

Clinical Report

Giant Platelets in a Case of Deletion 11q24-qter Confirmed by Fluorescence In Situ Hybridization

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Here we report the association of giant platelets and an increase in platelet volume in a 19-month-old black female with *de novo* del 11q24-qter. The deletion, which was visible on karyotype, was further confirmed and more precisely localized by fluorescence in situ hybridization studies (FISH) that showed the deletion to lie distal to the *MLL* gene region (11q23). Clinically, the case presented less severe symptoms than Jacobsen syndrome—the well known partial deletion of the distal end of chromosome 11. Platelet glycoproteins CD 41, CD 42a, C 42b, CD 61, and PAC-1 were also assayed and found to be normally expressed. To our knowledge, giant platelets are described for the first time in the relevant deleted region.

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KEY WORDS: platelets; deletion chromosome 11q; fluorescence in situ hybridization

INTRODUCTION

Partial deletion of the distal end of chromosome 11, also known as Jacobsen syndrome, is a well-recognized clinical entity [Jacobsen et al., 1973; Fryns et al., 1987; Obregon et al., 1992; Leegte et al., 1999]. Currently, several cases have been reported in which variable segments of 11q were deleted. In most instances the

deletion involves bands distal to 11q23. Few reports refer to interstitial deletions [Taillemite et al., 1975; Guc-Scekic et al., 1989; Wakazono et al., 1992]. The patients present postnatal growth retardation, mild psychomotor retardation, micro-trigonocephaly, strabismus, telecanthus, hypertelorism, antimongoloid slant, broad depressed nasal bridge, hand-foot abnormalities, and cardiac defects. Thrombocytopenia or pancytopenia has been detected in about half of the patients regardless of the extension of the deletion [O'Hare et al., 1984; Breton-Gorius et al., 1995; Gangarossa et al., 1996; Leegte et al., 1999]. Few reports mention thrombocyte morphology [Gangarossa et al., 1996]. To our knowledge, there have been no reports on the existence of giant platelets and on the characterization of platelet glycoproteins in these patients. Here we report on a patient with deletion 11q24-qter and discuss the clinical manifestations and results of thrombocyte studies with respect to the deleted region.

CLINICAL REPORT

The proband was a 19-month-old black female, the product of the seventh pregnancy of non-consanguineous parents. The mother and father were 35 and 39 years old, respectively. All the other sibs were alive and healthy. The patient was born at term after an uneventful pregnancy. At birth she weighed 3,000 g; the height and head circumference were not known. At the time of the referral, when she was eight months old, she presented severe hypotonia and was unable to hold her head still and to sit. She had a relatively large head (head circumference: 46 cm + 2 SD), dolicocephaly, a flat profile, hypertelorism, broad and depressed nasal bridge, small nose, long philtrum, and poorly formed low-set ears (Fig. 1). She also had a mild umbilical hernia and some pigment abnormalities on the right dorso-lateral skin area (Fig. 2). Her hands were normal but the digits of both feet showed malpositioning. There was syndactyly between the second and third fingers. When re-examined at 19 months, she could still not sit unsupported and had no speech.

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Fig. 1. Note the relatively large head with hypertelorism, depressed nasal bridge, small nose, long philtrum, and low set malformed ears.



Fig. 2. Pigment abnormalities on the trunk.

LABORATORY INVESTIGATIONS AND RESULTS

Cytogenetic Analysis

Chromosome studies were performed on whole peripheral blood. Thymidine was used for synchronization of the cells. The karyotype was described according to ISCN [Mitelman F, 1995]. High resolution chromosome analysis showed 46,XX,del(11)(q24-qter) (Fig. 3). The karyotypes of the parents were normal.

Fluorescence In Situ Hybridization (FISH) Analysis

FISH analysis was performed by region-specific Vysis *MLL* probe (Vysis Inc., Downers Grove, IL) in association with a chromosome 11-specific centromeric probe (Vysis CEP 11). The procedures were performed according to manufacturer's protocol. FISH results showed that the *MLL* gene locus (11q23) was retained on both chromosomes 11 (Fig. 4a). Sequential FISH analysis with the telomere probe (Cytocell D11S4974) and chromosome 11-specific centromere probe showed absence of the telomere hybridization signal on the deleted chromosome 11 (Fig. 4b).

