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Molecular Characterization of an 11q Interstitial Deletion in a Patient With the Clinical Features of Jacobsen Syndrome

Sharon L. Wenger,[1]* Paul D. Grossfeld,[3] Benjamin L. Siu,[2] James E. Coad,[1] Frank G. Keller,[2] and Marybeth Hummel[2]

[1]Department of Pathology, West Virginia University School of Medicine, Morgantown, West Virginia
[2]Department of Pediatrics, West Virginia University School of Medicine, Morgantown, West Virginia
[3]Department of Pediatrics, University of California, San Diego, California

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The 11q terminal deletion disorder or Jacobsen syndrome is a contiguous gene disorder. It is characterized by psychomotor retardation, cardiac defects, blood dyscrasias (Paris-Trousseau syndrome) and craniofacial anomalies. We report on a female patient with an approximately 10 Mb interstitial deletion with many of the features of Jacobsen syndrome: A congenital heart defect, dysmorphic features, developmental delay, and Paris-Trousseau syndrome. The karyotype of the patient is 46,XX,del(11)(q24.1q24.3). The interstitial deletion was confirmed using FISH probes for distal 11q, and the breakpoints were characterized by microarray analysis. This is the first molecularly characterized interstitial deletion in a patient with the clinical features of Jacobsen syndrome. The deletion includes *FLI-1*, but not *JAM-3*, which will help to determine the critical genes involved in this syndrome.

Key words: Jacobsen syndrome; contiguous gene syndrome; congenital thrombocytopenia; Paris-Trousseau

INTRODUCTION

Jacobsen syndrome is a contiguous gene disorder caused by a deletion in the long (q) arm of chromosome 11. Common clinical findings include thrombocytopenia, developmental delay, congenital heart disease, and short stature. Facial dysmorphology includes hypertelorism, ptosis, down slanting palpebral fissures, broad nasal bridge with short nose, thin upper lip and low-set malformed ears [Grossfeld et al., 2004]. The incidence of distal 11q deletions is estimated to be 1 in 100,000 [Penny et al., 1995]. Most cases arise from a de novo deletion or rarely as an unbalanced segregation from a balanced carrier. An early report suggested that the breakpoint was at the rare 11q23.3 fragile site (FRA11B) [Voullaire et al., 1987]. However, about 25% of cases occur as a result of a parent with the FRA11B fragile site. Molecular studies have demonstrated that the deletions range in size from ~7 Mb to greater than 20 Mb [Penny et al., 1995; Grossfeld et al., 2004]. To date, all cases analyzed using molecular techniques have demonstrated the presence of a terminal deletion, with retention only of telomeric sequences at the end of 11q [Grossfeld et al., 2004].

In this report we describe a patient with the clinical features of Jacobsen syndrome and demonstrate that she has an interstitial deletion. Molecular characterization of the deletion breakpoints should aid in the identification of disease-causing genes in 11q-.

CLINICAL REPORT

A female was born at 34 weeks gestation to a 29-year-old female delivered by Cesarian secondary to placental abruption. The patient presented at birth with a cardiac murmur, thrombocytopenia (37,000 platelets) and facial anomalies. An echocardiogram demonstrated double outlet right ventricle with normally related great arteries, a secundum atrial septal defect, a patent ductus arteriosus, and mild branch pulmonary artery stenosis. She underwent successful surgical repair of her heart defect at age of 2 months. A karyotype on a peripheral blood sample was reported as normal female, and blood was sent for Fanconi anemia testing, which was normal. At 3 months of age the patient was <5th

Sharon L. Wenger and Paul D. Grossfeld, these authors contributed equally to the manuscript.

*Correspondence to: Sharon L. Wenger, Ph.D., Department of Pathology, West Virginia University, P. O. Box 9203, Morgantown, WV 26506-9203.

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centile for height and weight and showed some developmental delay. She also had persistent thrombocytopenia, with a platelet count of 55,000 at 3 months of age. The platelets appeared normal in the peripheral blood. She has a tendency to have excessive bleeding, although to date platelet function studies have not been performed. A bone marrow biopsy showed atypical megakaryocytes (Fig. 1), consistent with those observed in Paris-Trousseau syndrome. Upon reevaluation of the karyotype at this time, a small 11q deletion was identified. At 1 year of age the patient had surgery for exotropia and ptosis for both eyes. At 2 years of age she is developmentally at the 10–12 months level, just pulling to a stand and no expressive language. Dysmorphic facial features include down-slanted palpebral fissures, ptosis, hypertelorism with broad nasal bridge, low posterior ears, micrognathia, mildly flattened philtrum, flat occiput, and small mouth (Fig. 2).

