We present a family with multiple cytogenetic abnormalities, identified through a girl with several dysmorphic features and cardiac problems, suspected for Jacobsen syndrome. Cytogenetic analysis showed a 46,XX,del(11)(qter) karyotype, which was confirmed by fluorescence in situ hybridization (FISH). Cytogenetic investigation of the parents showed a chromosome aberration in both: the father had a t(11;12)(p13;q22) translocation and the mother was carrier of an ins(4;11)(p14;q24q25). FISH analysis with an 11q-subtelomeric probe from the second-generation telomere clone set and BACs from 11q24-q25 suggested a complex maternal rearrangement. However, subsequent array analysis showed a single interstitial deletion in the proband, derived from the maternal insertion. The aberrant karyotypes in both parents implicated an increased risk of unbalanced fetal chromosome composition, thus high risk for a child with multiple congenital abnormalities. Therefore, during the next pregnancy, the couple opted for prenatal diagnosis by means of amniocentesis. An interphase FISH strategy for uncultured amniotic fluid cells predicted two possible unbalanced fetal chromosome constitutions. Karyotyping of cultured amniotic cells confirmed one of the predicted unbalanced cytogenetic options, demonstrating the value of a fast interphase strategy for parents who both are carriers of a chromosomal abnormality. In addition, we present an overview of patients with Jacobsen syndrome and an interstitial 11q deletion reported thus far in literature. © 2009 Wiley-Liss, Inc.

Key words: Jacobsen syndrome; interstitial 11q deletion; SNP array; review

INTRODUCTION

Jacobsen syndrome (11q terminal deletion disorder) is a rare genetic disorder, caused by deletions of the long arm of chromosome 11. Approximately 165 patients have been reported in the literature [Grossfeld et al., 2004]. The phenotype of Jacobsen patients is variable: almost all patients have (multiple) dysmorphic features and Paris-Trousseau syndrome, a defect in platelet development, resulting in thrombocytopenia and platelet dysfunction. Approximately 50% of the patients have serious congenital heart defects. Additionally, various associations include developmental delays, cryptorchidism, recurrent infections, and pyloric stenosis. Cognitive function in patients varies from normal intelligence to mental retardation, and, in some patients, there may be a correlation between deletion size and the severity of the mental retardation [Grossfeld et al., 2004].

In most patients, terminal deletions are observed with cytogenetic breakpoints in 11q23 or 11q24 [Penny et al., 1995; Grossfeld et al., 2004]. Only a few interstitial deletions have been reported to date, extending from the proximal long arm (11q13) to one of the distal cytogenetic bands (up to 11q25) [Taillemite et al., 1975; Sorensen et al., 1979; McPherson and Meissner, 1982; Sirota et al.,...
1984; Klep-de Pater et al., 1985; Carnevale et al., 1987; Guc-Scękic et al., 1989; Wakazono et al., 1992; Stratton et al., 1994; Ono et al., 1996; Pivnick et al., 1996; De Pater et al., 1997; Wenger et al., 2006].

Additionally, patients have been described with deletions resulting from familial balanced translocations or inversions [Pivnick et al., 1996; Zahn et al., 2005; Gadzicki et al., 2006], de novo unbalanced translocations [Van Hemel et al., 1992; Penny et al., 1995; Wenger et al., 2006; Courtens et al., 2007], and ring chromosomes [Pivnick et al., 1996]. In a subset of Jacobsen syndrome patients, the breakpoint has been identified at the folate-sensitive fragile site on chromosome 11q23.3, $\text{FRA11B}$ [Jones et al., 2000]. However, in most of the patients, the breakpoints are not at this fragile site, but located more distally. Tunnacliffe et al. [1999] have identified two critical regions in 11q23 distal to $\text{FRA11B}$, the first mapping between markers D11S924 and D11S2087/D11S696/D11S382, and the second between D11S1345 and D11S933. More recently, Grossfeld et al. [2004] defined critical regions for five groups of phenotypic features.

Here, we present two sibs with several dysmorphic features and cardiac problems, suspected for Jacobsen syndrome, showing an interstitial deletion of 11q, derived from a maternal chromosomal insertion. Additionally, we present a literature review of all reported patients with Jacobsen syndrome and interstitial 11q deletions.