Complete Blood Count

Complete blood count showed white blood cell count $12.3 \times 10^9/L$, hemoglobin value 10.6 g/dL, mean corpuscular volume (MCV) 84.1 fL, mean corpuscular hemoglobin (MCH) 26.4 pg, and a platelet count of $140 \times 10^9/L$. Peripheral blood smear showed that the thrombocyte size was approximately the diameter of an erythrocyte; so-called giant platelets existed (Fig. 5). Mean platelet volume was also increased to 12.8 fL (normal: 7–9.5 fL).

Characterization of Platelet Glycoprotein

Flow cytometric (FCM) analysis of the patient's platelets was performed on fixed platelets. Whole blood was fixed with 1% paraformaldehyde in phosphate buffered saline (PBS) immediately after drawing. The patient's platelets and control platelets were sampled the same way: they were washed and stained with monoclonal antibodies directed against GPIbV (CD 61 and CD 41) and against GPIbIIIa (PAC-1 Becton Dickinson, Franklin Lakes, NJ). The FCM analysis was performed on two different samples. We observed

no difference between the expression of patient's platelet glycoprotein and the control. Moreover, the glycoprotein expression was equivalent to our reference group composed of 10 normal healthy people.

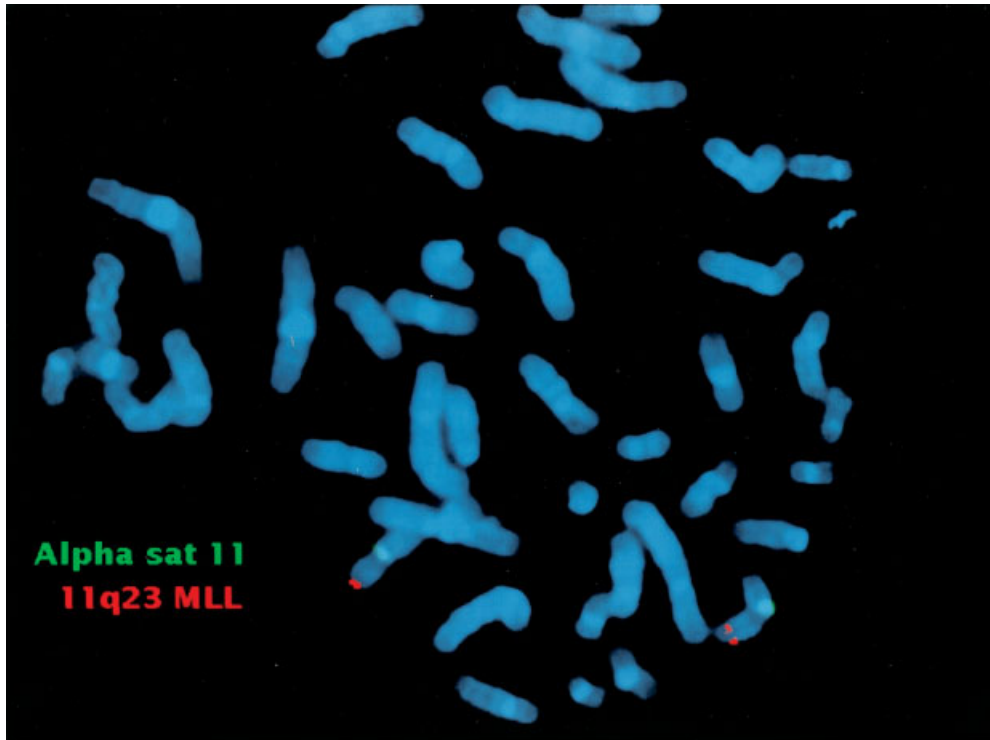
DISCUSSION

There is a broad phenotypic spectrum in patients with terminal deletions of chromosome 11q, the so-called Jacobsen syndrome [Pivnick et al., 1996]. Commonly observed features include psychomotor retardation, trigonocephaly, craniofacial anomalies, cardiac defects, hand-foot anomalies, and thrombocytopenia (Table I). There is controversy about the band responsible for the full expression of the syndrome. O'Hare et al. [1984] reported that the deletion of sub-band 11q24.1 was crucial for the typical features. Fryns et al. [1986] described a male patient with deletion of 11q24.2-qter who did not manifest the characteristic features of Jacobsen syndrome. Ono et al. [1996] reported 10 Japanese children with partial deletion of the long arm of chromosome 11 and suggested that the group with terminal deletion of 11q, including sub-band