Clinical diagnostic exome sequencing identified compound heterozygous *ASPM* gene alterations in a fetus with marked microcephaly.

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25 YEARS SINCE 1991 ACMG

Disclosure

I am a full time salaried employee at Ambry Genetics. Exome sequencing is among Ambry Genetics' commercially available tests.

Introduction

- 2012 ACMG Board of Directors policy statement

- Recent reports have described a number of prenatal cases in which DES was used to obtain a molecular diagnosis (Carss 2014; Hillman 2014; Drury 2015; Alamillo 2015).

- We report a postmortem case referred to our laboratory for DES due to marked microcephaly that was identified by ultrasound during the third trimester of pregnancy.

Alamillo C, *et al.* (2015) *Prenat Diagn* **35**(11):1073-8.

Carss KJ, *et al.* (2014) *Hum Molecul Genet* **23**(12):9.

Drury S, *et al*. (2015) *Prenat Diagn* **35**:1010-1017.

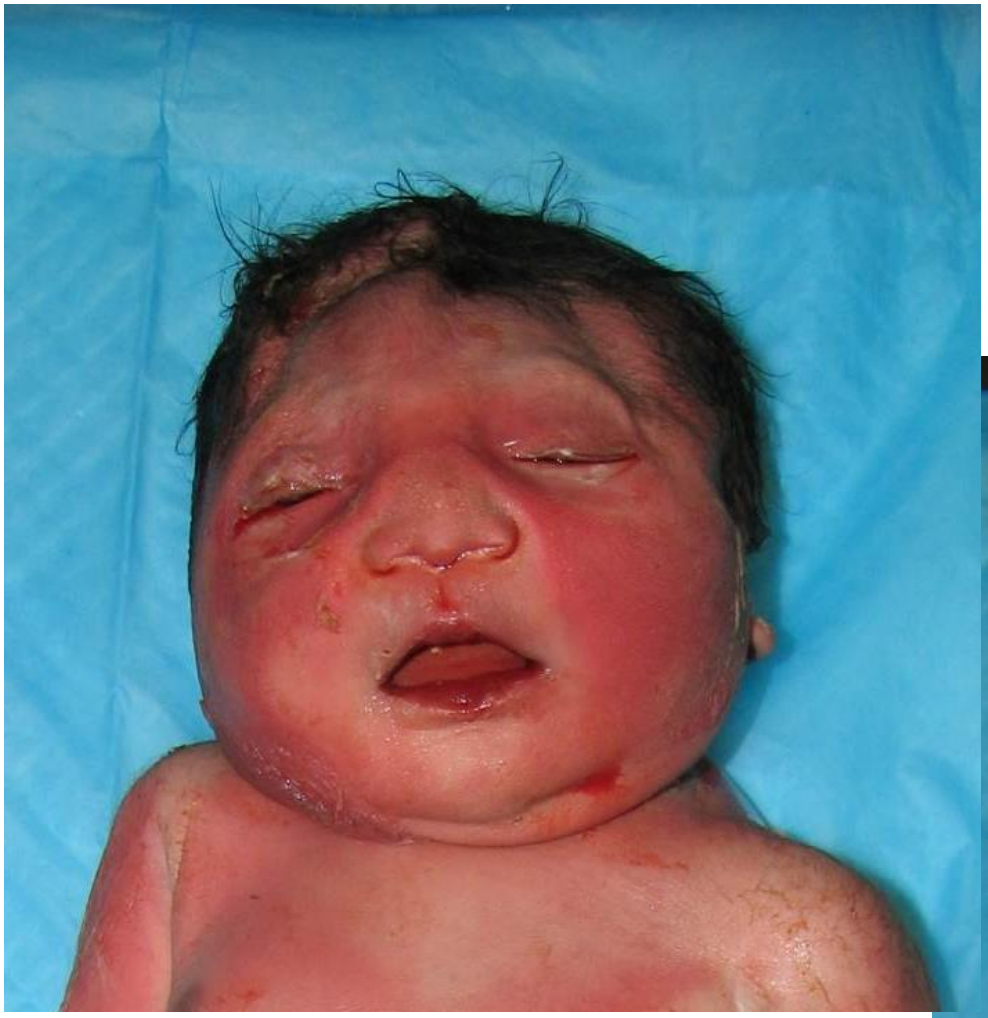
Hillman DW, et al. (2014) *Ultrasound in Obstet and Gynecol* **45**(1):6.