MATERIALS AND METHODS

Peripheral blood samples were processed using routine cytogenetic techniques and analyzed using G-banded chromosomes. Blood samples in the parents were cultured for 72 hr in 199 medium containing 5% dialyzed fetal bovine serum with the addition of 0.025 uM fluorodeoxyuridine during the last 24 hr of culture for fragile site induction. One hundred metaphase cells were evaluated for chromosome breakage.

The 11q subtelomere DNA probe (Vysis) was evaluated in metaphase cells using FISH techniques according to manufacturer's instructions. Human BAC clones spanning the distal 20 Mb region in 11q were used for FISH mapping as described previously [Grossfeld et al., 2004]. A genome-wide microarray analysis was performed by Spectral Genomics (Houston, TX).

RESULTS

Cytogenetic and Molecular Analysis

Chromosome analysis on the patient identified an 11q deletion (Fig. 3), which was determined by cytogenetics, FISH probes and microarray results to be 46,XX,del(11)(q24.1q24.3). FISH analysis using subtelomeric probes in distal 11q confirmed the interstitial deletion (Fig. 4, Table I). Parental karyotypes were normal and neither parent had a fragile site at 11q23.

To further delineate the boundaries of the deletion, a genome-wide microarray analysis was performed on the patient's genomic DNA (Spectral Genomics). The proximal breakpoint boundary was determined to be between BAC clones 344F5 and 164B14, (corresponding to positions 120.9 and 121.5), and the distal breakpoint boundaries between BAC clones 354O3 and 26N8 (corresponding to positions 129.7 and 130.6) (Table II). Thus, the deletion spans an approximately 9.7 Mb region.

DISCUSSION

Jacobsen syndrome is a rare cytogenetic disorder that has a well-characterized phenotype. The deletions are variable in size, ranging from as small as ~7 Mb to greater than 20 Mb [Penny et al., 1995].

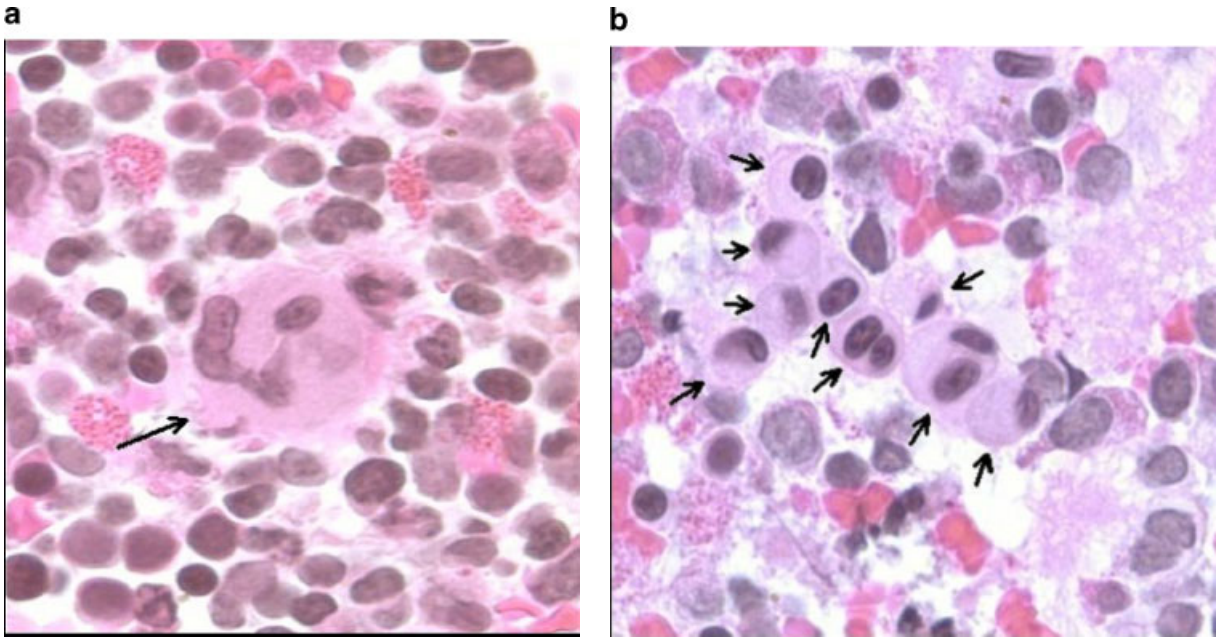

FIG. 1. Hematoxylin and eosin stain of the bone marrow aspirate showing (**a**) a normal megakaryocyte, and (**b**) a cluster of atypical megakaryocytes.