q24.1, presented typical features of Jacobsen syndrome. In Ono et al.'s study, one patient who presented deletion of 11q24.2-qter lacked mental retardation, and showed only congenital heart defect, failure to thrive, and recurrent infections [Ono et al., 1996]. In all these reports, the deletion breakpoint relied on karyotype analysis.

The present case had severe psychomotor retardation but lacked apparent trigonocephaly and there was relatively subtle dysmorphic features. Ocular abnormalities and congenital heart defect were not detected. Trigonocephaly is reported in 95% of deletion 11q patients. In the paper by Penny et al. [1995], a gene influencing calvarial suture closure is suggested to lie between D11S1351 and D11S912, a region probably corresponding to distal 11q23.3 and possibly proximal 11q24.1. In the study by Ono et al. [1996], trigonocephaly was seen in two patients, one with a deletion at 11q22.2 and the other distal to 11q23.2 (case 5 and 6). In 1996, Pivnick et al. [1996] reported a patient with trigonocephaly and notable dysmorphic features: extremely widely-spaced eyes (severe hypertelorism), bilateral inferior colobomas of the irides extending to the choroid and retina, and a carp-shaped mouth. She also had growth retardation, central nervous system



Fig. 3. Partial karyotype showing del 11q.



(a)



(b)

Fig. 4. **a:** FISH analysis showing signals for the locus-specific probe MLL (11q23) on both chromosomes 11. **b:** FISH analysis showing absence of signal for 11q telomeric probe (D11S4974) on the deleted chromosome 11.

abnormalities, growth hormone deficiency, and central hypothyroidism. In this patient, the deletion breakpoint was localized to q24.1 by FISH studies. All these data, including ours, suggest that a gene responsible for at least coronal and lambdoid suture closures lies

within or proximal to 11q24.1 region, and severe facial anomalies and ocular abnormalities are mostly seen in cases with deletions within/proximal to 11q24.1. It was difficult to explain the presence of the pigment abnormalities in our case. They presented as hypo- and

