

Two siblings with 11qter deletion syndrome that had been rescued in their mother by uniparental disomy

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ABSTRACT

Jacobsen syndrome refers to a congenital anomaly caused by deletion at 11q23.3-qter. We here describe two siblings with the same 11q23.3-qter deletion. Both parents were healthy with a normal karyotype. Cytogenetic microarray analysis revealed no mosaicism in either parent but the mother showed uniparental disomy encompassing the deleted region found in the two siblings. The pattern of X chromosome inactivation was almost completely skewed in the mother. These data suggested that the mother was a carrier of the 11q23.3-qter deletion but that this had been rescued by disomy formation during early embryogenesis except for her germinal cells.

1. Introduction

Jacobsen syndrome (MIM#147791) is a contiguous gene deletion syndrome caused by deletion of the 11qter region. The typical clinical features of Jacobsen syndrome include pre- and postnatal physical growth and psychomotor retardation, facial dysmorphic features, and thrombocytopenia. Some patients with this syndrome also have malformations of the heart, kidney, gastrointestinal tract, and central nervous system. Ocular and hearing problems can be also present. The estimated occurrence of Jacobsen syndrome is about 1/100,000 births (Mattina et al., 2009).

About 85% of Jacobsen syndrome cases are caused by a simple *de novo* terminal deletion. Other cases result from a variety of chromosomal abnormalities including segregation of a familial reciprocal balanced translocation, *de novo* unbalanced translocations, recombination of a parental pericentric inversion, or other rearrangements such as ring chromosomes. An 11q deletion has also been reported in the mosaic form of this condition. The breakpoints in these deletions occur within or distal to 11q23.3, and the deletions usually extend to the telomere (Grossfeld et al., 2004). The deletion size ranges from 7 to 20 Mb. The chromosomal region conferring specificity for the Jacobsen syndrome

phenotype is the 11q24.2 band, but the gene responsible for this phenotype is still unknown.

We here report on two siblings with the same 11q23.3-qter deletion, one with Jacobsen syndrome and the other detected by amniocentesis and terminated. The parents however showed a normal karyotype. Cytogenetic microarray analyses revealed that the healthy mother had uniparental disomy (UPD) encompassing the 11q22.3-ter region deleted in the siblings. A possible mechanism for the recurrence of this deletion is discussed.

2. Clinical report

A 4-year old Japanese male subject was the first child of a non-consanguineous healthy 36-year old father and 28-year old mother after having three miscarriages with no notable family history of disease (Fig. 1A). At 22 weeks of pregnancy, a congenital heart defect, mitral valve stenosis and aortic valve stenosis were suspected. He had been born after a 41 week gestation by an induced labor with a birth weight of 2644 g (−0.9SD), height of 47.5 cm (−0.7SD), head circumference of 34.5 cm (+0.8SD), and chest circumference of 30 cm. He showed a hypoplastic left heart, conductive auditory impairment in the left ear,

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3. Materials and methods

3.1. Subjects

Peripheral blood samples were obtained from the study subject and the parents. The research protocol for this study was approved by the local ethics committee of Fujita Health University, Japan. Written informed consent to participate in the study was obtained from the parents.

3.2. DNA extraction

Genomic DNA was extracted from whole blood using QuickGene 610 L (Fuji film, Tokyo, Japan). The concentration of the DNA was measured using an ND-1000 spectrophotometer (NanoDrop, Wilmington, DE) and the quality was determined by gel electrophoresis.

3.3. Cytogenetic microarray

High-resolution chromosomal microarray analysis using the CytoScan HD array (Affymetrix, Santa Clara, CA) was performed. DNA samples of 50 ng were used in this analysis in accordance with the manufacturer's instructions. The genomic coordinates were based upon genome build 37/hg19 (2009). Hybridization, data extraction and analysis were performed as per the manufacturer's protocols. Chromosome Analysis Suite software 3.0 (ChAS, Affymetrix Santa Clara, CA) was used for raw data analysis, review and reporting. Regions of copy-number changes were extracted with 20 probes of 50 kb. All of the extracted regions containing a copy-number change were confirmed by visual comparisons with the normal control data from Database of Genomic Variants (<http://dgv.tcag.ca>). UPD regions were extracted with 5 Mb. Regions with a sparse SNP density were carefully evaluated to exclude false calls.

3.4. FISH analysis

Peripheral blood lymphocytes and buccal samples were obtained by standard methods. FISH analysis was performed using standard techniques. The probes used for the FISH analysis were TelVysion 11p SpectrumGreen (D11S2071), TelVysion 11q SpectrumOrange (D11S1037) (Abbott Molecular, IL, USA). A hundred interphases nuclei were analyzed for pter/qter of chromosome 11.