Clinical Details

- Patient is a 32 year old G1P0
- Family history was unremarkable, no consanguinity
- Sequential screening results
- Ultrasound findings
    - First trimester scan: 12w4d
    - Anatomic survey: 19w3d
    - Follow-up ultrasound: 30w5d
- Fetal MRI

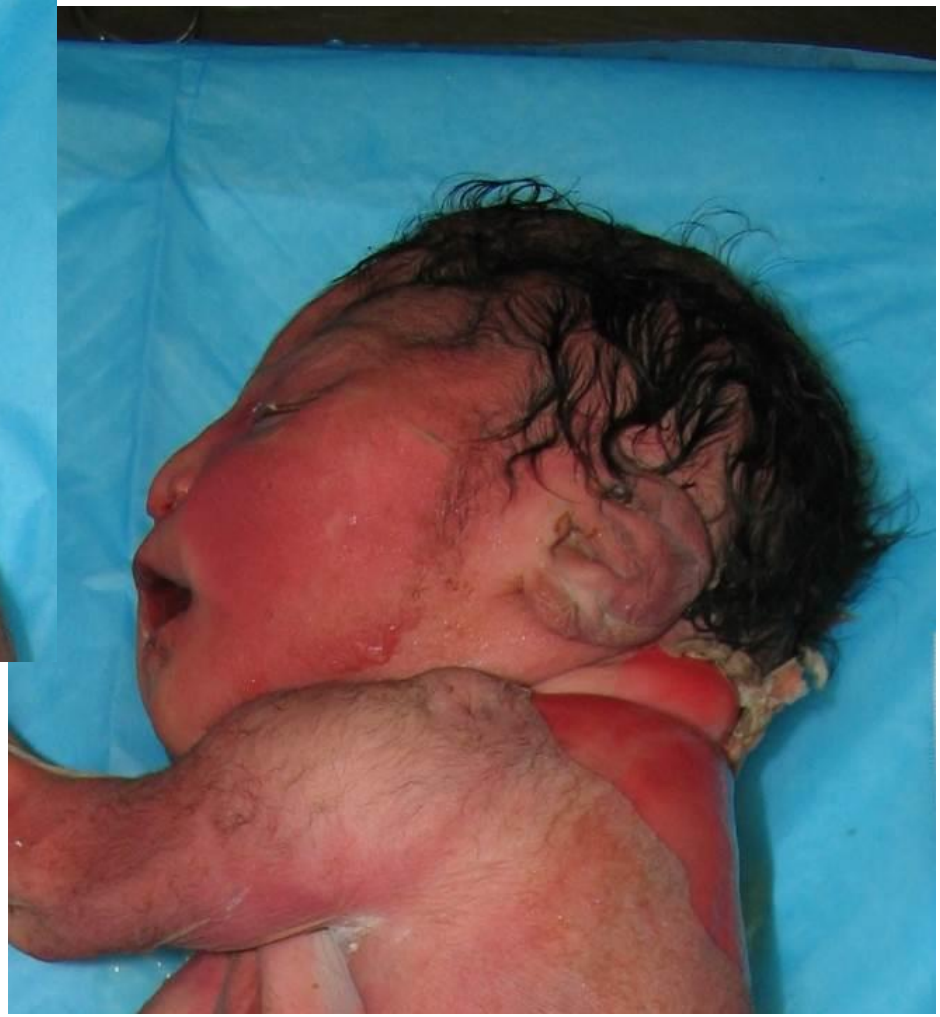
Clinical Details: Fetal Autopsy Findings

- Microcephaly, with head circumference <3rd percentile
- Multiple brain anomalies
- Dysmorphic features
- Karyotype and microarray

