welcome to an
AMBRY GENETICS
presentation

Diagnostic Exome Sequencing in a Prenatal Setting

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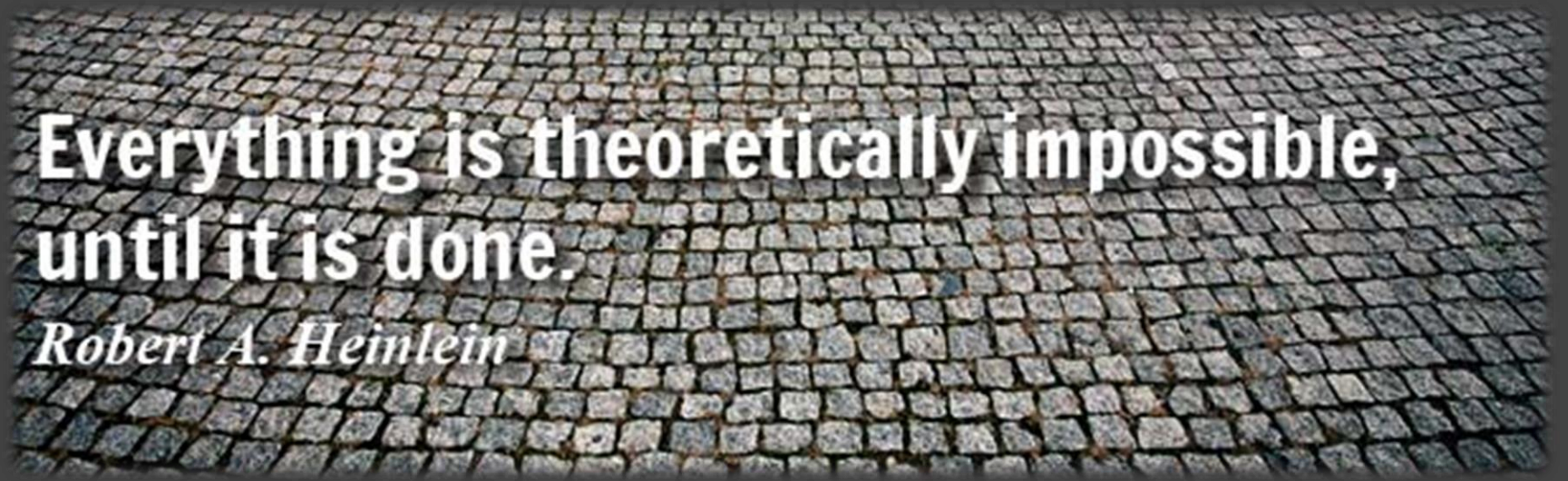
Disclosure

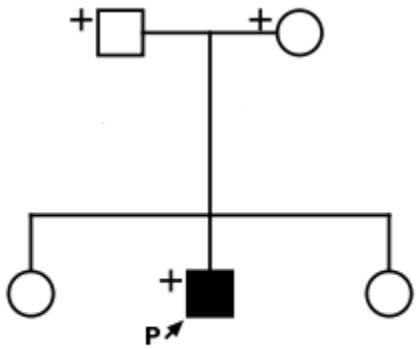
I am a full time salaried employee at Ambry Genetics. Exome sequencing is among Ambry Genetics' commercially available tests.


Everything is theoretically impossible,
until it is done.
Robert A. Heinlein

Overview of Diagnostic Exome Sequencing (DES)