3.5. HUMARA assay

To assess skewing of the X chromosome inactivation, we performed HUMARA assay according to the protocol described elsewhere (Beever et al., 2003). Briefly, we digested the genomic DNA with methylation-sensitive restriction enzyme *HpaII*. PCR primers, one of which was labeled with FAM, were designed across the polymorphic CAG repeat as well as two *HpaII* sites in the androgen receptor gene on the X chromosome. PCR amplification would be achieved only from the inactivated allele having the *HpaII* sites methylated. PCR products were analyzed by capillary electrophoresis (ABI3730 Genetic Analyzer) and quantified the area under the curve using GeneMapper software.

4. Results

We performed cytogenetic microarray analysis to demarcate the deleted region in our current case subject. A 15.4-Mb region was found to have been deleted at 11q23.3q25-qter in this patient (arr [hg19] 11q23.3q25 (119, 484, 933_134, 938, 470)×1), which is consistent with the typically deleted region in Jacobsen syndrome (Fig. 1B). The deleted region was found to contain 128 Refseq genes, and 70 OMIM genes. Single nucleotide polymorphism (SNP) genotyping indicated that the deleted chromosome was derived from the mother (data not

shown).

A possible explanation for the abnormal 46,XY,del (11) (q23.3) karyotype in two siblings from parents with a normal karyotype was that one of the parents harbored FRA11B, a (CCG)_n repeat expansion in the 5′ untranslated region of the *CBL2* gene. In more than 70% of normal individuals, this repeat is present in 11 copies but can be expanded to several hundred copies and lead to genomic instability and a susceptibility for terminal deletion (Mattina et al., 2009). However, the deletion breakpoint of our current patient was at chr11:119, 484, 933 (hg19), which is approximately 400 kb distal from FRA11B.

Neither of the parents showed deletion mosaicism at the 11q23.3-qter region. Interphase FISH on 100 peripheral blood lymphocytes and 100 buccal cells revealed no deletion for the 11q subtelomere-specific probe (data not shown). It was notable however that SNP array analysis of the patient's mother detected a 26.2-Mb region with a loss of heterozygosity at 11q22.3-qter consistent with uniparental disomy (UPD) (arr [hg19]11q22.3q25 (108, 657, 506_134, 942, 626)×2 hmz) (Fig. 1B). The deletion breakpoint in the son was 10-Mb distal from the UPD boundary in the mother.

A HUMARA assay was performed to determine when the UPD was generated in the mother. The patterns of X chromosome inactivation (XCI) showed 99.2% skewing in the mother (Fig. 1C), suggesting that she originally had the same deletion as her son and the chromosome copy number loss was corrected by UPD after XCI occurred in the early embryogenesis.

5. Discussion

Our analysis by cytogenetic microarray of our current case subject with 11qter deletion syndrome and his family suggests that segmental UPD corrected the chromosomal copy number of the deleted region and thereby rescued the phenotype in his healthy mother. To our knowledge, there have only been two previous reports of siblings showing a deletion of 11q23.3-qter despite a normal parental karyotype (Affi et al., 2008; Johnson et al., 2014). One of those reports also provided detailed molecular analyses showing a maternal UPD at the 11qter region (Johnson et al., 2014). A 22q13 deletion rescued by paternal UPD has also been reported (Bonaglia et al., 2009). Such deletion rescue event has not been reported for other terminal deletions. Our current case is therefore the third report to describe a deletion rescued by post-zygotic UPD generation.

It is likely that the mother of our current case subject originally carried the 11q23.3-qter deletion that had been transmitted from a gamete of a maternal grandfather or grandmother. After fertilization, this deletion was likely rescued during the post-zygotic stage via a DNA repair pathway for coincidental double-strand-breaks (DSBs) at the proximal region of the deletion breakpoint, thereby generating the segmental UPD. The UPD boundary in the mother is located 10 Mb more proximal than the breakpoint of deleted region of the patient, which is a strong evidence that the UPD developed after the deletion. The principal molecular mechanisms that have been postulated to explain segmental UPD are mitotic recombination or break-induced replication (BIR) (Costantino et al., 2014; Carvalho et al., 2015).

We observed an almost completely skewed XCI in the mother's DNA. Generally, an XCI pattern increases the extent of skewing with age as a consequence of hematopoietic stem cell senescence. At 20–39 years old, mean skewing level is reported to be 70.6% (Hatakeyama et al., 2004). Our current patient's mother was 30 years old at the time of genetic testing and showed very high skewing at 99.2%. This indicated that her blood cells were derived from a single clone after XCI (Kurahashi et al., 1991). We speculated that the mother originally harbored the 11q23.3-qter deletion as a zygote which was subsequently repaired in one of the somatic cells by mitotic recombination or BIR after XCI has been completed. The repaired cell likely obtained selective advantage during embryonic development and unrepaired cells were eliminated (Fig. 2). This resulted in a normal phenotype at birth and no evidence of

