Diagnostic Exome Sequencing Positively Identified Relevant Alterations in More Than Half of Prenatal Samples Tested

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BACKGROUND

- We performed a retroactive analysis of the first 7 cases referred to our laboratory for diagnostic exome sequencing following fetal demise or termination of pregnancy.
- All cases had multiple congenital anomalies identified by Level II ultrasound and were known to have normal karyotypes.
- All cases involved sequencing of the trio, including proband and parental samples. Parental samples were used for filtering and analysis. Samples were not available for any of the previously affected pregnancies or siblings.
- Chromosomal microarray results were available for 5 cases and were either normal or uncertain. In 1 case there was a continuous region of homozygosity identified that was consistent with reported consanguinity.
- Six of the 7 cases were the 2nd abnormal pregnancy of normal parents and in 2 cases there was a concerning history in more distant relatives of similar or possibly related findings.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from cultured amniocytes or products of conception which were sent to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **Exome library preparation**, sequencing, bioinformatics, and data analysis were performed as previously described (Farwell et al. 2014).

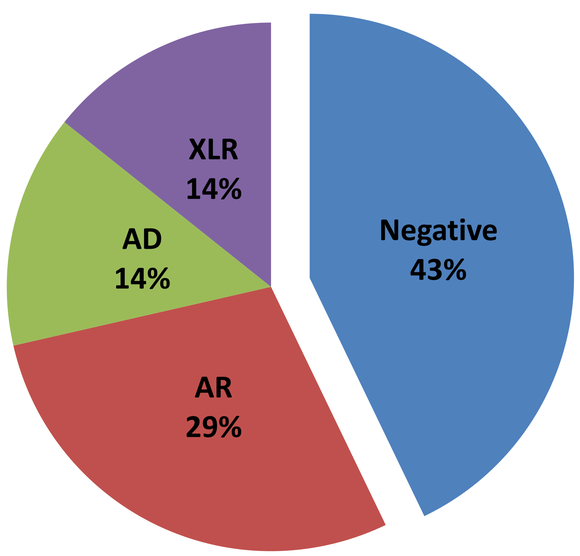
RESULTS

- Diagnostic exome sequencing positively identified relevant alterations in more than half (4/7) of the cases included in the study.
- Identified diagnoses included osteogenesis imperfecta II (*COL1A2)*, glycogen storage disease IV (*GBE1*), oral-facial-digital syndrome 1 (*OFD1*), and *RAPSN*-associated fetal akinesia deformation sequence.
- The cases of glycogen storage disease IV and *RAPSN*-associated fetal akinesia deformation sequence were inherited in an autosomal recessive fashion with both parents confirmed to be carriers of the inherited alterations.
- The fetus affected with osteogenesis imperfecta II was found to have a *de novo* autosomal dominant alteration.
- The fetus with oral-facial-digital syndrome 1 was a male found to have a maternally inherited X-linked recessive alteration.
- All three negative cases had positive family histories of similar findings.

MOLECULAR DETAILS OF POSITIVE CASES

Patient	Gene	Inheritance	Alteration	Zygosity	Diagnosis	Family History
1	*COL1A2*	AD	c.1361G>T (p.G454V)	Heterozygous *de novo*	Osteogenesis imperfecta II	No
2	*GBE1*	AR	c.1064G>A (p.R355H) c.1543C>T (p.R515C)	Heterozygous Inherited Heterozygous Inherited	Glycogen storage disease IV	Yes
3	*OFD1*	XLR	c.929T>C (p.F310S)	Hemizygous Inherited	Oral-facial-digital syndrome 1/ Simpson-Golabi-Behmel syndrome, type 2	Yes
4	*RAPSN*	AR	c.484G>A (p.E162K)	Homozygous Inherited	*RAPSN*-associated Fetal Akinesia Deformation Sequence	Yes

RESULTS AND INHERITANCE



CLINICAL FEATURES AND ORGAN SYSTEM INVOLVEMENT IN FETUSES UNDERGOING DIAGNOSTIC EXOME SEQUENCING

	Cardiovascular	Craniofacial	Dysmorphic Features	Gastrointestinal	Genitourinary	Musculoskeletal	Neurologic	Opthalmologic	Pulmonary	Renal	Intrauterine Growth Retardation	Increased NT/Cystic Hygroma	Edema/Hydrops	Ultrasound Soft Markers
Positive Result	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Negative Result	X			X					X	X				

TAKE-HOME POINTS

- Diagnostic exome sequencing may be a valuable diagnostic testing option for pregnancies with multiple congenital anomalies detected by prenatal ultrasound, however further research is needed to confirm these findings.
- The findings of this study underscore the importance of discussing the option of collecting and maintaining DNA samples following cases of pregnancy termination, fetal demise, or perinatal death in pregnancies affected with multiple congenital anomalies and/or a suspected genetic condition.
- Prenatal diagnostic exome sequencing enables providers to offer a viable testing option for families who wish to determine recurrence risks for future pregnancies and in future generations.

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