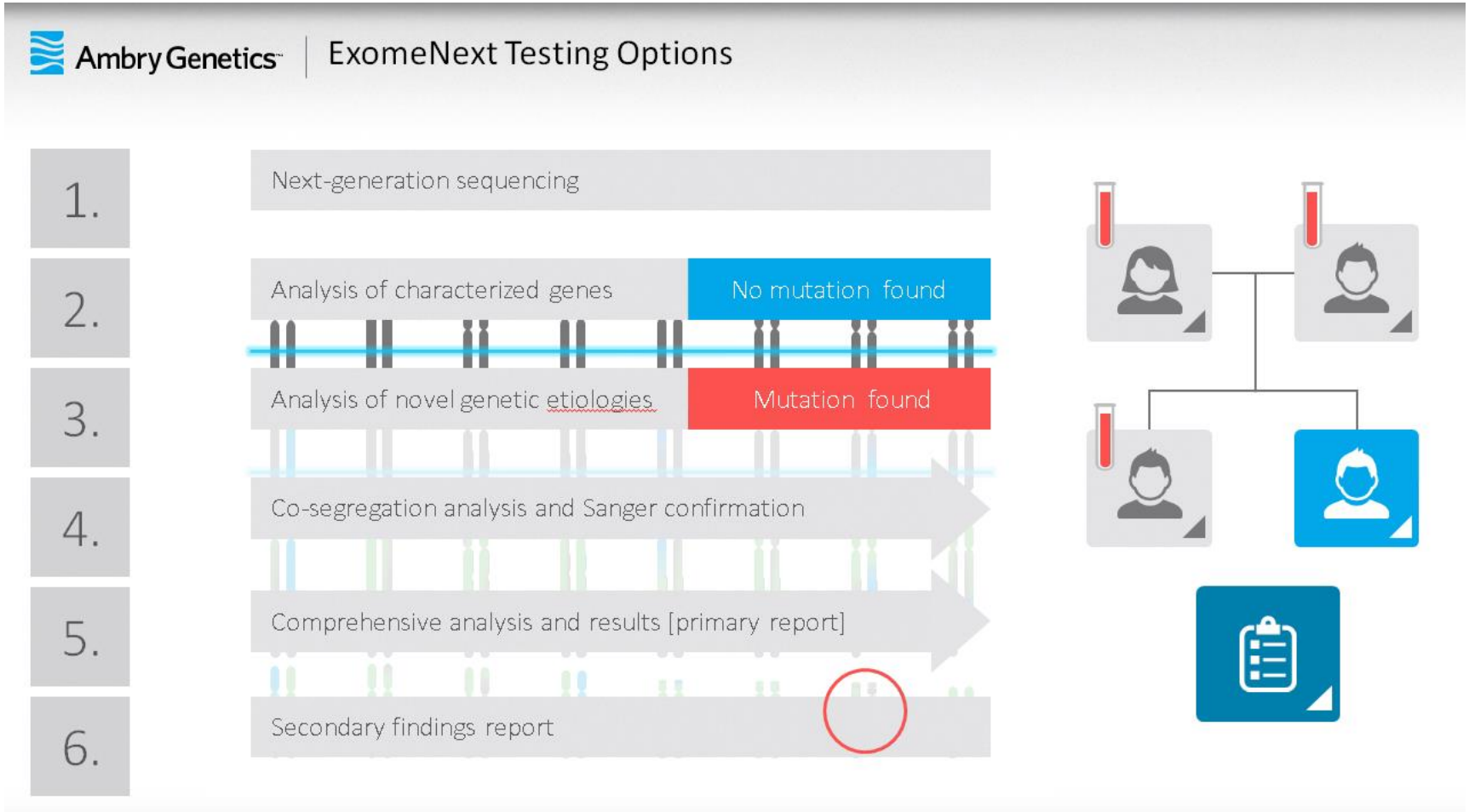
Family-Centered Sequencing and Analysis

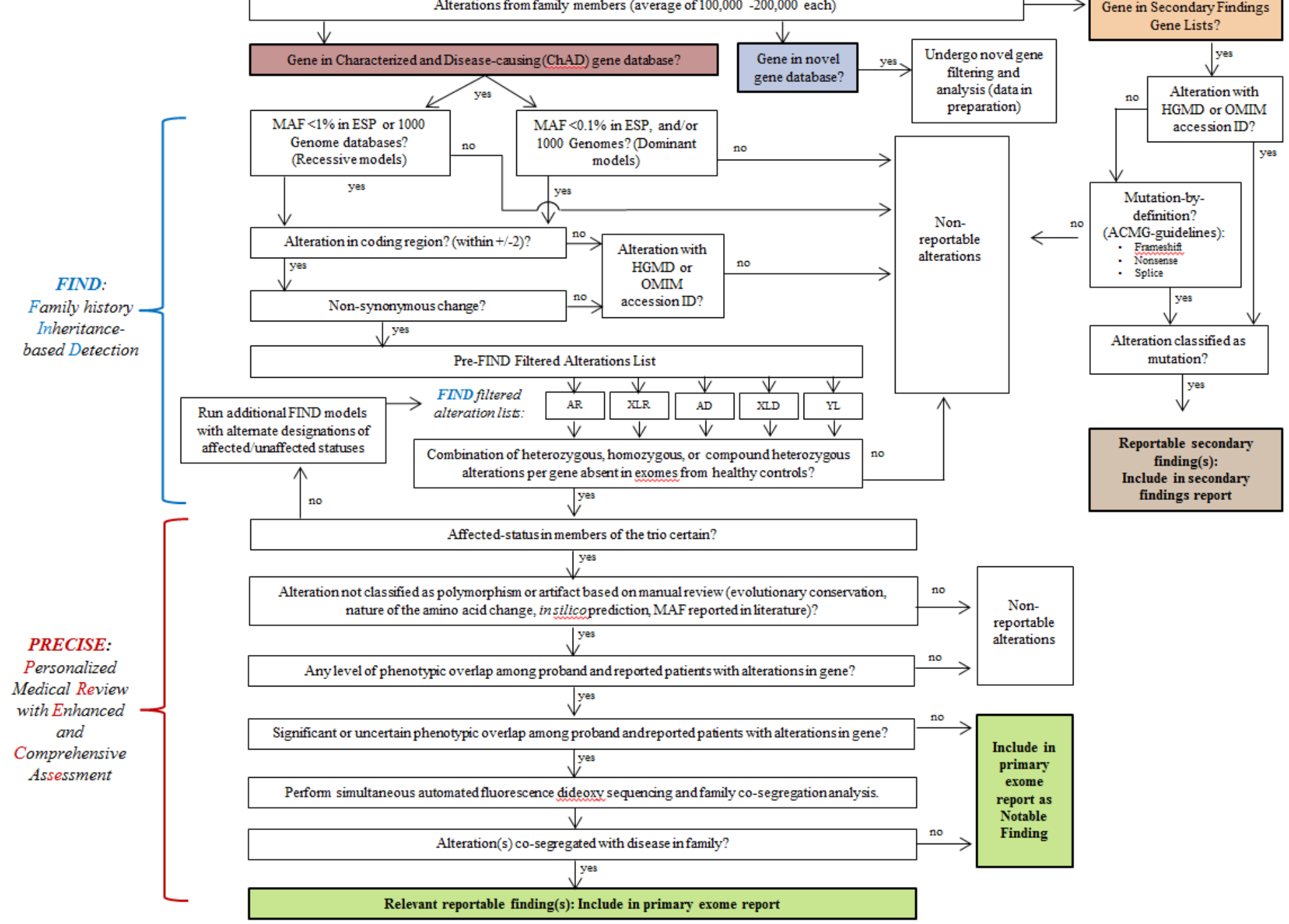
- Family-Centered Exome Sequencing and Analysis
- Trio sequencing: Whole exome sequencing of a group of three family members (generally parent-proband) performed simultaneously
- Family studies (AKA co-segregation analysis)



