfurgues G, Najean Y, Breton-Gorius J. A novel genetic thrombocytopenia (Paris-Trousseau) associated with platelet inclusions, dysmegakaryopoiesis and chromosome deletion at 11q23. *CR Acad Sci Paris* 1993;316:698–701.

3. Favier R. Paris-Trousseau thrombocytopenia: a new entity and a model for understanding megakaryocytopoiesis (editorial). *Pathol Biol (Paris)* 1997;45:693–696.
4. Jacobsen P, Hauge M, Henningsen K, Hobolth N, Mikkelsen M, Philip J. An (11;21) translocation in four generations with chromosome 11 abnormalities in the offspring. A clinical, cytogenetical, and gene marker study. *Hum Hered* 1973;23:568–585.
5. Michaelis RC, Velagaleti GV, Jones C, Pivnick EK, Phelan MC, Boyd E, Tarleton J, Wilroy RS, Tunnacliffe A, Tharapel AT. Most Jacobsen syndrome deletion breakpoints occur distal to FRA11B. *Am J Med Genet* 1998;76:222–228.
6. Penny LA, Dell'Aquila M, Jones MC, Bergoffen J, Cunniff C, Fryns JP, Grace E, Graham JM Jr, Kousseff B, Mattina T, et al. Clinical and molecular characterization of patients with distal 11q deletions. *Am J Hum Genet* 1995;56:676–683.
7. Pivnick EK, Velagaleti GV, Wilroy RS, Smith ME, Rose SR, Tipton RE, Tharapel AT. Jacobsen syndrome: report of a patient with severe eye anomalies, growth hormone deficiency, and hypothyroidism associated with deletion 11 (q23q25) and review of 52 cases. *J Med Genet* 1996;33:772–778.
8. Lewanda AF, Morsey S, Reid CS, Jabs EW. Two craniosynostotic patients with 11q deletions, and review of 48 cases. *Am J Med Genet* 1995;59:193–198.
9. Fryns JP, Kleczkowska A, Buttiens M, Marien P, van den Berghe H. Distal 11q monosomy: the typical 11q monosomy syndrome is due to deletion of subband 11q24.1. *Clin Genet* 1986;30:255–260.
10. Jones C, Slijepcevic P, Marsh S, Baker E, Langdon WY, Richards RI, Tunnacliffe A. Physical linkage of the fragile site FRA11B and a Jacobsen syndrome chromosome deletion breakpoint in 11q23.3. *Hum Mol Genet* 1994;3:2123–2130.
11. White JG. The morphology of platelet function. In: Harker LA, Zimmerman TS, editors. *Methods in hematology series 8: measurements of platelet function*. New York: Churchill Livingstone; 1983. p 1–25.
12. White JG. Fine structural alterations induced in platelets by adenosine diphosphate. *Blood* 1968;31:604–622.
13. White JG. Ultrastructural changes in stored platelets. *Blood Cells* 1992;18:461–475.
14. Breton-Gorius J. Megakaryocyte maturation and platelet release in normal and pathologic conditions. In: Harris JR, editors. *Blood cell biochemistry, megakaryocytes, platelets, macrophages and eosinophils*. New York: Plenum; 1991. p 1–25.
15. White JG. Platelet morphology. In: Johnson SE, editor. *The circulating platelet*. New York: Academic Press; 1971. p 45–121.