Case Reports in Women's Health 41 (2024) e00583

Contents lists available at ScienceDirect

Case Reports in Women's Health

journal homepage: www.elsevier.com/locate/crwh

Novel characterization of *CASK* variant *c.1963 A>G (p.Asn655Asp)* through whole-exome sequencing in a monochorionic diamniotic twin fetus with significant brain anomalies: A case report

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ARTICLE INFO

Keywords:
Calcium/calmodulin dependent serine protein kinase (*CASK*) gene
Whole-exome sequencing (WES)
Variant of uncertain significance
Monochorionic diamniotic
Case report

ABSTRACT

Whole-exome sequencing is an evolving technology in perinatal diagnosis which allows identification of genetic etiologies that would otherwise go undetermined. In this case report, a 38-year-old Hispanic woman, G5P3013, with a monochorionic diamniotic twin gestation with one fetus displaying significant cranial abnormalities on prenatal ultrasound and magnetic resonance imaging (MRI) of the brain is presented. Fetal anomalies included bilateral ventriculomegaly, absent cavum septum pellucidum, and absent corpus callosum. Diagnostic amniocentesis with chromosome analysis, chromosomal microarray, alpha-fetoprotein, cytomegalovirus, toxoplasmosis, and parvovirus had normal results. Whole-exome sequencing for the anomalous fetus detected a de novo mosaic variant of uncertain significance (VUS) in the calcium/calmodulin dependent serine protein kinase (*CASK*) gene: *c.1963 A > G (p.Asn655Asp)*. This variant was absent in the normal twin fetus, the mother, and the father. Pathogenic CASK gene mutations are associated with three syndromes: FG syndrome 4, intellectual developmental disorder and microcephaly with pontine and cerebellar hypoplasia (MICPCH), and intellectual developmental disorder with or without nystagmus. Whole-exome sequencing identified a potential etiology for the anomalies detected. The variant likely arose de novo and was the potential cause of the identified cranial abnormalities in one fetus of this monochorionic diamniotic twin gestation. Whole-exome sequencing may provide additional diagnostic utility when standard diagnostic testing is noncontributory.

1. Introduction

Prenatal genetic technology is advancing rapidly. It is now possible to identify sub-chromosomal abnormalities with chromosomal microarray analysis (CMA). The American College of Obstetrics and Gynecology (ACOG) and Society of Maternal Fetal Medicine (SMFM) recommend using CMA testing in the evaluation of an anomalous fetus and the technology should be made available to any desiring individual [1]. G-banded karyotypes have a resolution of 7–10 million base pairs while CMA can detect abnormalities as small as 50 kilobases [2]. CMA does not require living tissue and is less efficient at detecting mosaicism [2]. Whole-exome sequencing (WES) is a more novel modality which examines coding exons but is not currently recommended for routine use [1]. The following case represents a situation where WES provided insight into an anomalous fetus with otherwise normal diagnostic studies.

2. Case Presentation

A 38-year-old woman, G5P3013, at 13 weeks and 0 days gestational age, with a monochorionic diamniotic twin gestation, presented for care. Pregnancy dating was by last menstrual period and confirmed by

Abbreviations: ACOG, American College of Obstetrics and Gynecology; AFP, alfa-fetoprotein; CASK, calcium/calmodulin dependent serine protein kinase; CMA, chromosomal microarray analysis; MRI, magnetic resonance imaging; SMFM, Society of Maternal Fetal Medicine; VUS, variant of uncertain significance; WES, whole-exome sequencing.

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https://doi.org/10.1016/j.crwh.2024.e00583
Received 16 December 2023; Received in revised form 12 January 2024; Accepted 15 January 2024
Available online 17 January 2024

an 8-week gestational age ultrasound. She was of South American heritage. Her three previous full-term spontaneous vaginal deliveries had been uncomplicated. She had had a spontaneous abortion 18 years previously managed non-surgically. Her last vaginal delivery was 7 years prior. Her youngest child and the pregnancy described were conceived with the same partner. The three previous pregnancies were conceived with a different partner. She had a past medical history of chronic hypertension controlled without medication and class I obesity with a body mass index (BMI) of 33 kg/m^2. She had an otherwise unremarkable medical history. She denied an infectious history including toxoplasmosis, syphilis, cytomegalovirus, mumps, rubella, and influenza. She was started on daily prenatal vitamins, iron supplementation, folic acid, and calcium and vitamin D supplementation. The patient's second son (conceived with a different partner) was diagnosed with autism spectrum disorder. Family history was negative for intellectual disability, genetic or chromosomal disorders, maternal metabolic disorders, or history of birth defects.