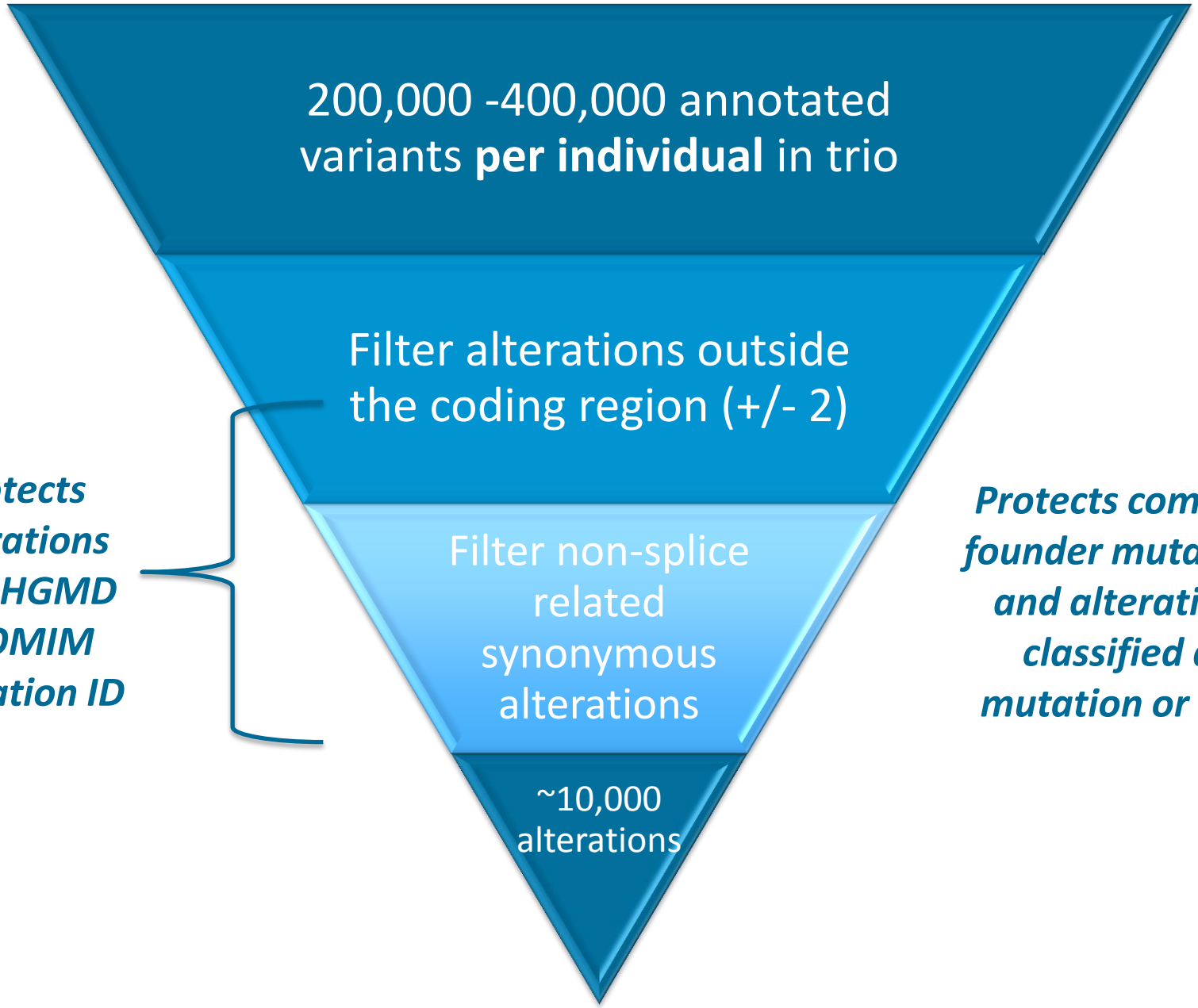
- **Increases diagnostic yield**
- **Decreases the rate of uncertain results**

Diagnostic Exome Sequencing: The Process




Alterations from family members (average of 100,000 -200,000 each)
Gene in Secondary Findings Gene Lists?
yes
Gene in Characterized and Disease-causing (ChAD) gene database?
Gene in novel gene database?
yes
Undergo novel gene filtering and analysis (data in preparation)
yes
no
Alteration with HGMD or OMIM accession ID?
yes
MAF <1% in ESP or 1000 Genome databases? (Recessive models)
no
MAF <0.1% in ESP, and/or 1000 Genomes? (Dominant models)
no
yes
yes
Non-reportable alterations
no
Mutation-by-definition? (ACMG-guidelines):
• Frameshift
• Nonsense
• Splice
yes
Alteration in coding region? (within +/-2)?
no
Alteration with HGMD or OMIM accession ID?
no
yes
Non-synonymous change?
no
yes
Alteration classified as mutation?
yes
FIND: Family history Inheritance-based Detection
Pre-FIND Filtered Alterations List
FIND filtered alteration lists:
AR
XLR
AD
XLD
YL
Run additional FIND models with alternate designations of affected/unaffected statuses
Combination of heterozygous, homozygous, or compound heterozygous alterations per gene absent in exomes from healthy controls?
no
yes
Reportable secondary finding(s): Include in secondary findings report
no
Affected-status in members of the trio certain?
yes
PRECISE: Personalized Medical Review with Enhanced and Comprehensive Assessment
Alteration not classified as polymorphism or artifact based on manual review (evolutionary conservation, nature of the amino acid change, in silico prediction, MAF reported in literature)?
no
Non-reportable alterations
yes
Any level of phenotypic overlap among proband and reported patients with alterations in gene?
no
yes
Significant or uncertain phenotypic overlap among proband and reported patients with alterations in gene?
no
Include in primary exome report as Notable Finding
yes
Perform simultaneous automated fluorescence dideoxy sequencing and family co-segregation analysis.
Alteration(s) co-segregated with disease in family?
no
yes
Relevant reportable finding(s): Include in primary exome report

Bioinformatics Filtering

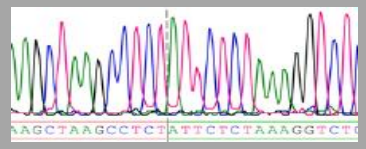


Analysis Algorithm: Postnatal Cases

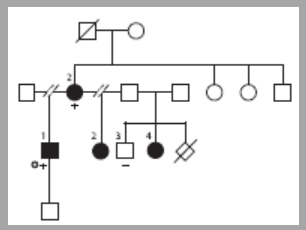
Analysis of Characterized Genes and mtDNA

Positive findings

Concurrent:



Sanger Sequencing Confirmation



Familial co-segregation Analysis

Alteration confirmed & co-segregated

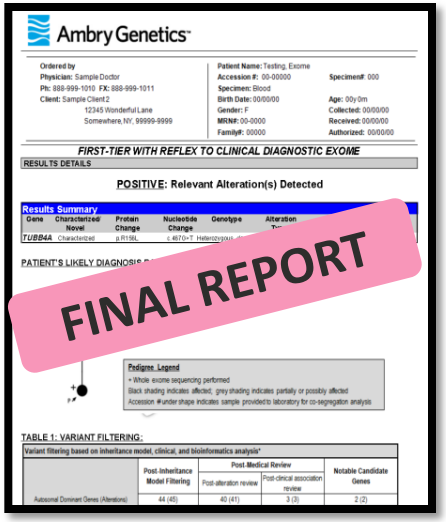
No positive findings

Analysis of Novel Genetic Etiologies

Positive findings

Alteration not confirmed and/or failed to co-segregate

No positive findings



Results Categories

CHARACTERIZED GENETIC ETIOLOGIES	
Positive	Clinically relevant alteration(s) detected
Likely Positive	Alteration(s) with likely clinical relevance detected
Uncertain	Alteration(s) of uncertain clinical relevance detected
Negative	No clinically relevant alteration(s) detected

