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Triploidy Mosaicism (45,X/68,XX) in an Infant Presenting with Failure to Thrive

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Abstract

Triploid mosaicism is a rare aneuploidy syndrome characterized by growth retardation, developmental delay, 3-4 syndactyly, microphthalmia, coloboma, cleft lip and/or palate, genitourinary anomalies, and facial or body asymmetry. In the present report, we describe a 3-month-old female presenting with failure to thrive, growth retardation, and developmental delay. A chromosomal microarray demonstrated monosomy X, but her atypical phenotype prompted further evaluation with a chromosome analysis, which demonstrated 45,X/68,XX mixoploidy. To our knowledge, this is the first report of a patient with this chromosome complement. Mosaicism in chromosomal aneuploidies is likely under-recognized and may obscure the clinical diagnosis. At a time when comparative genomic hybridization and genome sequencing are increasingly used as diagnostic tools, this report highlights the clinical utility of chromosome analysis when a molecular diagnosis is not consistent with the observed phenotype.

Keywords

Mixoploidy; Triploidy; Mosaicism; Turner syndrome

INTRODUCTION

Chromosomal aneuploidies represent one of the most common classes of genetic diagnoses, and are often discerned by their distinctive clinical features. Chromosome analysis is used to confirm the clinical diagnosis and inform assessments of recurrence risk. Numerical chromosome abnormalities result from errors in meiosis and are detected in approximately 5% of clinically recognized pregnancies and 0.3% of live births [Hassold *et al.*, 1996; Hassold and Hunt 2001; Nagaoka *et al.*, 2012]. Mosaicism involving a euploid cell line provides a viable chromosome complement and can mitigate the severity of the phenotype [Boonen *et al.*, 2011; Hook and Warburton 2014].

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Monosomy X (45,X) affects as many as 1-1.5% of all pregnancies, though only 1% of these conceptuses survive to birth and are given a diagnosis of Turner syndrome (TS) [Hook and Warburton 2014]. TS patients have characteristic features of short stature, congenital cardiovascular defects, and ovarian failure [Bondy 2007]. A webbed neck or peripheral edema may suggest a cardiovascular or lymphatic system defect. Although triploidy is somewhat more common in pregnancies (2%), it is almost always incompatible with life [van de Laar *et al.*, 2002; Wellesley *et al.*, 2012]. Individuals with triploidy share features of pre- and post-natal growth retardation, syndactyly involving one or more extremities, microphthalmia, coloboma, cleft lip and/or palate, renal anomalies, and ambiguous external genitalia [Boonen *et al.*, 2011; Doshi *et al.*, 1983; van de Laar *et al.*, 2002]. Facial and body asymmetry is also typical.

In this report, we describe a patient with failure to thrive, poor growth, hypotonia, and a history of neonatal hypoglycemia who was ultimately found to have Turner syndrome/triploidy mosaicism: 45,X/68,XX. This case underscores the role of chromosome analysis in the evaluation of patients with aneuploidy, particularly when the initial molecular diagnosis is inconsistent with the patient's phenotype.

CLINICAL REPORT

The patient was born at 34 6/7 weeks gestation to a 29-year-old G3P2002 mother and a 32-year-old father, both of mixed European ancestry. An ultrasound at 6 weeks gestation had revealed a suspected molar pregnancy, but subsequent ultrasounds demonstrated an intrauterine pregnancy and minimal fetal growth toward the end of gestation. Birth weight was 2.10 kg (10-50th centile for gestational age) and length was 40.64 cm (<3rd centile for gestational age). Although neonatal records were not available, parents reported a 15-day hospitalization complicated by thrombocytopenia, hypoglycemia and poor feeding. Immediately after discharge, the patient developed emesis following breast milk feeds. These episodes progressed in frequency and severity, leading to a hospital admission for failure to thrive at three months of age.

The parents reported irritability, poor nighttime arousal for feeds, and fatigue and sweating during feeds. Developmental delay with limited head control and no cooing was noted. Physical exam revealed a length of 53 cm (3.5 SD below mean for age), a weight of 3.17 kg (5 SD below mean for age), and fronto-occipital circumference of 36 cm (3 SD below mean for age). Dysmorphic features included frontal bossing, plagiocephaly, down-slanting palpebral fissures, ears with prominent antihelices, a bulbous nose, and a high-arched palate (**Figure 1A-C**). Other features included hypoplastic nails, medial displacement of bilateral first toes, mottled skin with cool extremities, a sacral dimple, labial adhesions, and hypotonia.