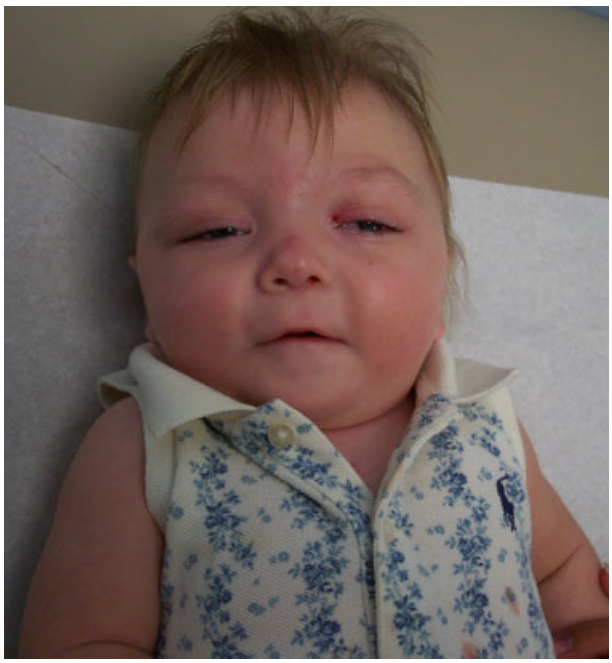

FIG. 2. Facial dysmorphology of patient at 2 years of age showing hypertelorism, flat bridge of the nose, down-slanted palpebral fissures, ptosis, flattened philtrum and small mouth.

Previous studies have demonstrated that the deletion breakpoints cluster around CCG repeat sequences. About one fourth of the patients have the largest size deletion, whose breakpoint is at the FRA11B fragile site. Jones and colleagues [1995, 2000] have demonstrated that this deletion arises as a result of a fragile site caused by CCG repeat expansion. The remaining smaller deletions are not associated with a fragile site [Grossfeld et al., 2004], and thus are likely to arise from a different mechanism.

Prior to the current report, all previous patients with Jacobsen syndrome who have been characterized by molecular techniques did not demonstrate the presence of an interstitial deletion, and were consistent with a terminal deletion. One case with an interstitial deletion 11q23.3q24.2 by karyotype analysis alone was due to recombination of an inherited intrachromosomal insertion. The patient had facial manifestations similar to that seen in Jacobsen syndrome, although he had no heart defect and there was no mention of thrombocytopenia [Forsythe et al., 1988]. Several other patients with interstitial deletions in 11q whose distal breakpoint was reported by karyotype to be in 11q23.3 were determined, by FISH, not to overlap the Jacobsen syndrome region [Grossfeld, unpublished results]. Although one report described a patient with Jacobsen syndrome with a possible interstitial deletion, telomeric rather than a subtelomeric probe was used to map the deletion [Pivnick et al., 1996]. Previous studies have demonstrated that all "terminal" deletions contain telomeric sequences, which are essential for stable replication and transmission of the deleted chromosome [Jones et al., 1994]. Thus, that patient was likely to have a terminal deletion. In the present study, we used subtelomeric probes that demonstrated unequivocally the presence of an interstitial deletion, which was confirmed subsequently by microarray analysis.

FIG. 3. Partial karyotypes of chromosome 11 demonstrating the interstitial deletion. The abnormal chromosome is on the right in each pair.

The identification of an interstitial deletion in a patient with 11q- is important to be able to further localize disease-causing genes in this region. The clinical phenotype in 11q- is variable. For some phenotypes, such as developmental delay, previous studies have demonstrated a strong correlation between deletion size and severity of phenotype. However, for other phenotypes, such as congenital heart disease, the genotype/phenotype relationship is less clear.