**PATIENT REPORT**

**Patient 1**

The proband, a 3-day-old girl, was the first child of healthy, unrelated parents. There was no history of miscarriage or congenital heart disease. She had a healthy maternal half-brother and two healthy paternal half-brothers. She was born after a normal delivery at 41 weeks of gestation with a birth weight at $-2\ SD$. She presented with several dysmorphic features: mild upslanting palpebral fissures, broad nasal bridge, long philtrum, thin lips, oval-shaped mouth, short and mildly webbed neck, low-set dysplastic ears (supporting information Fig. 1A,B may be found in the online version of this article) and widely spaced nipples. There was thrombocytopenia and hyperbilirubinemia. Cardiac ultrasound showed a ventricular and an atrial septum defect, and patent ductus arteriosus. Abdominal ultrasound showed unilateral renal dysplasia.

At the age of 3 weeks pulmonary vascular resistance was elevated and rapidly progressive. Medical treatment consisting of inhaled nitric oxide, sildenafil, bosentan, and prostacyclin was started, but the pulmonary hypertension was not responsive. Extra corporal membrane oxygenation (ECMO) was instituted for 2 weeks, but had no effect. Due to unexplained anuria and weak leg pulses a previously undiagnosed coarctation of the aorta was suspected. Repeat cardiac catheterization showed a hypoplastic transverse arch

**FIG. 1.** Aberrant chromosomes of (A) proband, (B) father, and (C) mother. D,E: FISH using probes $\text{RP1-26N8}$ (11qter, red) and $\text{RP1-44H16}$ (11pter, green) confirmed the deletion in the proband (D) and insertion in the mother (E). Arrows show the normal and derivative chromosome 11, the arrowhead shows the derivative chromosome 4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
with a significant pressure gradient. Balloon dilatation was attempted, after which the patient experienced severe bleeding. Due to therapy-resistant hypovolemic shock the patient died at the age of 5 1/2 weeks. Parents did not give permission for autopsy.

Patient 2

In the subsequent pregnancy, prenatal diagnosis predicted another affected child. The parents chose to continue the pregnancy. Polyhydramnios was noted and at 20 weeks of gestation a hypoplastic left heart was seen by ultrasound. The boy was born at term with birth weight of 3,320 g, Apgar scores of 8 and 8 after 1 and 5 min, and thrombopenia. He had a broad and high forehead, hypertelorism, small ears with an overfolded upper helix, long philtrum, epicanthal folds, widely spaced nipples, broad hands with a unilateral single transverse palmar crease, a hypoplastic left heart with a partially abnormal venous return, and a restrictive foramen ovale. The cardiac defect was considered inoperable and he died at 3 days of age.

MATERIALS AND METHODS

Routine Cytogenetic Analysis

For postnatal cytogenetic investigation, karyotyping was performed according to standard cytogenetic procedures on GTG banded metaphase spreads obtained from peripheral blood cultures from the proband and her parents. Remaining methanol:acetic acid (3:1) fixed cell suspensions were stored at -20°C.

For prenatal cytogenetic analysis, 2 ml of amniotic fluid (AF) was used for the preparation of direct slides for fluorescence in situ hybridization (FISH) as described previously [Van Opstal et al., 1993]. The remainder was cultured using the in situ method in BD Falcon Culture slides (VWR, Amsterdam, The Netherlands). Twelve AF cell clones were analyzed after pancreatin–trypsin–Giemsa staining.

Fluorescence In Situ Hybridization

BAC clones were selected from the UCSC genome browser (UC Santa Cruz, Santa Cruz, CA, http://genome.ucsc.edu/cgi-bin/hgGateway, assembly March 2006) and purchased from BACPAC Resources (Oakland, CA). Five to 10 μg of DNA was semi-automatically isolated with an AutoGenPrep 3000 robot (Autogen, Holliston, MA) and after whole genome amplification (WGA, Repli-G; Qiagen, Hilden, Germany), the DNA was digested and labeled (Random Prime labeling system; Invitrogen, Carlsbad, CA) with Bio-16-dUTP or Dig-11-dUTP (Roche, Penzberg, Germany). The BAC probes (Table I and Fig. 2) were validated on control metaphases. The FISH experiments were performed according to standard protocols with minor modifications [Verkerk et al., 2003]. Cultured and uncultured AF slides were pretreated as previously described [Van Opstal et al., 1995]. FISH slides were analyzed with an Axioplan 2 Imaging microscope (Zeiss, Siedlerecht, The Netherlands) and images were captured using Isis software (MetaSystems, Altlussusheim, Germany).