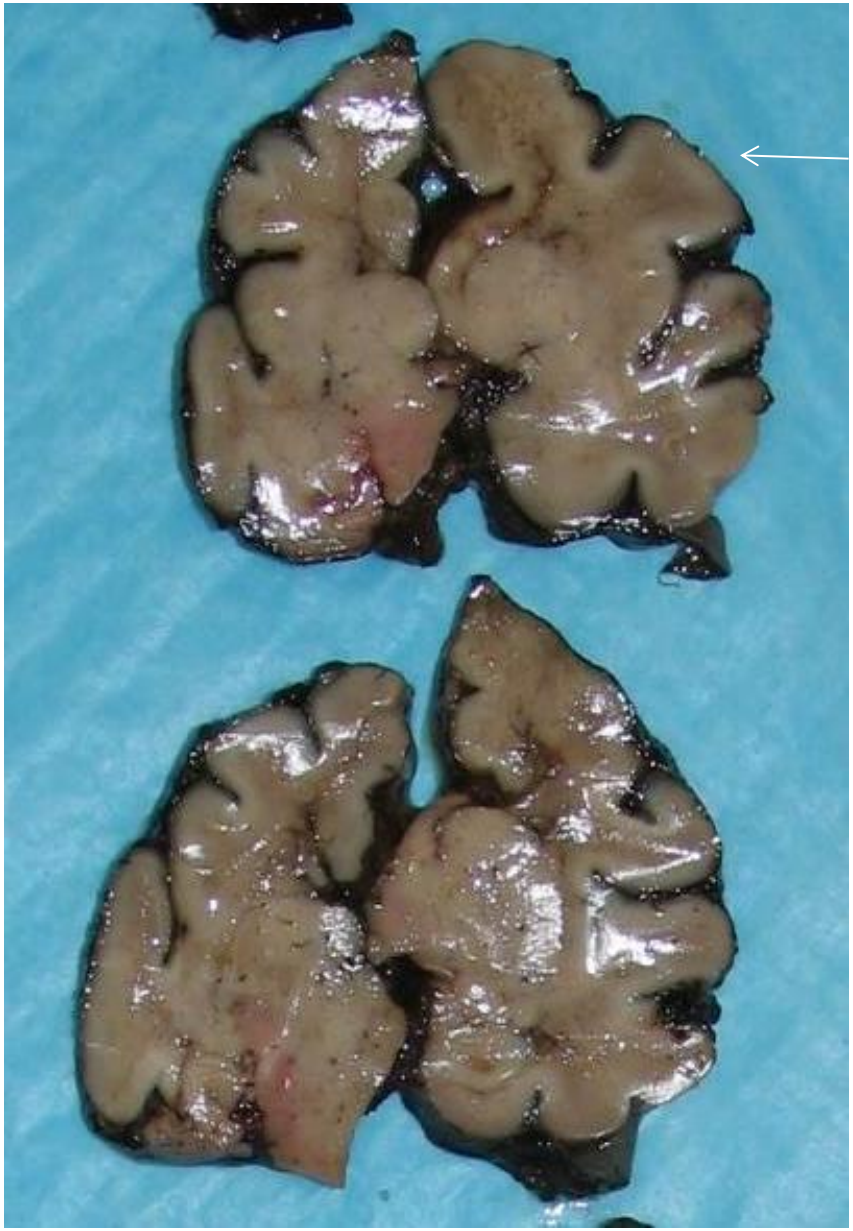


37 weeks gestation stillborn female
Dysmorphic facial features
Small cranial vault

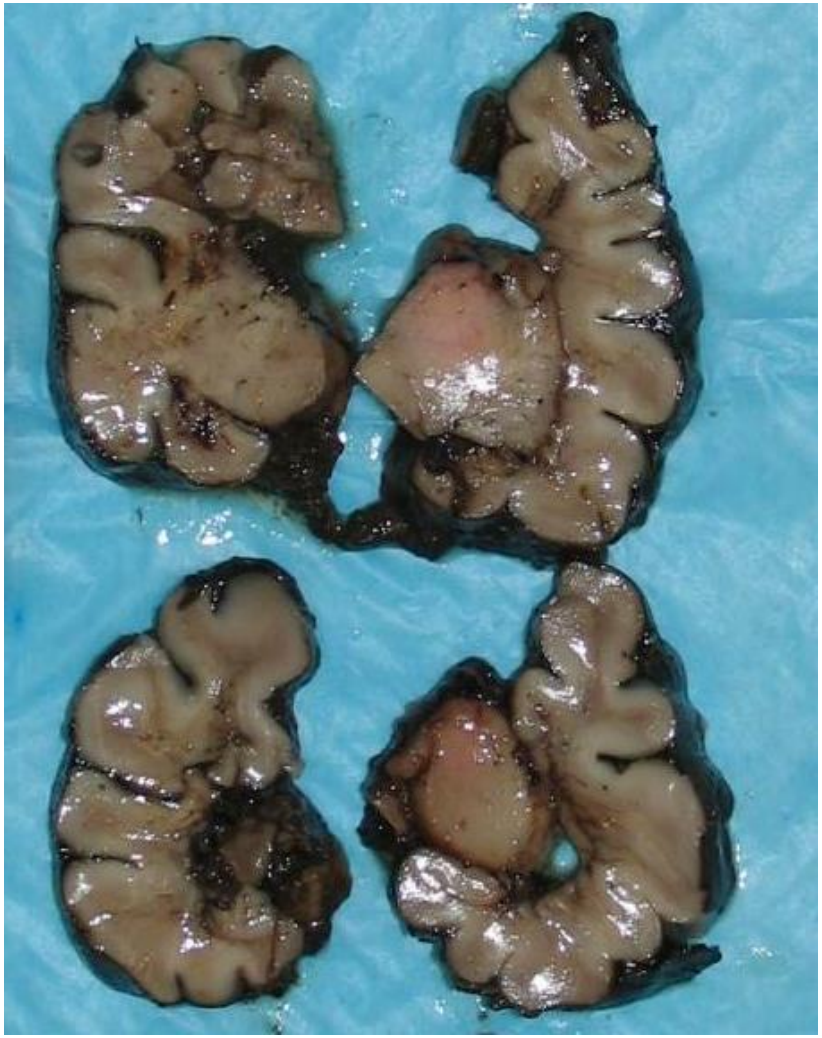
(postmortem changes are evident)