Over half of 11q- patients have a congenital heart defect. One-third of these patients have so-called "flow" defects, which include left-sided obstructive lesions and isolated ventricular septal defects. The other two-thirds of patients described have a wide spectrum of defects, including double outlet right ventricle that our patient has. One possibility is that there is a single gene responsible for all of the congenital heart defects in 11q-. A second possibility is that there is more than one causative gene, and that each gene may contribute to a subset of the spectrum of heart defects that occur in 11q-.

Phillips et al. [2002] used molecular techniques to define a 7 Mb "critical" region for at least a subset of cardiac defects in 11q, based on mapping three patients with heart defects: Two with hypoplastic left heart syndrome and one with an isolated ventricular septal defect. Grossfeld et al. [2004] identified the same cardiac "critical region" by mapping 35 11q- patients with heart defects. Phillips et al. [2002] proposed JAM-3, a cardiac junction adhesion molecule located in the cardiac critical region, to be a candidate gene for causing heart defects in 11q-. The distal breakpoint of our patient's deletion was at least 1.4 Mb centromeric to *JAM-3*. There are at least three possible explanations for this observation. First, the region deleted in these patients shares a region of

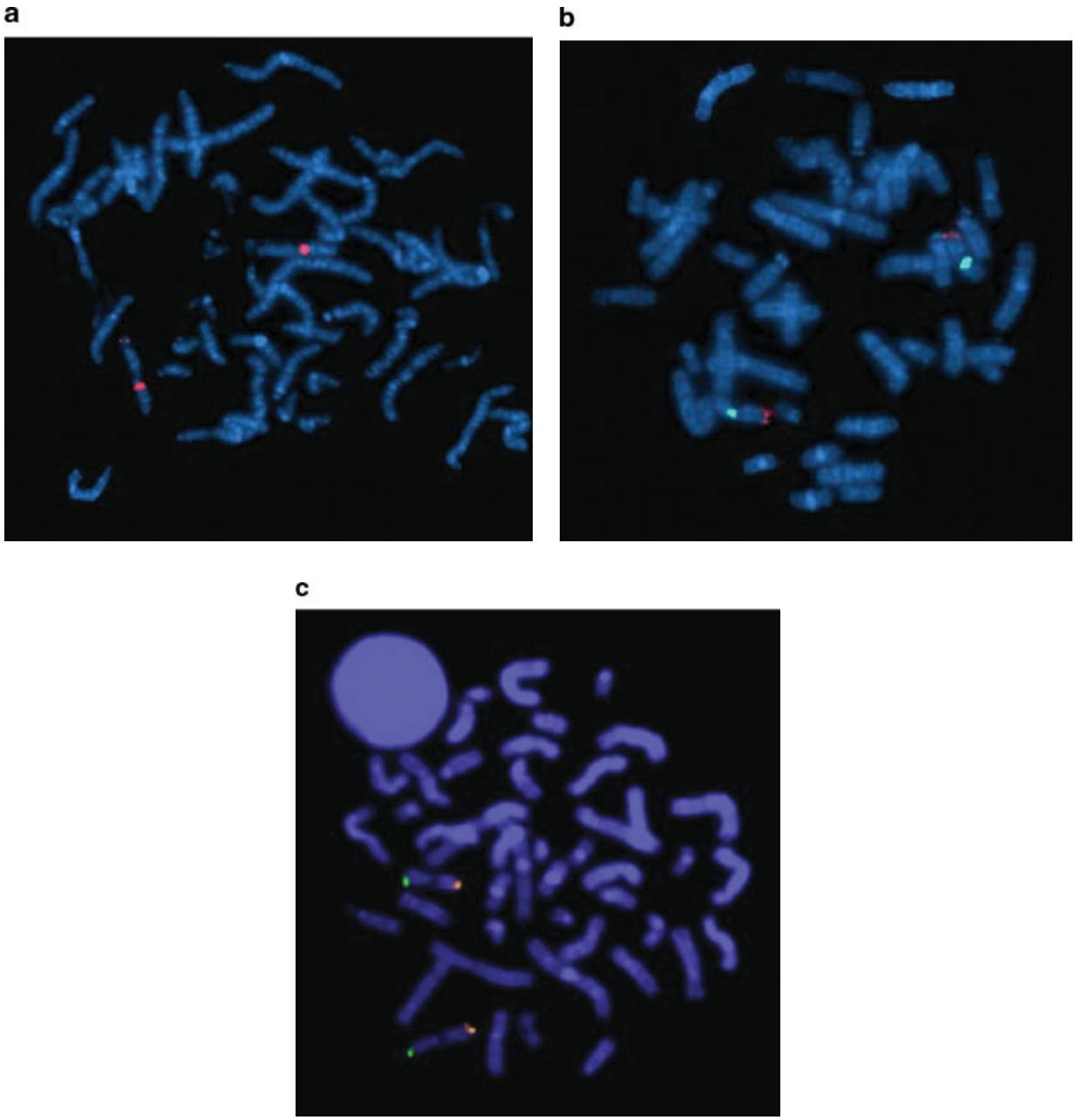

FIG. 4. (**a**) FISH probe F0751 (D11S1351) absent on one chromosome, (**b**) FISH probe 217L21 present on both chromosomes near the telomere, (**c**) FISH probe for 11q subtelomere present on both chromosomes.

overlap with the previously defined cardiac critical region that is ~6 Mb, containing ~25 known genes. Thus, a causative gene (or genes) may be within this region, which would exclude *JAM-3*. An alternative model would invoke more than one causative gene for heart defects in 11q-. In the present patient, a causative gene (or genes) would be more centromerically located, and responsible for causing conotruncal heart defects such as double outlet right ventricle. A more distally located gene, which would include *JAM-3*, could cause the flow defects including ventricular septal defects and left-sided obstructive lesions. Thirdly, the deletion could affect the expression of *JAM-3*, even though the coding region of the gene was not deleted. Future studies involving gene-targeted knockouts