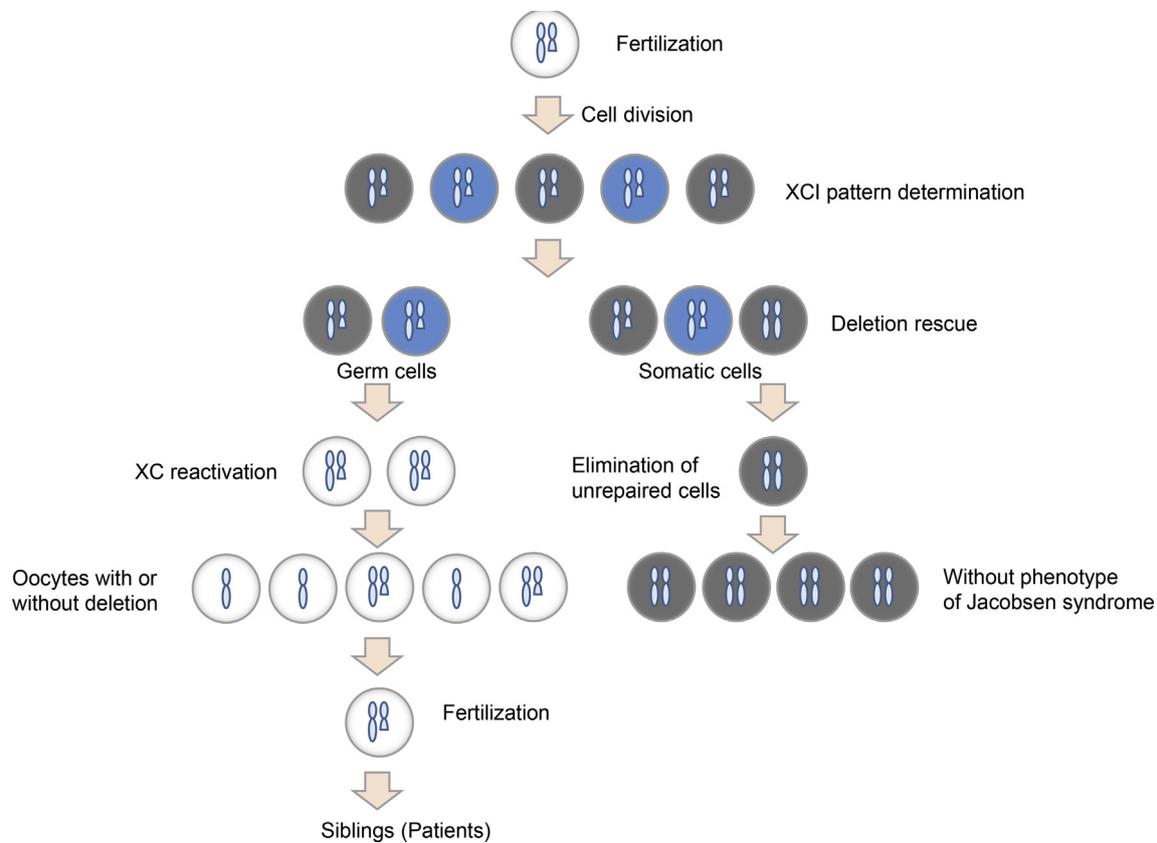


Fig. 2. An illustration for the status of the mother and the two siblings.

Jacobsen syndrome. It appears however that although her somatic cells had all been rescued by UPD her germ cells retained the 11q23-qter deletion. Such monoclonality has been described previously in trisomy rescue of chromosome 15 (Butler et al., 2007). Thus, this mother had no Jacobsen syndrome phenotype but transmitted the causative deletion to her two sons.

As far as we are aware, there have been only two other case reports of ‘deletion rescue’ (Bonaglia et al., 2009; Johnson et al., 2014). It would be intriguing if 11q23.3-qter was found to be a hotspot for deletion rescue. One possible explanation for this phenomenon is a strong negative selection process as a result of gene loss. An alternative possibility is that there might be a DSB hotspot that induces mitotic recombination or BIR at the region proximal to the 11q23.3 breakpoint. If this is indeed the case, the recurrence risk in the affected siblings would be slightly higher than in the general population. SNP array analysis of the parents might be advisable even in an apparent *de novo* case of Jacobsen syndrome.

In conclusion, we speculate that the maternal 11q23.3-qter deletion was repaired in our current study family via mitotic recombination or BIR leading to UPD generation. As a consequence of this DNA repair, the chromosomal copy number was corrected in the mother resulting in a normal phenotype. On the other hand, some of maternal germline cells retained 11q23-ter deletion, leading to a recurrence of Jacobsen syndrome in her offspring. Careful genetic counseling is therefore warranted regarding the recurrence of Jacobsen syndrome.

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