NOVEL GENETIC ETIOLOGIES	
Uncertain, Strong Evidence	Alteration(s) of potential clinical relevance detected
Uncertain, Moderate Evidence	Alteration(s) of potential clinical relevance detected
Negative	No alteration(s) with potential clinical relevance detected

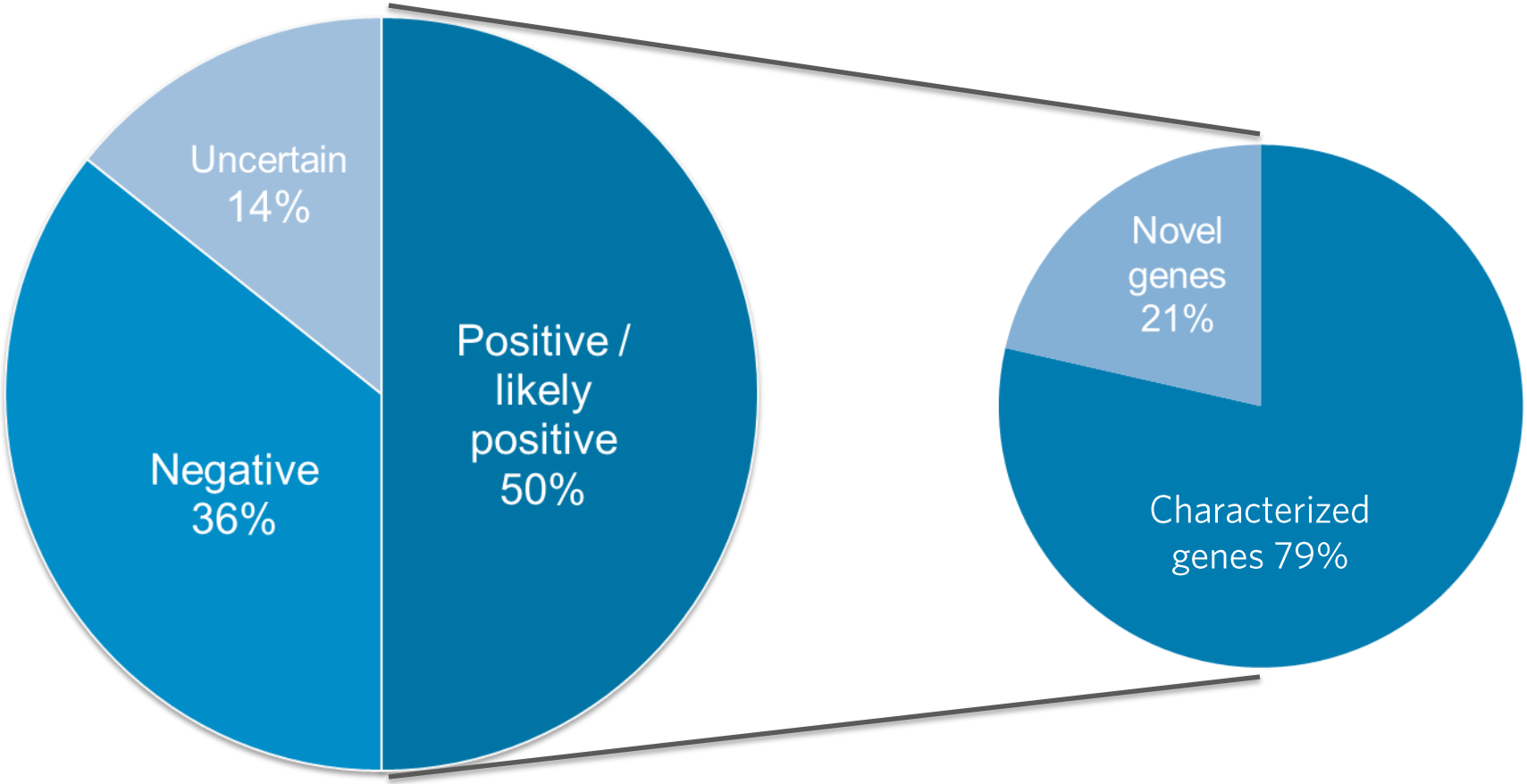
Notable findings: these are alterations of potential interest in characterized genes that do not currently meet criteria for reporting as a primary result but cannot be ruled out entirely. If identified, notable findings will be included in the supplemental pages of the Primary Report.

Diagnostic Exome Sequencing: A Successful for Mendelian Genetic Diagnosis

Disease Category	Total No. of Patients	Diagnostic Rate	Reference
Unselected Clinical Cohorts:			
Ambry Genetics	500	30%	Farwell *et al.* 2014 *Genet in Med*
Baylor College of Medicine	250	26%	Yang *et al.* 2013 *N Engl J Med*
Baylor College of Medicine	2000	25%	Yang *et al.* 2013 *JAMA*
UCLA	814	26%	Lee *et al.* 2014 *JAMA*
Single Clinic Cohorts:			
Kennedy Kreiger Institute (Neurogenetics Clinic)	78	41%	Srivastava *et al.* 2013 *Ann Neurol*
Columbia University Medical Center	115	32%	Iglesias *et al.* 2014 *Genet in Med*
Duke University Medical Center	119	24%	Zhu *et al.* 2015 *Genet in Med*

*Diagnostic rate is 38% including novel genes

Diagnostic Exome Sequencing in the Neonatal Setting



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Diagnostic Exome Sequencing: Limitations

- Coverage is not 100%: 90-95% at 20X
  - All reported cases meet ACMG quality parameters:

    Mean coverage of 100X Proband, 70X Trio

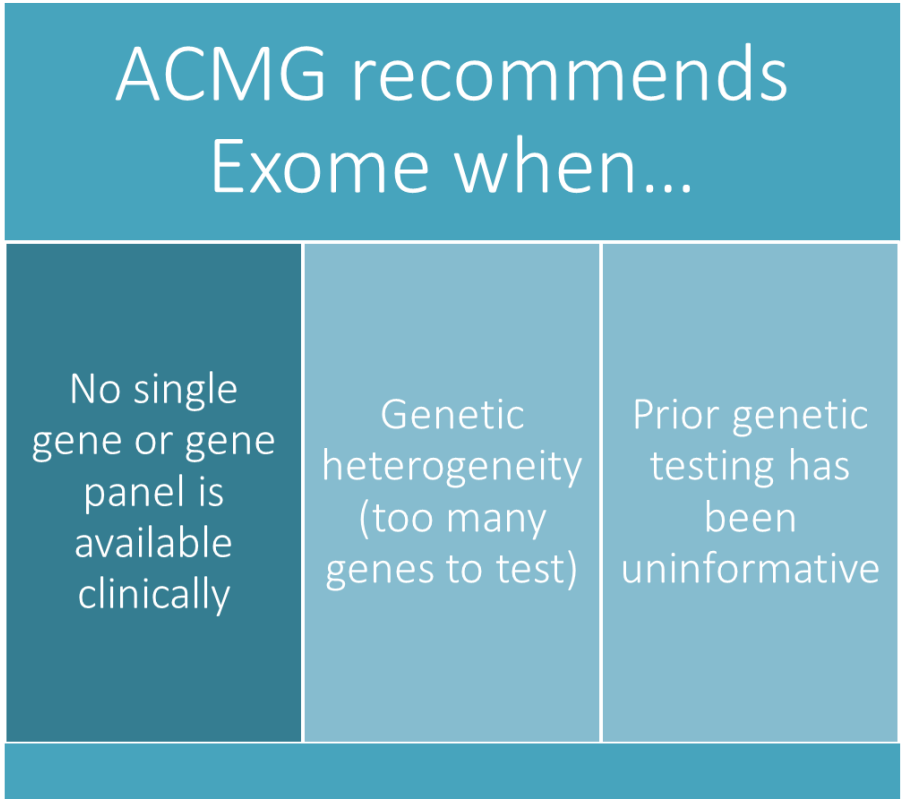
- Some mutation types
  - Large copy number variants
  - UPD
  - Trinucleotide expansions
  - Highly homologous regions of the genome
  - Methylation abnormalities

Prenatal Diagnostic Exome Sequencing (PDES)

The difficulty lies not so much in developing new ideas as in escaping from old ones.

— John Maynard Keynes —

PDES: ACMG Recommendations



ACMG Board of Directors (2012) *Genet in Med* **17**:2**.**

In 2012, the ACMG Board of Directors released a policy statement on appropriate uses of DES, which included “a fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests available for that phenotype, have failed to arrive at a diagnosis”.

Why is a Molecular Diagnosis Important?

Guide clinical management

Treatments
Medical interventions
Appropriate medical referrals to specialists
Pre-symptomatic screening for associated complications
Appropriate educational planning and patient advocacy
Anticipatory guidance and support group referrals

Establish a molecular diagnosis

Determine/establish inheritance pattern- for recurrence risk counseling

Family reproductive planning

Carrier testing
Prenatal diagnosis

Research

Novel therapies
Further understanding of disease natural history- especially features that present prenatally

End the Diagnostic Odyssey

Have an answer to "Why" for families
End costly, time-consuming, and invasive procedures

Carss et al. (2014) and Hillman et al. (2014)

- Reported the results of exome sequencing performed on 30 prenatal and neonatal samples.
- The cases had various structural abnormalities identified by ultrasound.
- All had normal karyotype results.
- Results:
    - “Very likely causative variants”: 3/30 (10%)
        - All were *de novo*
    - “Likely causative variants”: 5/30 (17%)

Carss KJ, Hillman SC, Parthiban V, *et al.* (2014) *Hum Molecul Genet* **23**(12):9.
Hillman DW, Carss KJ, McMullan DJ, et al. (2014) *Ultrasound in Obstet and Gynecol* **45**(1):6.

Drury et al. (2015)

- Reported the results of exome sequencing performed on DNA extracted from chorionic villi or amniocytes from a total of 24 pregnancies.
- The cases were referred due to an increased nuchal translucency and/or another ultrasound abnormality.
- All pregnancies were previously found to be “cytogenetically normal” by karyotype and/or array CGH.
- Cohort 1: The first 14 cases
  - Sequencing was performed on the proband only
  - Sanger was performed on parent samples
- Cohort 2: The last 10 cases
  - Trio sequencing (proband/parents) was performed
  - Variants thought to be causative were Sanger confirmed

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

Drury et al. (2015): Results

- "Definitive diagnoses" in 5/24 (21%)
- "Plausible diagnosis" in 1/24 (4%)
- In 2/24 (8%) cases, results were "highly suggestive of an autosomal recessive disorder"
  - Clinical features in the fetus were consistent with the phenotype associated with the gene
  - Only one mutation was identified, however alterations in these genes are generally inherited in an autosomal recessive fashion
- In 2/24 (8%) cases, mutations suggested conditions that were unrelated to the ultrasound findings

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

Drury et al. (2015): Results

- Definitive diagnoses:
  - Milroy disease (*FLT4)*
  - Hypophosphatasia (*ALPL*)
  - Achondrogenesis type 2 (*COL2A1*)
  - Freeman-Sheldon syndrome/distal arthrogryposis 2A (*MYH3*)
  - Baraitser-Winter syndrome (*ACTB*)
- Plausible diagnosis:
  - Orofaciodigital syndrome type VI (*C5orf42*)
- Highly suggestive of a recessive condition:
  - Short-rib thoracic dysplasia with or without polydactyly *(DYNC2H1)*
  - Fraser syndrome (*FREM2*)
- Unrelated findings:
  - Homozygous *ATP7B* alteration
  - *De novo NF1* alteration

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

ACMG Genomics Case Conference December 16, 2015

- Hosted by Baylor College of Medicine
- Presented the results of 43 clinically consecutive cases of DES performed on fetal samples or products of conception.
- Testing of both parents was also performed (trio testing).
- The majority of reports were returned within a 3 week turnaround time, with 70% reported between 1-2 weeks.