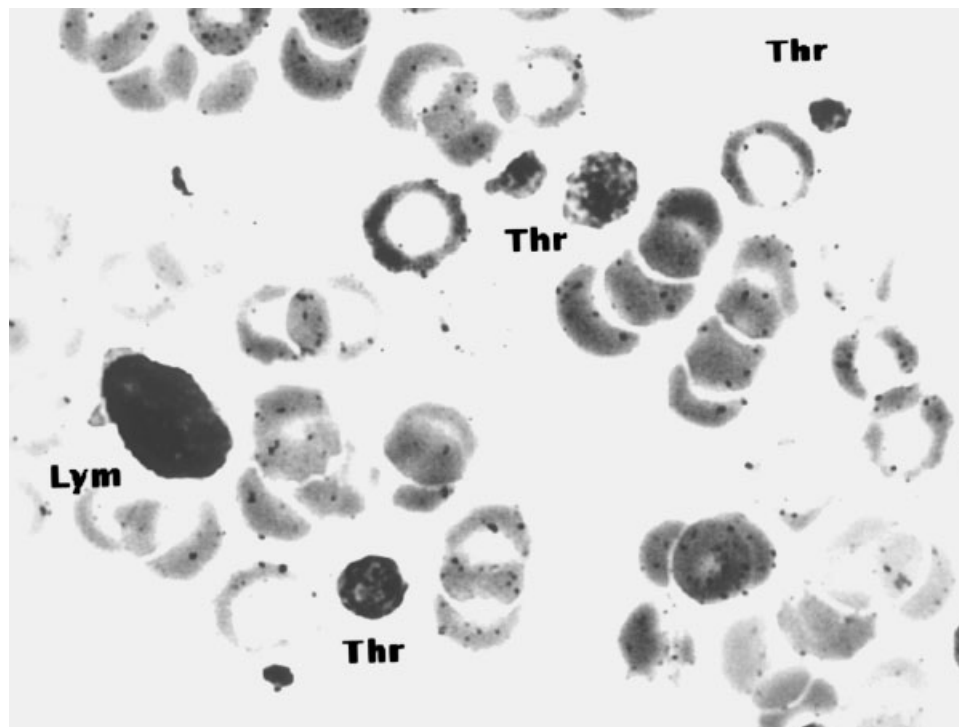


Fig. 5. Giant platelets in the peripheral blood smear.

hyperpigmentation in lateral-lumbar skin regions. Such a feature, to our knowledge, is never mentioned before in Jacobsen syndrome (Table I).

Thrombocytopenia or pancytopenia is observed in almost half of the 11q-syndromes. They are mostly mentioned in deletions relevant to 11q23 region but also to 11q24. In the paper by Breton-Gorius et al. [1995], a new congenital dysmegakaryopoietic thrombocytopenia associated with giant platelet alpha granules and chromosome 11q23 deletion have been mentioned. Gangarossa et al. [1996] reported chronic

thrombocytopenic purpura and micromegakaryocytes in a patient with del 11q24.2-qter. Penny et al. [1995] suggested that the gene(s) responsible may lie distal to D11S1351. Our case presented a very mild thrombocytopenia; however, giant platelets and an increased platelet volume were obvious. As there were no other complaints (e.g., bleeding symptoms), no neutrophil inclusions were observed, and the relevant glycoprotein expressions were normal, many giant thrombocyte diseases such as May-Hegglin anomaly and Bernard-Soulier syndrome were not considered [Mhawech and Saleem, 2000]. It is difficult at this stage to judge whether this finding of giant platelets is associated with the del 11q24-qter or if it is just a coincidence; however, due to the data in the literature about the association of thrombocyte quantification and morphology, we suggest this is related to the deletion. Furthermore, due to the breakpoint of the deletion in our case, we suggest that, if any, a gene(s) responsible for thrombocyte differentiation lies within or distal to 11q24. Fli-1 gene can be a candidate gene as in a recent paper by Hart et al. [2000] hemizygous loss of Fli-1, which lies within chromosome 11q24 region, is suggested to be responsible for dysmegakaryopoiesis.

The present case is one of the few literature reports defining the deletion in the long arm of chromosome 11 by FISH techniques. Although no further FISH analyses other than those mentioned above could be realized, a more precise definition of the breakpoint is presumed to lie within 11q24.1 due to clinical and hematological findings. The case is unique in the sense that giant platelets and increase in platelet volume are

TABLE I. Clinical Manifestations of Jacobsen Syndrome, Comparison with Present Case

	Del 11q23	Our case
Growth retardation	+	-
Psychomotor retardation	+	+
Trigonocephaly	+	Macrocephaly
Strabismus	+	-
Telecanthus	+	+
Hypertelorism	+	+
Coloboma of irides	+	-
Antimongoloid slant	+	-
Depressed nasal bridge	+	+
Cardiac abnormalities	+	-
Low-set malformed ears	+	+
Pigment abnormalities	-	+
Hand abnormalities	+	-
Foot abnormalities	+	+
Thrombocytopenia	+	Mild
Micromegakaryocytes	One case ^a	-
Giant platelets	?	Present case

^aGangarossa et al. [1996].

described for the first time in the relevant deleted region. Further studies with respect to thrombocyte morphology and differentiation in distal 11q deletion are needed to clarify this issue.

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