Autopsy and slides prepared by
Aida Cviko, M.D., Ph.D. and Galen Schauer, M.D.
Regional Fetal Pathology Service
The Permanente Medical Groups, Inc.
Oakland, California



Small brain: weight 152 gm
(normal range for 37 wks gestation is 228-368 gm)
Reduced gyration, worst in frontal lobes
Thin cortex averages 3 mm thick
Subcortical white matter is severely reduced



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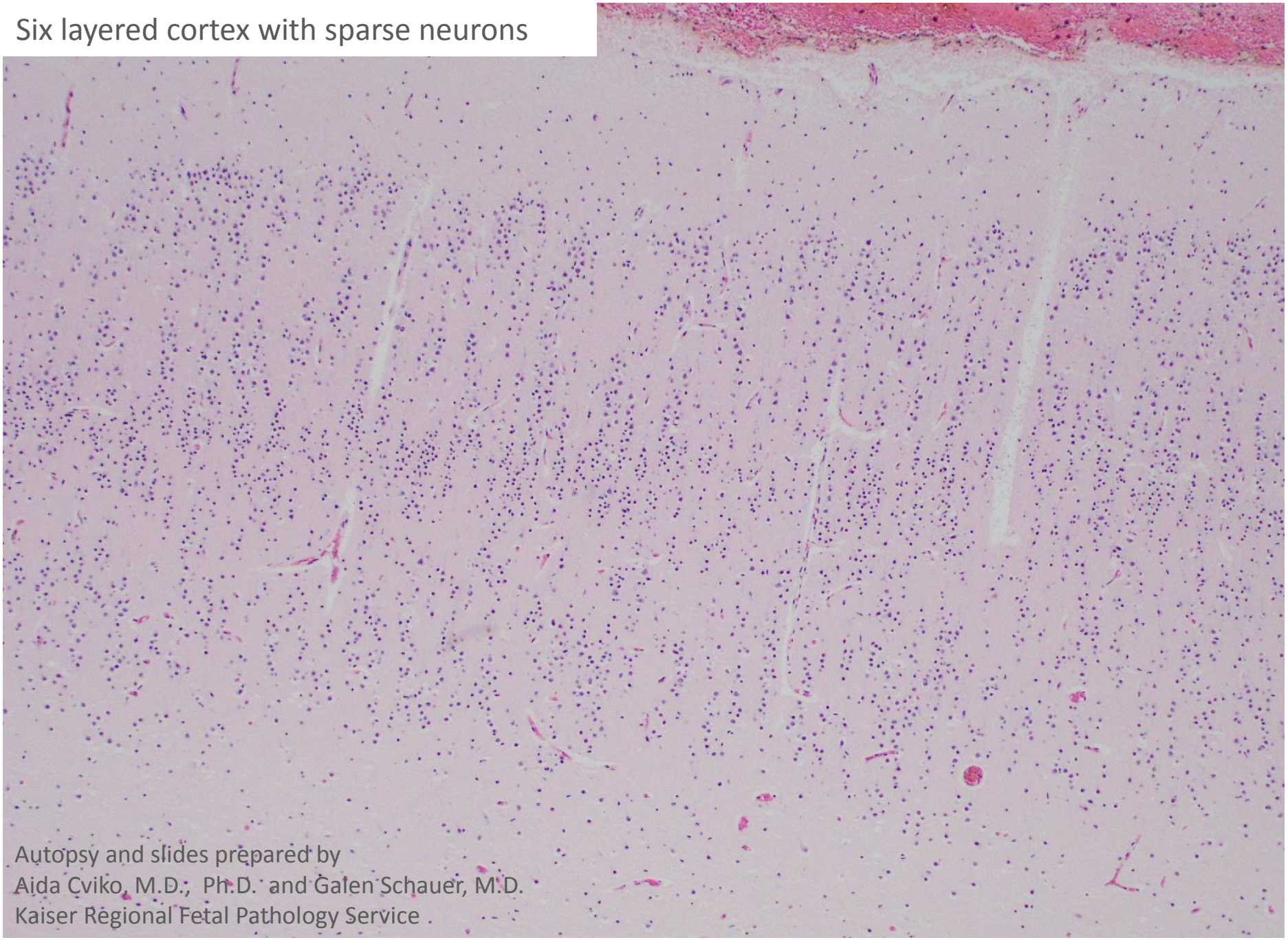
Six layered cortex with sparse neurons