For each sample, at least five metaphases per probe were analyzed. For the uncultured AF slides, 50 interphase nuclei were investigated.

ARRAY

High-resolution whole genome analysis was performed using the 250K Nsp1 array (Affymetrix, Santa Clara, CA) according to the manufacturer’s protocol. This array has a mean resolution of 262K. The SNP call in this experiment was 94.97% with a standard deviation of 0.206289. The array was scanned using the GeneChip® Scanner 3000 7G System with AutoLoader (Affymetrix) and data were analyzed using the copy number analysis tool for gene chips (CNAG version 2.0) provided by www.genome.umin.jp.

RESULTS

Postnatal Investigations

Routine cytogenetic analysis of the proband showed a female karyotype, with a terminal deletion of 11q (Fig. 1A), which was confirmed by FISH with the DNA probe RP1-26N8 (11q subtelomere) (Fig. 1D). The karyotype was 46,XX,del(11)(q24–25). Cytogenetic analysis of both parents revealed that father’s karyotype was 46,XY,t(11;12)(p13;q22) (Fig. 1B), the mother showed a 46,XX,ins(4;11)(p14;q24q25) karyotype (Fig. 1C). The der(11)ins(4;11)(p14q24q25) was identical to the abnormal chromosome 11 in the proband. Both parental chromosome abnormalities were also confirmed using FISH (mother (Fig. 1E); father (data not shown)).

Additional FISH probes (RP11-367N15, 11q24.2 and RP11-102M23, 11q25, the latter overlapping with JAM3) were used to investigate the mother’s chromosomes for determination of the chromosome 11 breakpoints. Signals of RP11-367N15, a more proximal probe, were observed on the der(4)ins(4;11) and on the normal chromosome 11, but not on the der(11)ins(4;11). On the other hand, signals of RP11-102M23, a more distal probe, were visible on the normal chromosome 11 and der(11)ins(4;11), but not on the der(4)ins(4;11) (Fig. 2). In the proband, RP11-367N15 was visible on the normal chromosome 11, but absent on other chromosomes, whereas RP11-102M23 showed signals on the normal chromosome 11 and der(11) (Fig. 2). FISH to verify the localization of both probes on normal metaphase spreads showed that RP11-367N15 is located proximal to RP11-102M23 (data not shown), confirming the localization from the UCSC genome browser. This, together with the deletion of RP1-26N8, suggested that an inversion had occurred in the mother’s chromosome 11 before insertion of the telomeric part of this rearranged chromosome 11 into the short arm of a chromosome 4.

Additional BAC clones in the region 11q23–11qter were used on samples of the proband and mother to investigate the inversion (Fig. 2). RP11-778O17 and RP11-158K18 (both 11q23.3) showed signals on the normal chromosome 11 and der(11) in both mother and child. RP11-10N17, RP11-20M1 (both 11q24.2, like RP11-367N15), RP11-80J16 (11q24.3), and RP11-15J5 (11q25) showed signals on the normal chromosome 11 and the der(4) of the mother’s chromosomes, and showed only one signal on the normal chromosome 11 of the child. Finally, RP11-469N6 and RP11-265F9 (both 11q25, like RP11-102M23) showed signals on the normal chromosome 11 and der(11) again. Thus, the proximal inversion
TABLE I. Review of De Novo Interstitial 11q Deletions and Clinical Features in Patients With Jacobsen Syndrome

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<td>High narrow or stiff palate</td>
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<td>Recurrent infection</td>
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Filled black boxes: extent of deletion; filled light gray boxes: alternative possibility of extent of deletion; filled dark gray boxes: overlap between alternative deletion possibilities; clinical features in bold: classical Jacobsen syndrome features; blank: no information available on clinical feature.

*Based on information in abstract only.
breakpoint was between RP11-158K18 (11q23.3) and RP11-10N17 (11q24.2), a region of approximately 4 Mb. The distal inversion breakpoint was between RP11-265F9 (11q25, the most telomeric BAC clone in the UCSC genome browser) and the subtelomeric PAC RP1-26N8.