ACMG Genomics Case Conference: Results

Overall: 14/43 cases were positive (33%)

Positive Rates by Indication:

- 3/5 cases with only brain anomalies (60%)
- 6/16 cases with brain anomalies + anomalies affecting other organ systems (38%)
- 5/22 cases with ultrasound anomalies not including the brain (23%)
- 3/7 cases with cardiac, brain, + other anomalies (43%)
- 1/4 cases with cardiac + other (non-brain) anomalies (25%)
- 4/11 total cases with cardiac involvement (36%)
- 5/19 cases with a positive family history (26%)
- 9/24 cases with no family history (38%)

Relevant Alterations in More Than Half of Cases with an Indication of Prenatal Ultrasound Anomalies

Alamillo CL, Powis Z, Farwell K, Shamirzadi L, Weltmer E, Turocy J, Lowe T, Kobelka C, Chen E, Basel D, Ashkinadze E, D'Augelli L, Chao E, and Tang S (2015) *Prenatal Diagnosis* **35**(11):1073-8.

Alamillo et al. (2015): Clinical Details

- Performed a retrospective analysis of the first 7 prenatal cases referred to our laboratory with an indication of congenital anomalies identified by ultrasound.
- None of the pregnancies were ongoing at the time of testing.
- 6/7 probands were fetuses of couples who had more than one affected pregnancy.
- One case had a positive history of consanguinity (parents were first cousins)
- One case had a parent with possibly related findings.
- All 7 cases previously had a normal karyotype analysis.
- 5/7 cases previously had microarray results that were either normal or uncertain.

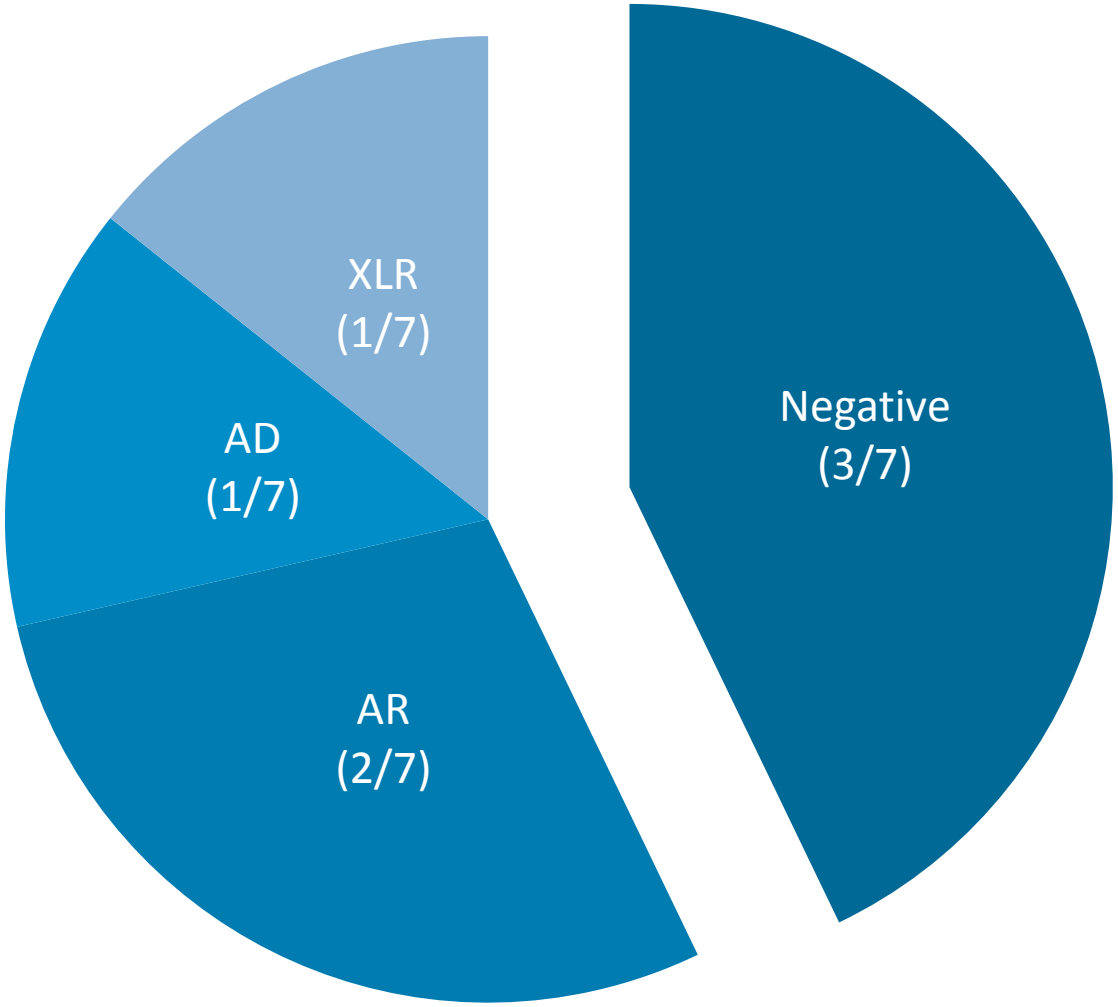
Alamillo et al. (2015): DES

- Submitted samples included cultured amniocytes, extracted DNA from amniocytes, extracted DNA from products of conception.
- Exome sequencing was performed on parent/proband trios.
- Exome sequencing, bioinformatics, variant analysis, co-segregation analysis, and Sanger confirmation of candidate alterations were performed as previously described.

Alamillo et al. (2015): Summary of Results

- PDES positively identified relevant alterations in more than half (4/7; 57%) of cases.
- 3 of the 4 positive cases were the second similarly affected pregnancy of the parents.
- Parents of all 3 of the negative cases had also had additional affected pregnancies.
- 1 of the 4 positive cases was *de novo.*
- Of the positive results, all of the reported alterations were classified as “pathogenic” or “likely pathogenic”.
- No secondary findings were analyzed nor reported for any of the cases.