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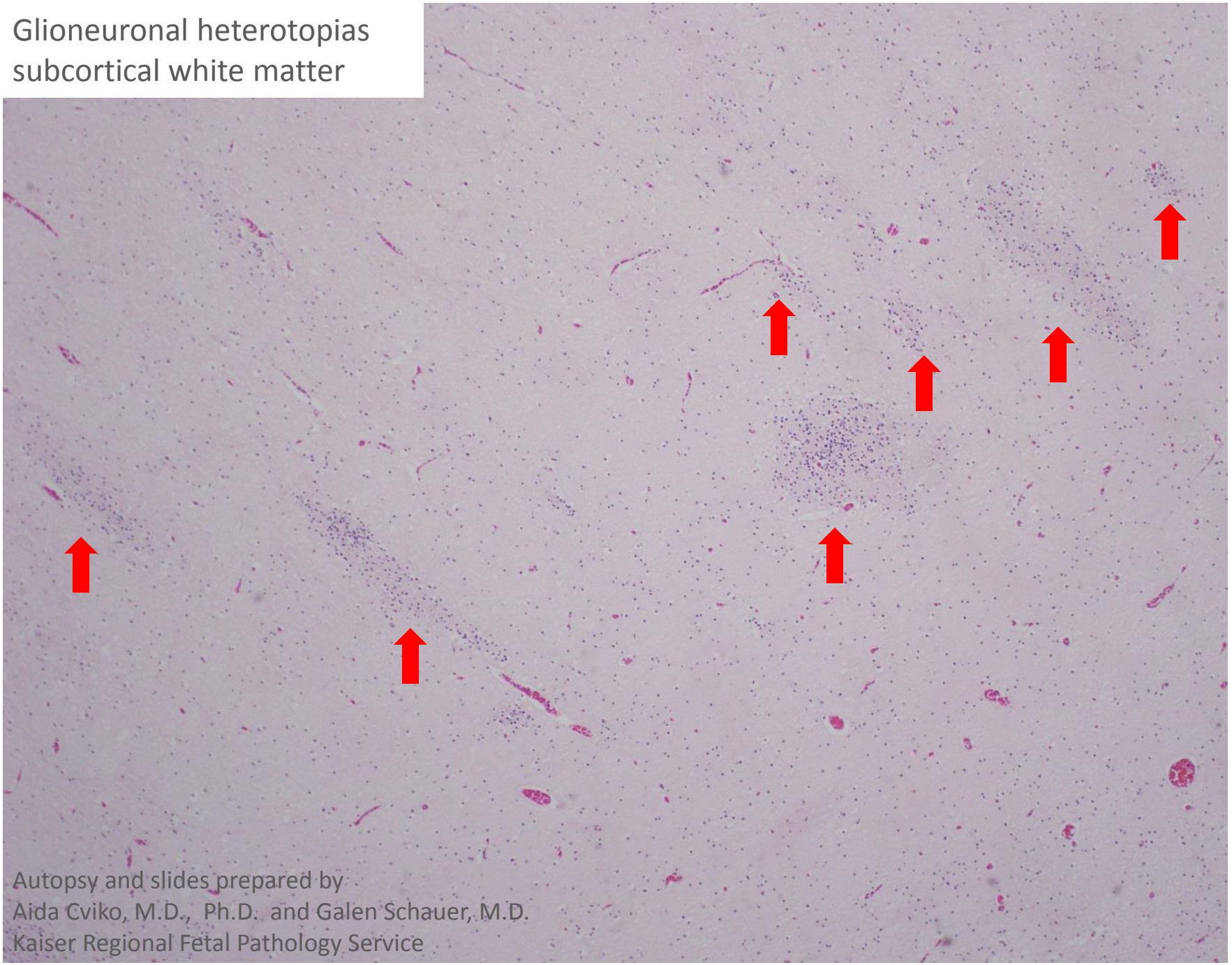
Glioneuronal heterotopias
subcortical white matter