First-trimester screening reported a low risk for trisomy 21, trisomy 18, and trisomy 13. However, the PAPP-A level was reported to be low, at the 5th percentile. Due to the monochorionic diamniotic twin gestation, serial ultrasound examinations to assess for twin-to-twin transfusion syndrome were planned as well as a detailed fetal anatomy ultrasound and fetal echocardiogram. The patient declined prenatal diagnostic testing after her initial genetic counseling consultation for advanced maternal age and monochorionic diamniotic twin gestation. Ultrasound evaluation at 15 weeks and 5 days gestational age revealed fetus B to have a dangling choroid plexus and suspected ventriculomegaly. Subsequent fetal magnetic resonance imaging (MRI) at 16 weeks and 5 days gestational age noted bilateral ventriculomegaly, absent cavum septum pellucidum, and absent corpus callosum in fetus B. MRI of fetus A revealed no abnormalities. Ultrasound evaluation at 18 weeks gestational age revealed bilateral ventriculomegaly of fetus B with lateral ventricles measuring 17 mm on the left and 14 mm on the right. The cavum septum pellucidum and corpus callosum in fetus B were not visualized. No extracranial abnormalities were noted on the fetal anatomy scan of fetus B. Fetus A had no identifiable abnormalities in all organ systems. After counseling, the patient elected to proceed with a diagnostic amniocentesis at 19 weeks and 2 days gestational age. Amniocentesis testing included chromosomal analysis, CMA, AFP, cytomegalovirus, toxoplasmosis, and parvovirus. All test results were negative for both fetuses and showed two normal females. She was further counseled that it was unknown whether fetus B could have an undetected syndrome. At that time the option of WES was reviewed. A selective reduction of fetus B at 22 weeks and 1 day gestational age was performed after counseling on the observed central nervous system abnormalities by maternal fetal medicine and pediatric neurology. The patient requested WES for fetus B after the selective reduction. The turnaround time for the test was approximately 6 weeks. The WES for fetus B detected a de novo mosaic variant of uncertain significance (VUS) in the calcium/calmodulin dependent serine protein kinase (*CASK*) gene: *c.1963 A > G (p.Asn655Asp)*. The variant was noted to be present in 12.5% of 32 sequencing reads. WES of fetus A, the mother, and the father revealed that this variant was not present. The patient had an uncomplicated term vaginal delivery of fetus A. This fetus had no identifiable neurological deficits and central nervous system imaging was not deemed necessary.

3. Discussion

CMA and WES are new and evolving technologies. CMA detects both large changes in chromosomes and submicroscopic abnormalities, including copy number variants. Drawbacks of CMA are that it cannot detect balanced chromosomal rearrangements or low-level tissue mosaicism and generally does not assess for single-gene disorders or single-nucleotide variants [1]. The vast majority of anomalous fetuses with a normal karyotype will also have a normal CMA [1]. WES is a next-generation sequencing technology analyzing only coding regions of DNA or exons. Exons represent only 1–2% of the genome but account for 85% of disease-causing mutations [3]. WES utilizes "trio sequencing", where sequencing of both parents' DNA and the proband's DNA is done for comparison [3]. WES technology is considered investigational and both ACOG and SMFM discourage routine use of WES except in select circumstances such as recurrent or lethal anomalies [1]. The results may also take weeks or months to be available.

The *CASK* gene, located on the X chromosome, is known to be expressed highly in the mammalian nervous system coding for a membrane scaffolding protein important in synaptic interactions [4]. Pathogenic *CASK* gene mutations are associated with three syndromes: FG syndrome 4, intellectual developmental disorder and microcephaly with pontine and cerebellar hypoplasia (MICPCH), and intellectual developmental disorder with or without nystagmus [5]. FG syndrome 4 (FGS4) was first described by Opitz and Kaveggia (1974) and named with the Opitz system of using the initials of patients' surnames [6]. This syndrome is associated with intellectual disability, congenital hypotonia, constipation, behavioral abnormalities, and dysmorphic features. FGS4 and intellectual developmental disorder with or without nystagmus, are both X-linked recessive disorders. Females with a heterozygous pathogenic mutation in the *CASK* gene are generally expected to develop MICPCH [5]. The MICPCH syndrome is associated with varying degrees of pontocerebellar hypoplasia and severe intellectual disability, among other symptoms [7].

In this case, WES detected a disorder related to the *CASK* gene, specifically a de novo mosaic VUS, the *c.1963 A > G (p.Asn655Asp)* variant. The diagnostic testing laboratory, GeneDx, noted the variant to be present in 12.5% of 32 sequencing reads. This variant of the *CASK* gene has not been previously published as pathogenic. For this reason, the variant was labeled as a VUS. As both parents and the non-anomalous fetus tested negative for this variant, it was suggested by the testing laboratory that the variant developed de novo. Additionally, the laboratory reported that the in silico analysis supported that this missense variant would have a deleterious effect on protein structure/function and that it has not been observed at significant frequency in large population cohorts [8]. Fetal neurological abnormalities and other congenital anomalies may be attributed to maternal risk factors, such as infectious diseases, genetic disorders, and other multifactorial conditions. However, there was no evidence of these maternal conditions or risk factors in this patient.