Alamillo et al. (2015): Results/Inheritance



Alamillo et al. (2015): Alterations Identified

Patient	Gene	Diagnosis	Inheritance	Alteration	Classification	Origin	ESP/ ExAC/ 1000Genomes	Previously Reported
1	*COL1A2*	Osteogenesis imperfecta II	AD	c.1361G>T (p.G454V)	pathogenic	de novo	NO	NO
2	*GBE1*	Glycogen storage disease IV	AR	c.1064G>A (p.R355H)	likely pathogenic	inherited	NO	NO
				c.1543C>T (p.R515C)	pathogenic	inherited	NO	YES
3	*OFD1*	Oral-facial-digital syndrome 1 Simpson-Golabi-Behmel syndrome, type 2	XLR	c.929T>C (p.F310S)	likely pathogenic	maternally inherited	NO	NO
4	*RAPSN*	RAPSN-associated Fetal Akinesia Deformation Sequence	AR	c.484G>A (p.E162K)	pathogenic	inherited	NO	YES

Alamillo et al. (2015): Case 1

- Clinical Features: Male fetus with a skeletal dysplasia of unknown etiology
- Only affected pregnancy of the parents
- Differential Diagnosis: skeletal dysplasia
- Result: Heterozygous *de novo COL1A2* alteration
- Diagnosis: Osteogenesis imperfecta II

Alamillo et al. (2015): Case 2

- Clinical Features: Male fetus with growth retardation, hydrops, flexion contractures, and dysmorphic features. The pregnancy ended in demise
- Previous pregnancy with non-immune fetal hydrops with massive edema and bilateral large cystic hygromas
- Differential Diagnosis: Lethal multiple pterygium syndrome, possibly lysosomal
- Result: Compound heterozygous *GBE1* alterations
- Diagnosis: Glycogen storage disease IV

Alamillo et al. (2015): Case 3

- Clinical Features: Male fetus with omphalocele and bilateral cleft lip and palate
- Affected male sibling
- Differential Diagnosis: Fraser syndrome, Miller-Dieker syndrome, Smith-Lemli-Opitz syndrome
- Result: Hemizygous alteration in *OFD1*
- Diagnosis: Oral-facial-digital syndrome 1/Simpson-Golabi-Behmel syndrome type 2

Alamillo et al. (2015): Case 4

- Clinical Features: Male fetus with nuchal fold thickening/edema and skeletal anomalies.
- Similar findings were observed in their previous pregnancy, which was terminated at 22 weeks gestation
- The parents are consanguineous (first cousins)
- Differential Diagnosis: Arthrogryposis/akinesia syndrome
- Result: Homozygous *RAPSN* alteration
- Diagnosis: *RAPSN*-associated fetal akinesia deformation sequence

Alamillo et al. (2015): Negative Cases

Patient	Clinical Details	Differential Diagnosis	Sample Type	>1 Affected Fetus
5	Male fetus with congenital heart defect, renal and lung anomalies, and dysmorphic features. Previous affected male fetus had similar features. Family history of multiple miscarriages.	Autosomal recessive or X-linked syndrome	Extracted DNA	Yes
6	Female fetus with complex congenital cardiac abnormalities, heterotaxy and left-right patterning defect. Additional affected male fetus with complex congenital heart defects. Mother affected with bicuspid aortic valve.	Patterning defect or cardiac malformation sequence	Extracted DNA	Yes
7	Male fetus with renal anomalies. Previous affected pregnancy had similar features. Parents both unaffected.	Hereditary renal adysplasia spectrum	Extracted DNA	Yes

Alamillo et al. (2015): Organ Systems Involved

	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				

Alamillo et al. (2015): Discussion

- Counseling patients about possible diagnoses, expected pregnancy outcomes, and recurrence risks can be challenging in a prenatal setting.
- In most cases, a genetic etiology cannot be predicted based on the ultrasound findings alone.
- Testing offered to patients may include screening tests, karyotype, microarray, and/or testing for single gene disorders.
- Testing options for single gene disorders can be limited unless there are single gene or clinical testing panels available for the specific type of ultrasound finding(s) identified.

Alamillo et al. (2015): Discussion

- PDES may be a useful option for certain prenatal cases, given that it can simultaneously test for a wide range of genetic etiologies.
- The diagnostic yield of PDES ranges from 10-57% in published studies.
- The lengthy turnaround times for DES results have shortened considerably in the past few years, making it a reasonable option for testing of ongoing pregnancies.
- There are no formal recommendations regarding the reporting of secondary findings in prenatal cases.

Take-Home Messages

- These cases illustrate 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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QUESTIONS

Let's Find the Answer.

Another Case Example:
PDES in a fetus with marked microcephaly

Introduction

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.

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

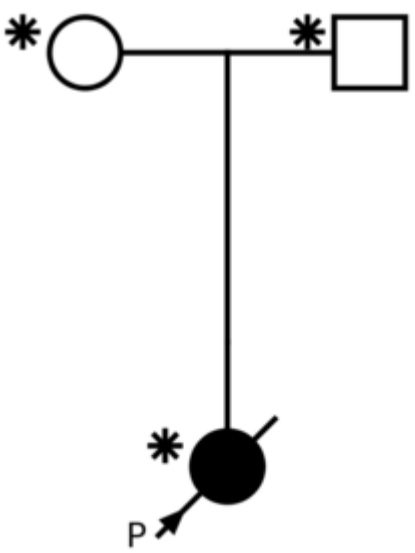
- Microcephaly, with head circumference <3rd percentile
- Multiple brain anomalies
- Dysmorphic features
- Karyotype and microarray