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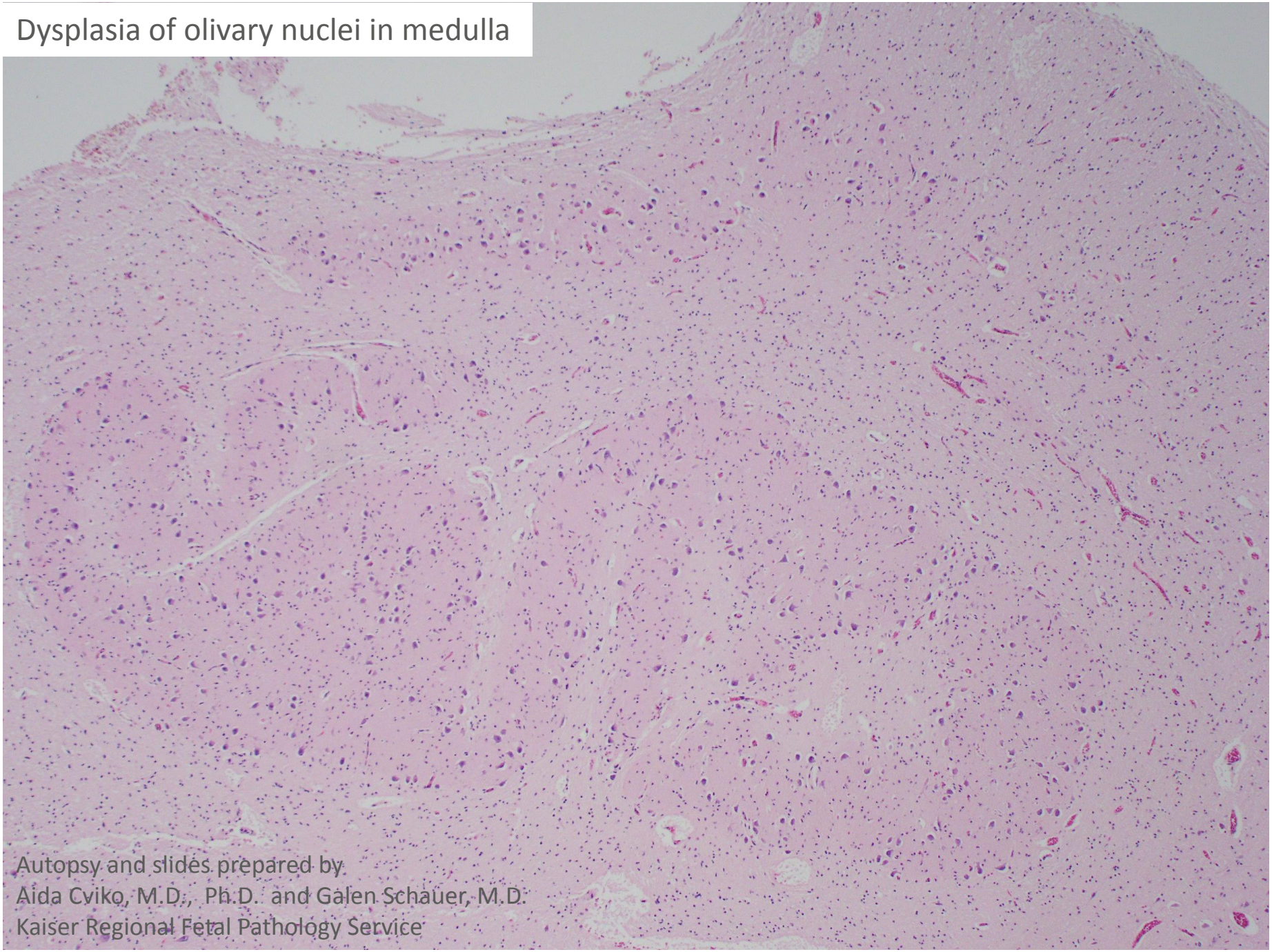
Dysplasia of olivary nuclei in medulla