TABLE I. FISH Mapping Results

Clone	Map Position (Mb)	Result
133H4(D11S577)	119.0	Retained
F0751(D11S1351)	127.2	Deleted
FLI-1	127.7	Deleted
217L21	130.3	Retained

TABLE II. Microarray Results of Distal 11q Clones*

Clone	Cytogenetic pos.	Map pos.	Result
RP11-344F5	11q24.1a-11q24.1b	120.9	N
RP11-164B14	11q24-11q24	121.5	D
RP11-87O12	11q23-11q24	122.4	D
RP11-11C15	11q23.2-11q24.1	122.7	D
RP11-164A10	11q24-11q24	123.4	D
RP11-10N17	11q24.2a-11q24.2b	124.2	D
RP11-50B3	11q24-11q24	125.1	D
RP11-20M1	11q24-11q24	125.5	D
RP11-41K5	11q24-11q24	125.8	D
RP11-112M22	11q24-11q24	127.3	D
AP003775.3	11q24.3	128.4	D
RP11-354O3	11q24.3c-11q24.3c	129.7	D
RP1-26N8	11q25	130.6	N
RP4-770G7	11q25	130.7	N
RP11-77C9	11q25a-11q25a	130.8	N
RP11-17M17	11q25-11q25	131.8	N
AP000903.6	11q25	132.2	N
RP11-27H17	11q25-11q25	133.2	N
RP11-469N6	11q25d-11q25d	133.7	N

*N, normal copy number; D, one copy deleted.

in mice should help distinguish the one versus multigene hypothesis.

Paris-Trousseau "syndrome" is characterized by thrombocytopenia, abnormal platelet function, abnormal megakaryocytes (from bone marrow), and abnormal appearing "giant" platelets in the peripheral blood. This phenotype is highly penetrant, affecting at least 92% of patients with 11q- [Grossfeld et al., 2004]. The *FLI-1* gene is a transcription factor that plays a role in megakaryopoiesis, and there is strong evidence supporting its role in causing Paris-Trousseau syndrome. In a transgenic mouse study, over-expression of *FLI-1* resulted in increased lymphopoiesis, while a null allele was lethal during mid-fetal development [Hart et al., 2000]. This has been confirmed by overexpressing the gene through in vitro transfection studies in cells from two patients with 11q terminal deletion syndrome [Raslova et al., 2004]. Our patient was found to have at least some of the features of Paris-Trousseau, and a deletion that spans *FLI-1*. Interestingly, she did not have giant platelets, suggesting a second gene may contribute to this aspect of Paris-Trousseau phenotype.

In summary, we report the first ever molecular characterization of a patient with de novo interstitial deletion in 11q (11q24.1q24.3) with the clinical features of Jacobsen syndrome, using subtelomeric FISH probes and microarray analysis. Identification of such patients will help contribute to our understanding of the genetic basis of the 11q-phenotype.

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