Subsequently, array analysis revealed a single interstitial deletion ranging from rs10750286 (123.9 Mb from pter) to rs1540206 (132.1 Mb) (Fig. 3A). FISH using BAC clones situated around these breakpoints (RP11-822K24, CTD-2247D20 (both 11q24.2), RP11-715016, RP11-17M17 (overlapping the distal breakpoint), and RP11-192G18 (11q25)) confirmed the size of the deletion (Fig. 3B–F). We did not observe an additional terminal deletion using array analysis, arguing against our theory of two subsequent events and in favor of a more proximal location of RP1-26N8. Indeed, verification of the SNP locations around the breakpoints and those overlapping with RP1-26N8 in the Database of Genomic Variants (DGV, human genome build 36, March 2006) showed RP1-26N8 to be located at 130.8–131.0 Mb and not at the subtelomere (134.4 Mb) (Fig. 2). Thus, the maternal chromosome abnormality was ins(4;11)(p14;q24.2q25) instead of the complex chromosome rearrangement suggested by our initial FISH experiments. Using the refined breakpoints defined by array analysis, we revised the karyotype of the proband to 46,XX,der(11)ins(4;11)(p14;q24.2q25)mat.

Prenatal Investigations

During our cytogenetic investigations, the mother became pregnant again. Because both parents had an abnormal karyotype, there was a high risk (75%) of a cytogenetically unbalanced pregnancy outcome. Although chorionic villus sampling was offered, the couple opted for second trimester amniocentesis. For timely diagnosis, we designed an interphase FISH strategy for uncultured AF cells using the probes for 11pter (RP1-44H16, 11pter (RP1-26N8), 12pter (CTB-124K20), and 12qter (RP1-221K18) to obtain an early indication of the fetal karyotype. All possible zygote combinations regarding the paternal chromosomes 11 and 12, and maternal chromosomes 4 and 11 were determined, including details not only for chromosomal composition but also for the number and localization of FISH signals (supporting information Table I may be found in the online version of this article).

Fifty interphase nuclei of uncultured AF cells were scored per probe. With the 12pter (CTB-124K20, red) and 12qter (RP1-221K18, green) probe combination, we observed two green and two red signals in the majority of interphase nuclei, suggesting a balanced pattern with respect to chromosome 12 (supporting information Fig. 2B may be found in the online version of this article). Using the probe combination for 11pter (RP1-44H16, green) and 11qter
(RP1-26N8, red), we observed two green signals and one red signal in the majority of interphase nuclei, suggesting an unbalanced situation with loss of 11qter (supporting information Fig. 2A may be found in the online version of this article). Cytogenetic analysis of the cultured preparations revealed the fetal karyotype 46,XY,der(11)ins(4;11)-(p14;q24.2q25)mat, which was confirmed using metaphase FISH (supporting information Fig. 2C–E may be found in the online version of this article).
DISCUSSION

Cytogenetic, FISH, and array analyses in a Jacobsen-like patient showed an interstitial 11q deletion resulting from the unbalanced inheritance of a balanced maternal insertion. Based on our initial FISH analyses using probes RP1-26N8, RP11-102M23, and RP11-367N15, we interpreted the chromosomal abnormality of the proband as a deletion derived from a complex maternal chromosomal rearrangement in which multiple rearrangements of chromosome 11 had occurred: first, an inv(11)(q24q25), and then an insertion of the most distal part of this inverted chromosome into 4p14. However, subsequent array analysis revealed a single 8.2 Mb interstitial deletion: the most telomeric SNPs on the array were present normally, suggesting a more proximal location of PAC RP1-26N8 rather than the subtelomeric location described by Knight et al. [2000]. In the DGV, RP1-26N8 is located approximately 3.5 Mb from the subtelomere. We observed that also other probes from the second-generation set of telomere-specific clones [Knight et al., 2000] are located more proximal than originally determined (data not shown). Therefore, these probes should be used with caution and probe locations should be examined in the latest updates of genomic maps before drawing conclusions about telomeric deletions or (complex) rearrangements.

We designed an interphase FISH strategy on uncultured AF cells to predict the possible fetal chromosomal constitution of the mother’s new pregnancy. We observed a balanced pattern regarding chromosome 12, but an unbalanced pattern for chromosome 11, with loss of 11pter (supporting information Fig. 2A,B may be found in the online version of this article). According to our scheme (supporting information Table I may be found in the online version of this article), two different fetal chromosome constiutions were possible: a fetus with the normal chromosome 4 and der(11) of the mother and either the normal chromosomes 11 and 12 (combination 1B) or both the der(11) and der(12) (combination 4B) of the father (supporting information Table I may be found in the online version of this article). All other combinations would have resulted in normal, balanced, or otherwise unbalanced chromosomal constitutions. Conventional cytogenetic analysis and metaphase FISH on cultured AF cells indeed confirmed one of the two constitutions, 46,XY,der(11)ins(4;11)(p14;q24.2;q25)mat, representing fetal chromosomal constitution 1B. The parents chose to continue the pregnancy.