This case is unique for several reasons. First, while most WES studies use trio sequencing in which exomes are compared to both parents of the proband, the presence of a monochorionic diamniotic twin gestation allowed a third comparison for the anomalous fetus: the twin fetus. The mother, father, and twin gestation (Twin A) did not harbor the identified variant in the *CASK* gene. Post-twinning somatic de novo mutations can occur during early mitotic events, resulting in genomic variation between monozygotic twins. This early presence of a mutation may then result in a significant level of mosaicism [9]. It can be hypothesized that an early mitotic error may be the cause of the de novo *CASK* mutation in twin B, which, at a significant level of mosaicism, would result in phenotypic abnormalities. The absence of this mutation in the phenotypically normal co-twin and parents adds credence to the mutation as the cause of the structural abnormalities. The mutation has been characterized as a VUS. Second, the case represents a situation where WES may have identified a causative abnormality that was not identified by standard prenatal diagnostic technology. Strict interpretation of ACOG and SMFM recommendations for WES would have resulted in not performing this testing. This case suggests that a more liberal approach to ordering prenatal WES than recommended by ACOG and SMFM may be warranted, as both societies indicate such technology should not be routinely used. This de novo mosaic VUS in the *CASK* gene: *c.1963 A > G (p.Asn655Asp)* was a potential cause of the significant neurological abnormalities in one twin fetus. This case provides an argument for further consideration of WES technology when other diagnostic tests are non-

contributory.

Contributors

Nathan A. Keller contributed to conception of the case report, completion of the literature review, acquiring and interpreting the data, drafting the manuscript, and revising the manuscript for important intellectual content.

Luis A. Bracero contributed to conception of the case report, completion of the literature review, acquiring and interpreting the data, drafting the manuscript, and revising the manuscript for important intellectual content.

Insaf Kouba contributed to patient care, conception of the case report, acquiring and interpreting the data, drafting the manuscript, and revising the manuscript for important intellectual content.

Abigail Steinberg contributed to patient care, conception of the case report, completion of the literature review, acquiring and interpreting the data, drafting the manuscript, and revising the manuscript for important intellectual content.

Jolene Muscat contributed to patient care, drafting the manuscript, and revising the manuscript for important intellectual content.

David Bergman contributed to patient care, drafting the manuscript, and revising the manuscript for important intellectual content.

All authors approved the final submitted manuscript.

Funding

No funding from an external source supported the publication of this case report.

Patient consent

Written informed consent was obtained from the patient for publication of this case report.

Provenance and peer review

This article was not commissioned and was peer reviewed.

Conflict of interest statement

The authors declare that they have no conflict of interest regarding the publication of this case report.

References

[1] American College of Obstetrics and Gynecology Committee Opinion 682, Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology, December 2016. Reaffirmed 2020.

[2] J.L. Giordano, M. Stosic, B. Levy, R. Wapner, Chapter 12: Chromosomal microarray analysis, in: M.E. Norton, J.A. Kuller, L. Dugoff (Eds.), Perinatal Genetics, Inc, Elsevier, 2019, pp. 125–136.

[3] I.B. Van Den Veyver, Chapter 13: Exome and genome sequencing, in: M.E. Norton, J. A. Kuller, L. Dugoff (Eds.), Perinatal Genetics, Inc, Elsevier, 2019, pp. 137–148.

[4] Y.P. Hsueh, The role of the MAGUK protein CASK in neural development and synaptic function, Curr. Med. Chem. 13 (16) (2006) 1915–1919, https://doi.org/10.2174/092986706777585040.

[5] Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 300172: 5/05/2023: World Wide Web URL: https://www.omim.org/entry/300172.

[6] Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 300422: 5/4/2023: World Wide Web URL: https://www.omim.org/entry/300422.

[7] Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 300749: 11/11/2022: World Wide Web URL: https://www.omim.org/entry/300749.

[8] M. Lek, K. Karczewski, E. Minikel, et al., Analysis of protein-coding genetic variation in 60,706 humans, Nature. 536 (2016) 285–291, https://doi.org/10.1038/nature19057.

[9] M. Mohiuddin, R.F. Kooy, C.E. Pearson, De novo mutations, genetic mosaicism and human disease, Front. Genet. 13 (2022) 983668, https://doi.org/10.3389/fgene.2022.983668.