When standard genetic testing does not solve the mystery: a rare case of preimplantation genetic diagnosis for campomelic dysplasia in the setting of parental mosaicism

Biren Patel, M.D.,[a] Jan L. B. Byrne, M.D.,[a] Amber Phillips, M.S.,[b] James M. Hotaling, M.D.,[c] and Erica B. Johnstone, M.D.[a]

[a] Obstetrics and Gynecology, [b] Genetic Counseling, and [c] Surgery, University of Utah, Salt Lake City, Utah

Objective: To report a rare case of somatic mosaicism with a germline component of campomelic dysplasia in a woman undergoing in vitro fertilization with preimplantation genetic diagnosis (IVF-PGD).
Design: Case report.
Setting: Clinic.
Patient(s): A 28-year old G2P0110 and her 34-year old husband had two previous pregnancies complicated by fetal campomelic dysplasia with suspected germline mosaic mutation. The couple, both phenotypically normal, underwent IVF-PGD to reduce their chances of transmission. None of the embryos could initially be determined to be disease free, because all embryos shared either a maternal or a paternal short tandem repeat haplotype with the products of conception from her last pregnancy.
Intervention(s): Peripheral-blood cytogenomic single-nucleotide polymorphism (SNP) microarray to identify the carrier of the mutation, and IVF-PGD to identify the disease-free embryo.
Main Outcome Measure(s): Disease-free embryo.
Result(s): Only one of the five euploid embryos was identified as disease free.
Conclusion(s): A woman with suspected germline mosaicism for campomelic dysplasia was found to be a somatic mosaic with a germline component via a peripheral blood SNP microarray test. This identified her solitary disease-free embryo, which was transferred to her uterus but did not result in a viable pregnancy. (Fertil Steril® 2018;110:732–6. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Mosaicism, campomelic dysplasia, PGD, germline, somatic

Mosaicism refers to the presence of two genetically distinct populations of cells within an individual. They can be either somatic or germline. Somatic mosaicisms are developmental-stage post-zygotic mutations that affect only a portion of the total cells in the body. Their phenotypic outcomes are highly varied, depending on the time in embryologic development of the mutation and the cells throughout the body that end up carrying the mutation (1). The true frequency of somatic mosaicism is unknown because large parts of the genome are noncoding and many mutations lack clinical significance and are therefore not detected.

Germline mosaicism occurs when there are two or more genetically distinct populations of cells strictly limited to sperm or oocyte lines. Typically, germline mosaic mutations have no phenotypic consequence in the parent and are discovered only when there are multiple affected progeny. Rarely, there can be mosaicism in both somatic and germline cells. This is termed somatic mosaicism with a germline component and, depending on the degree of affected somatic cells, may have a phenotypic presentation. The true frequency of any type of germline mosaicism is also not known. The full spectrum of mosaicism is potentially confounding for clinicians given the

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Reprint requests: Biren Patel, M.D., University of Utah Hospital, 30 N. Medical Drive, Dept of Ob/Gyn, Salt Lake City, UT 84132 (E-mail: birenvpatel@gmail.com).

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varied presentations. Most initial cases are misclassified as a de novo mutation in the offspring.

CASE REPORT

The patient's consent was obtained before submitting this case report. A phenotypically normal 21-year old woman became pregnant with her also normal 25-year old husband. The pregnancy was complicated by fetal skeletal abnormalities observed in utero, and the female infant died at 3 months of life. Given the phenotypic appearance and classic radiographic findings, a clinical diagnosis of campomelic dysplasia (CD) was made.

CD is a rare genetic disorder, characterized by typical skeletal abnormalities, respiratory insufficiency, male-to-female sex reversal and often demise in utero or in infancy (2). The disease is caused by alterations in the *SOX9* transcription factor on chromosome 17, which is essential for sex determination and cartilage differentiation. It is autosomal dominant and is very rare, with an incidence of 1 individual per 100,000–200,000 (3).

Chromosomal analysis was completed on the infant and revealed a normal female chromosome complement (46,XX) without translocation, a commonly reported cause of CD (4, 5). Molecular (Sanger) sequence testing looking for point mutations was then performed to confirm diagnosis. The testing was normal for the critical disease-causing *SOX9* gene. Based on the strength of the clinical presentation, however, testing was expanded to include a gene duplication/deletion study, which identified a heterozygous complete *SOX9* gene deletion.

Neither the patient nor her husband had a significant medical history. Their family histories were also negative, so a de novo mutation in the infant was strongly suspected. The possibility of germline mosaicism in one of the parents was considered, but the lack of available testing for germline mutations prevented further work-up. The patient decided to conceive again naturally.

Several years later, the patient conceived again and a first-trimester ultrasound showed skeletal abnormalities concerning for recurrence of CD. The patient underwent a termination of the pregnancy, and testing on the products of conception confirmed CD. A single-nucleotide polymorphism (SNP) microarray showed a 625-kb deletion within the 17q24.3 segment containing the *SOX9* gene. The patient was counseled that one of the parents was most likely a germline mosaic carrier. The idea of a somatic mosaicism with germline component was also discussed. The couple were offered testing via peripheral blood with additional testing if negative to other bodily sources (buccal swabs, hair, etc.) but declined. At this time it was thought that a germline mutation without any somatic involvement was responsible, and the couple did not want to know which one was the carrier. They were advised to undergo in vitro fertilization (IVF) with preimplantation genetic diagnosis for future pregnancies.

In preparation for the preimplantation genetic diagnosis, haplotype testing with linked short tandem repeats (STRs) was performed on the patient, her husband, and the products of conception from the second pregnancy, so that it could be determined which copy of each parent's chromosome 17 might be affected. A sample was unavailable from the first pregnancy. The patient underwent IVF the following year but had her cycle cancelled for premature ovulation. She repeated IVF 3 years later and had seven blastocyst embryos biopsied for genetic testing (Table 1) with the use of SNP microarray (Roche NimbleGen 135K oligonucleotide array, with resolution of 40 kb) for disease localization and Next Gen Sequencing (Illumina Veriseq v1.0) for aneuploidy. Five of the embryos were euploid, and three of the embryos shared both paternal and maternal haplotypes around the *SOX9* segment with the affected pregnancy and were deemed to be at risk for the CD mutation.

Of the remaining two embryos, one had the maternal and the other the paternal haplotype from the affected pregnancy. Thus disease could not be ruled out without knowing which parent carried the germline mutation. After much counseling with the patient and her husband about the options, they elected to undergo experimental germline testing. Because sperm collection is both less costly and has fewer risks than oocyte collection, the husband volunteered to have his sperm tested. Multiple laboratories and genetic testing companies were contacted and one of the companies agreed to take on the project. The husband's sperm sample was sent for *SOX9* sequencing.

The company testing the husband's sperm encountered unexpected delays and after waiting several weeks without progress, the patient and her husband cancelled the germline testing on the sperm. Instead, they decided to test their

TABLE 1

Analysis comparing available embryos with affected fetal haplotypes.

Embryo	Paternal C17q24.3 segment	Maternal C17q24.3 segment	NGS aneuploidy	Transfer potential?
1	Different haplotype	Same as affected fetus	No	No
2	Same as affected fetus	Same as affected fetus	No	No
3	Same as affected fetus	Same as affected fetus	No	No
4	Same as affected fetus	Different haplotype	No	No
5	–	–	Yes	No
6	Same as affected fetus	Same as affected fetus	No	No
7	–	–	Yes	No

Note: NGS = next-generation sequencing.

Patel. Campomelic dysplasia and mosaicism. Fertil Steril 2018.