Approximately 165 patients with Jacobsen syndrome have been reported in the literature, most displaying terminal 11q deletions [Grossfeld et al., 2004]. Using molecular breakpoint mapping in patients with a terminal 11q deletion previously identified by karyotyping analysis, Grossfeld et al. [2004] have not identified any interstitial deletion. To the best of our knowledge, only 15 patients with de novo interstitial deletions have been published thus far [Taillemite et al., 1975; Sorensen et al., 1979; McPherson and Meissner, 1982; Sirota et al., 1984; Klep-de Pater et al., 1985; Carnevale et al., 1987; Guc-Scekic et al., 1989; Wakazono et al., 1992; Stratton et al., 1994; Ono et al., 1996; Pivnick et al., 1996; De Pater et al., 1997; Wenger et al., 2006]. The size and breakpoints of the deletions are highly variable (Table I). Some of first interstitial deletions published show breakpoint uncertainty, as molecular techniques were not available yet to characterize the deletion [Klep-de Pater et al., 1985]. Consequently, it is not possible to determine whether a single minimal region of overlap exists. Besides breakpoint heterogeneity, the clinical features are very heterogeneous as well (Table I), thus complicating genotype–phenotype correlations. Grossfeld et al. [2004] identified critical regions for five groups of phenotypic features, including a high palate (121.9–134 Mb), oval-shaped mouth (124.4–134 Mb), and platelet disorder (127.4–134 Mb), in Jacobsen syndrome patients with terminal 11q deletions. The deletion in our patient overlapped with the deleted region in the patient reported by Wenger et al. [2006] from 123.9 to 130.6 Mb. Both patients displayed thrombopenia, thus narrowing the critical region for platelet disorder further down to 127.4–130.6 Mb from the p-telomere. On the contrary, several patients with interstitial 11q deletions and a carp-shaped mouth [Taillemite et al., 1975; Wakazono et al., 1992; Pivnick et al., 1996] or a high/cleft palate [Klep-de Pater et al., 1985; Guc-Scekic et al., 1989; Wakazono et al., 1992; Ono et al., 1996] showed deletions outside the critical regions identified by Grossfeld et al. [2004]. The differences between the published critical regions and the regions reviewed here might be explained by the different methods used to characterize the deletions. Grossfeld et al. [2004] included molecular analysis of the terminal deletion breakpoints, whereas the breakpoints of most interstitial deletions reported thus far were determined by karyotyping. However, breakpoint mapping by karyotyping alone is limited, since cytogenetic bands 11q14 and 11q22 are of similar width and intensity; therefore, it is difficult to discriminate between these two cytogenetic bands. For that reason we performed array analysis to identify the interstitial 11q deletion presented here. To our knowledge, this is the second patient with an interstitial 11q deletion characterized by array analysis. The first patient, with a congenital heart defect, showed a distal deletion breakpoint centromeric to JAM3, arguing against JAM3 as a candidate gene for heart defects associated with 11q deletions [Wenger et al., 2006]. In our patients, who had heart defects as well, the distal deletion breakpoint was also centromeric to JAM3 (located at 133.4–133.5 Mb), thus providing further evidence to exclude deletions of this gene as the cause of heart defects in 11q. To help refining the critical region for Jacobsen syndrome clinical features, breakpoints of published interstitial deletions should be reanalyzed using the molecular techniques available now.

In conclusion, when both parental karyotypes show chromosome abnormalities, an increased risk for recurrent abortions, and/or conception of a child with an unbalanced karyotype exists. We demonstrate that a FISH strategy can be a useful diagnostic tool to investigate rapidly whether the fetal karyotype is balanced or unbalanced. However, probes from the second-generation set of telomere-specific clones should be used cautiously. Additionally, the predicted fetal chromosome constitution can be used to correlate the fetal genotype with a possible phenotype. This information can be very valuable for appropriate genetic counseling. Breakpoint heterogeneity in patients with interstitial 11q deletions poses a problem for correlating genotype with phenotypic features characteristic for Jacobsen syndrome. Molecular characterization of previously published and new interstitial deletions might provide better insight into the genetic causes of clinical features associated with Jacobsen syndrome.
ACKNOWLEDGMENTS

We thank the parents for their collaboration in this study.

REFERENCES