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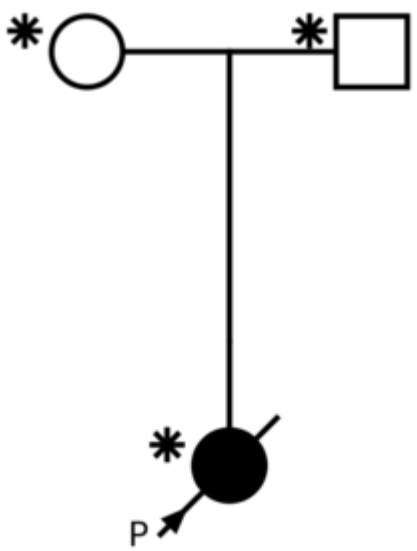
Genetic Counseling: Pre-Test

- *In-person pre-exome counseling session*
- *Appropriately upset by the autopsy findings*
- *Concerned about recurrence risk*
- *The limitations of exome testing were discussed*
- *Discussed possibility of results being positive, negative, or of uncertain significance*
- *The couple stayed in touch with the genetic counselor while results were pending*

Diagnostic Exome Sequencing

- **Primary Indication: Disorder primarily affecting the brain**
- There was no reported family history of similar findings.
- DES was performed on a DNA sample isolated from fetal tissue.
- Parental blood samples were submitted so that trio exome sequencing could be performed.
- Secondary findings were declined by the parents.



Diagnostic Exome Sequencing: The Process

Diagnostic Exome Sequencing: Candidates

Results revealed compound heterozygous *ASPM* alterations in the fetal sample:

One maternal nonsense mutation: (c.1286C>G; p.Y462*)

One paternal splice site mutation: (c.8988-1G>C)

- This alteration is predicted to abolish the native acceptor splice site
- This alteration is not observed in healthy cohorts
- The altered nucleotide is conserved throughout vertebrates
- The alteration is predicted to be deleterious by in silico models

These alterations were both classified as pathogenic mutations.

Diagnostic Exome Sequencing: *ASPM*

- The *ASPM* gene encodes the abnormal spindle-like microcephaly-associated protein.

- Alterations in this gene are generally inherited in an autosomal recessive fashion in association with primary microcephaly-5 (MCPH5).

- MCPH5 is characterized by:
    - Decreased occipital-frontal circumference
    - Intellectual disabilities, speech delay
    - Seizures
    - Short stature
    - Abnormal brain MRI

Diagnostic Exome Sequencing: *ASPM*

- A number of sibships in separate consanguineous families have been reported to have homozygous mutations in *ASPM* (Darvish, 2010; Desir, 2008; Sajid Hussain, 2013; Shen, 2005).

- Desir et al. (2008) reported on a consanguineous family with one daughter affected with primary microcephaly in addition to an ongoing affected pregnancy.

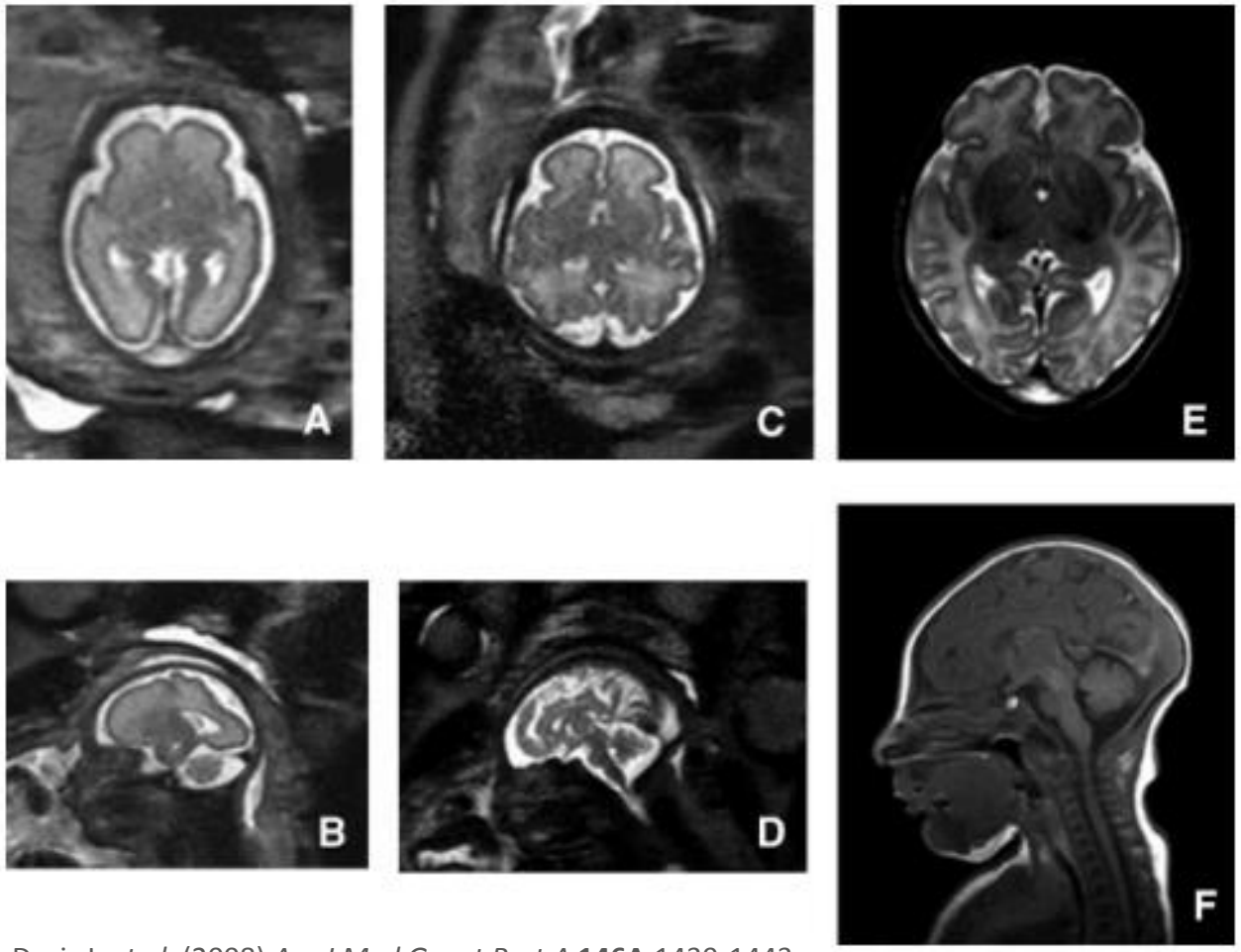
Darvish H, *et al.* (2010) *J Med Genet* **47**:823-828.