Genetic Counseling: Pre-Test

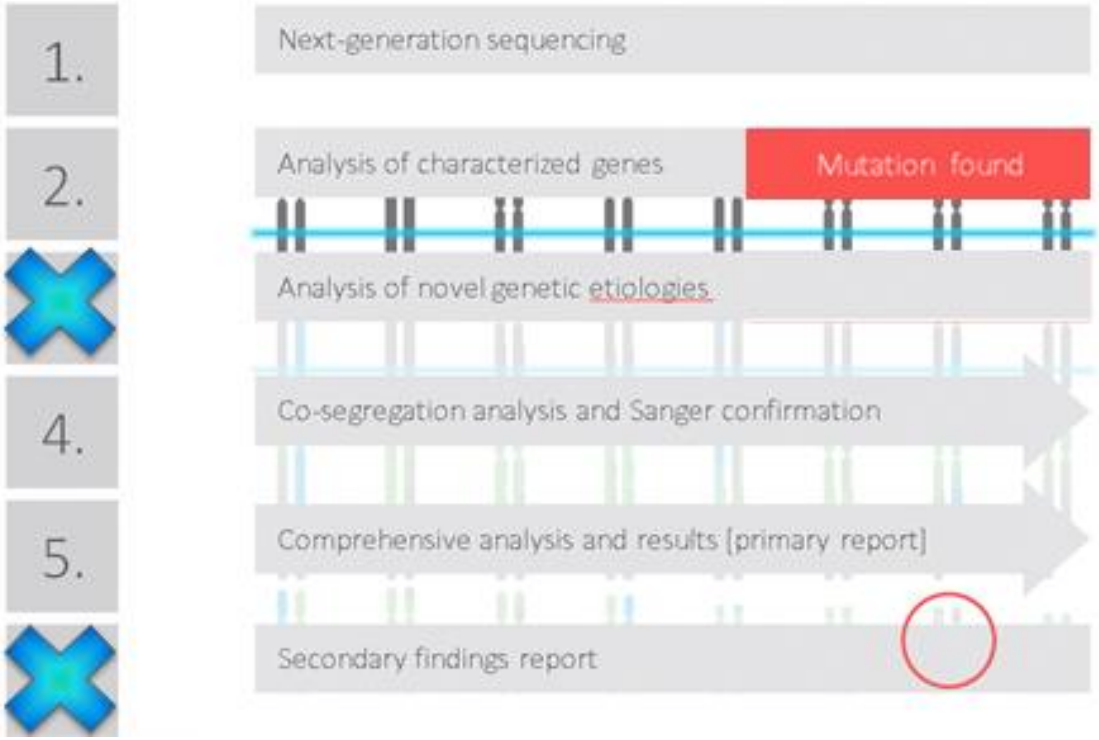
- *In-person pre-exome counseling session*
- *Appropriately upset by the autopsy findings*
- *Concerned about recurrence risk*
- *The limitations of exome testing were discussed*
- *Discussed possibility of results being positive, negative, or of uncertain significance*
- *The couple stayed in touch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

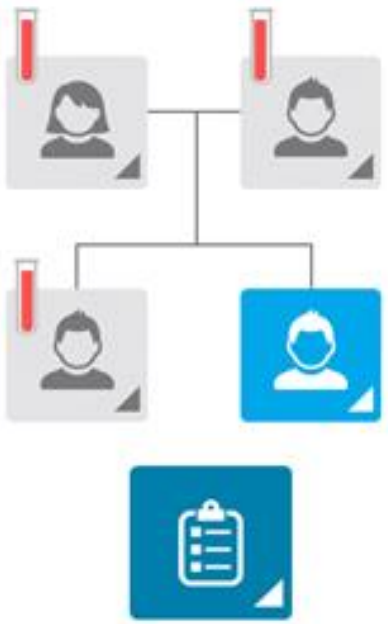
Diagnostic Exome Sequencing

- **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

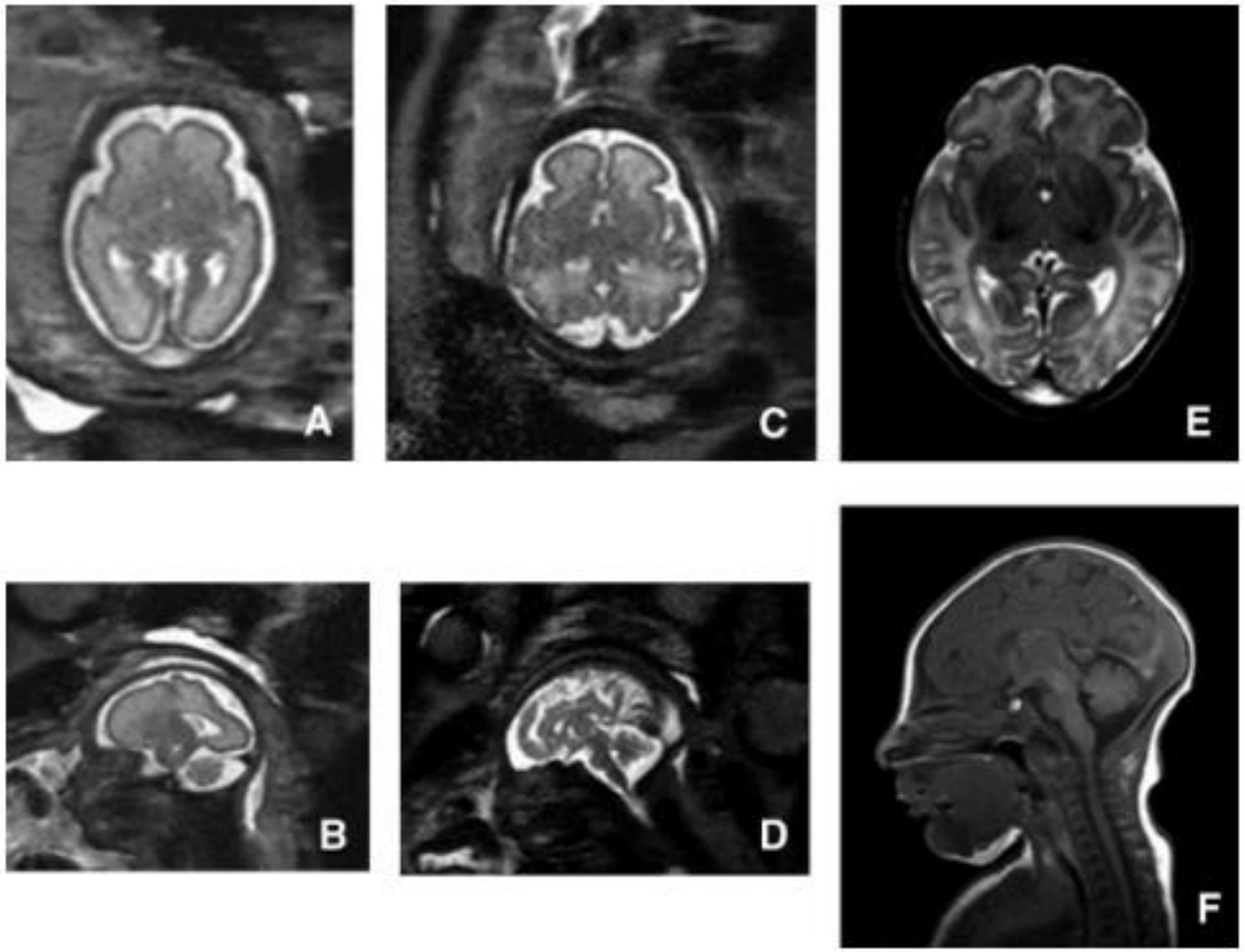
Diagnostic Exome Sequencing: *ASPM*

- 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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QUESTIONS

Let's Find the Answer.