Initial evaluation included an echocardiogram that demonstrated coarse trabeculations involving the inferior and posterior apex of the left ventricle, a spine MRI suggestive of a tethered cord, and a normal pyloric ultrasound and brain MRI. Bilateral conductive hearing loss was diagnosed by auditory brainstem response. Thyroid stimulating hormone was mildly elevated (8.49 μ IU/mL, reference 0.5-6.0 μ IU/mL), and free T4 was normal (1.5

ng/dL, reference 0.8-2.0 ng/dL). Biochemical evaluation, including plasma amino acids, acylcarnitine profile, and qualitative urine organic acids were normal. The CMA (Baylor College of Medicine, Version 9.1.1), which includes 60,000 probes used for single nucleotide polymorphism (SNP) analysis, identified a copy number loss detected by all chromosome X-specific probes, consistent with a diagnosis of Turner syndrome. *SRY* fluorescence *in situ* hybridization (FISH) analysis (Baylor College of Medicine) was obtained to rule out the presence of a Y chromosome, and analysis of 10 metaphases and 300 nuclei did not demonstrate the presence of *SRY*; however, FISH analysis with an X chromosome centromere probe revealed 290 of 310 nuclei (94%) with a single X chromosome signal and 20/310 nuclei (6%) with two signals for the X chromosome. Because the patient's phenotype was not typical for Turner syndrome, high resolution chromosome analysis (Baylor College of Medicine) was pursued. Analysis of 30 cultured lymphocyte cells revealed the presence of two cell lines, with 80% demonstrating monosomy X and 20% containing an additional haploid set of chromosomes not detected by SNP analysis: 45,X[24]/68,XX[6] (**Figure 1D,E**). Molecular short tandem repeat (STR) studies were conducted to determine the parental origin of the additional haploid set of chromosomes using the AmpFLSTR® Identifier® Plus PCR Amplification Kit (Life Technologies); however, there was no detectable evidence of a triploid cell line in DNA extracted from either whole blood or the fixed cell culture pellet (**Supplemental Table 1**). A subsequent FISH analysis on the original uncultured blood sample using the AneuVysion Multicolor DNA Probe Kit (Abbott Molecular) revealed 6/200 (3%) cells with a signal pattern consistent with a 68,XX chromosome complement while the remaining 97% of cells showed a signal pattern consistent with a 45,X chromosome complement (**Figure 1F-I**). Thus, the normal SNP array and STR data most likely reflect the low level of mosaicism for the triploid cell line in the patient's peripheral blood, despite the cultured lymphocytes showing a 20% frequency of triploid cells.

DISCUSSION

In the present report, we describe an infant with multiple clinical features including failure to thrive, short stature, hypotonia, dysmorphic craniofacial features, left ventricular non-compaction and a tethered cord. The initial CMA testing demonstrated a loss of all X-chromosome specific probes with normal SNP data, suggestive of Turner syndrome, while confirmatory chromosome analysis of peripheral blood demonstrated two abnormal cell lines, consistent with mosaicism for both monosomy X and triploidy. To our knowledge, this is the first patient reported with 45,X/68,XX mosaicism, although patients with triploid mosaicism have been described [Fryns *et al.*, 1980; Quigley *et al.*, 2005].

Our patient had short stature, a high-arched palate, and hearing loss, all frequently present in Turner syndrome patients. Growth delay, which was the most prominent feature of her presentation, is also a feature of Turner syndrome, yet is typically not as severe as was observed in our patient. Rather, the severe growth delay in our patient is more typical of patients with triploidy. Our patient had a birth length of 40.64 cm, which is more than two standard deviations below reported mean birth length for Turner syndrome patients of Northern European or German ancestry (47.9 $\pm$ 2.8 cm, 48.3 $\pm$ 3.2 cm, respectively) [Ranke *et al.*, 1983; Rongen-Westerlaken *et al.*, 1997]. At 3 months of age, her length was

53 cm, which is more than one standard deviation below reported mean birth length for Turner syndrome patients of Northern European ancestry [Rongen-Westerlaken *et al.*, 1997]. Additional features such as her prominent forehead, and hypotonia are also more typical of infants with triploidy.