Desir J, *et al.* (2008) *Am J Med Genet Part A* **146A**:1439-1443.

Sajid Hussain M, *et al.* (2013) *Clin Genet* **83**:446-451.

Shen J, *et al.* (2005) *J Med Genet* **42**:725-729.

Diagnostic Exome Sequencing: *ASPM*



Desir J, *et al.* (2008) *Am J Med Genet Part A* **146A**:1439-1443.

Diagnostic Exome Sequencing: *ASPM*

- **The proband's clinical presentation is consistent with that of previously-reported patients with ASPM alterations**

- **Based on the available evidence, the clinical overlap of this gene with the patient's reported phenotype is positive. The patient's overlapping features include microcephaly, small brain, hypoplasia of corpus callosum, and sloping forehead.**

Diagnostic Exome Sequencing: The Result

CHARACTERIZED GENES: **POSITIVE** **RELEVANT ALTERATIONS DETECTED**

Results Summary

Gene Symbol	Gene Inheritance	Characterized/Novel Gene*	Protein Change	Nucleotide Change	Genotype	Alteration Type	Alteration Classification	Gene Overlap
ASPM	Autosomal recessive	Characterized	p.Y462*	c.1386C>G	Heterozygous, maternal	Nonsense	Pathogenic	Positive
				c.8988-1G>C	Heterozygous, paternal	Splice	Pathogenic	

Patient's likely diagnosis based on molecular results:

Primary microcephaly-5 (MCPH5) (MIM_605481)

Genetic Counseling: Post-Test

- *In-person results disclosure*
- *Discussed the two ASPM mutations in the fetus*
- *Discussed that they are both carriers of this condition*
- *25% recurrence risk in future pregnancies*
- *The couple was pleased about the positive result*
- *The couple is relieved that they can perform prenatal diagnosis in future pregnancies*
- *They are currently trying to conceive*
- *Will contact their genetic counselor when pregnant to discuss testing options*

Take-Home Messages

- **This case illustrates the importance of discussing the option of collecting and maintaining DNA samples following pregnancy termination, fetal demise, or perinatal death in pregnancies affected with multiple congenital anomalies and/or a suspected genetic condition.**

- **DES is likely to be a valuable diagnostic testing option for pregnancies with multiple congenital anomalies detected by prenatal ultrasound.**

- **Pathogenic DES results allow for recurrence risk counseling and provide the option for targeted prenatal diagnosis in future pregnancies.**

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Thank You

Aida Cviko, MD, PhD and Galen Schauer, MD
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We would like to send a special thank you to the family presented in this talk for allowing us to share their story with the scientific community.