Although non-mosaic triploidy is almost always incompatible with life, over 30 patients with diploid/triploid mosaicism have been described in the literature [Boonen *et al.*, 2011; Fryns *et al.*, 1980; Oktem *et al.*, 2007; Rittinger *et al.*, 2008; van de Laar *et al.*, 2002]. In many cases, cytogenetic analysis of peripheral blood leukocytes is not sufficient to detect a triploid cell line, and skin fibroblast studies are needed for diagnosis. In the present case, analysis of cultured lymphocytes did detect the triploid cell line, albeit at a higher percentage (20%) than analyses of uncultured lymphocytes (3-6%). The uncultured lymphocyte studies are likely more reflective of the true level of triploidy mosaicism in the reported patient's peripheral blood, as cell culture techniques can lead to biased selection of a subpopulation of cells. Patients with triploid mosaicism have features that are similar to those of full triploidy, albeit less severe, and frequently have facial and body asymmetry reflecting their mosaic state. Sandal gap deformity and post-natal feeding difficulties, both present in our patient, have been reported in a significant proportion of triploidy patients [van de Laar *et al.*, 2002]. The phenotype is further influenced by the parent-of-origin of the extra haploid set of chromosomes and may resemble certain imprinting disorders. Rittinger *et al* described a 14-year-old female whose early features of growth retardation, a broad forehead, clinodactyly and camptodactyly, and developmental delay were suggestive of Russell Silver syndrome. She subsequently developed truncal obesity, raising the possibility of Prader-Willi syndrome or maternal UPD14. Analysis of cultured skin fibroblasts demonstrated a triploid cell line with a maternal origin of the extra haploid set of chromosomes. Boonen *et al* also described a 14-year-old female with similar phenotypic features who was diagnosed with maternal origin triploidy. Conversely, one male infant described as having coarse facies, a bulbous nose, hepatic hamartomas, and congenital hyperinsulinism resembling Beckwith-Wiedemann syndrome was found to be mosaic for a paternal origin triploidy cell line detected in pancreatic tissue but absent in peripheral leukocytes or skin fibroblasts [Giurgea *et al.*, 2006]. In the present case, features typical of any particular imprinting disorder were not observed, and this likely reflects the relative contributions of 45,X and 68,XX cells to her development. Molecular short tandem repeat (STR) studies were unable to determine the parental origin of the extra haploid set of chromosomes, likely due to the low percentage of triploid cells (**Supplemental Table 1**), and other sources of tissue were unavailable for study. The STR result does, however, indicate normal bi-parental inheritance for the chromosome complement represented in the predominant (45,X) cell line. This is consistent with the absence of phenotypic features suggestive of an imprinting disorder.

There are several hypothesized mechanisms for the occurrence of mixoploidy [Jacobs *et al.*, 1978; Jacobs *et al.*, 1982; Uchida and Freeman 1985]. Delayed digyny and delayed dispermy each involve incorporation of a haploid genome into an early stage diploid zygote [Brems *et al.*, 2003; Dewald *et al.*, 1975; Muller *et al.*, 1993]. A preponderance of cases in which the extra chromosome set is of maternal origin has led some to suggest that digynic

fetuses have a better prognosis than those of diandric origin [van de Laar *et al.*, 2002]. Additional hypotheses involve independent and simultaneous formation of diploid and triploid zygotes that fuse to form a chimera, or postzygotic diploidization through which a triploid zygote loses a haploid chromosome complement [Daniel *et al.*, 2003; Golubovsky 2003].

There is one published report of a patient with a 45,X/69,XXY chromosome complement [Quigley *et al.*, 2005]. No diploid cell lines were observed, and the authors proposed that delayed dispermy was followed by loss of a sex chromosome from the diploid line. This patient presented at birth with 3-4 syndactyly, ambiguous genitalia, and atrial and septal defects [Quigley *et al.*, 2005]. In the present case, no diploid cell line was observed by chromosome analysis of 30 cells. Furthermore, STR studies performed on DNA from both cultured and uncultured blood showed no evidence of an additional haploid set of chromosomes. Notably, STR analysis did not demonstrate evidence of uniparental disomy for any of the chromosomes analyzed, suggesting that the mechanism of postzygotic diploidization is unlikely in the present case. The mechanism most consistent with our patient's unique 45,X/68,XX karyotype is a monosomy X conceptus, followed by delayed dispermy or polar body inclusion. However, in the absence of available tissue for further molecular studies of polymorphic markers, the origin of her mixoploidy cannot be ascertained.

CONCLUSION

In the present report, we describe a 3-month-old female with failure to thrive, dysmorphic craniofacial features, hypotonia, and left ventricular non-compaction who was found to have a unique 45,X/68,XX mixoploidy. Mosaicism for chromosomal aneuploidies is likely under-recognized and can obscure a clinical diagnosis. This case underscores the role of chromosome analysis in cases for which a molecular diagnosis is discordant with the observed phenotype. Despite increasing diagnostic use of molecular techniques such as genome sequencing and comparative genomic hybridization, chromosome analysis remains an essential clinical tool.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CMA	chromosomal microarray
FISH	fluorescence <i>in situ</i> hybridization

SD	standard deviation
SNP	single nucleotide polymorphism
TS	Turner syndrome

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Figure 1.

Images at 7 months (A), and 19 months of age (B, C) demonstrate frontal bossing, down-slanting palpebral fissures, bulbous nose, and prominent antihelices. High resolution chromosome analysis reveals two cell lines: 45,X (D) and 68,XX (E). FISH analysis of chromosomes 18 (blue) and X (green; F,G) and chromosomes 13 (green) and 21 (red; H,I) confirm 45,X/68,XX mixoploidy.