welcome to an AMBRY GENETICS presentation
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

1. Next-generation sequencing
2. Analysis of characterized genes
3. Analysis of novel genetic etiologies
4. Co-segregation analysis and Sanger confirmation
5. Comprehensive analysis and results [primary report]
6. Secondary findings report
200,000 - 400,000 annotated variants per individual in trio

Filter alterations outside the coding region (+/- 2)

Filter non-splice related synonymous alterations

~10,000 alterations

Protects alterations with HGMD or OMIM alteration ID

Protects common founder mutations and alterations classified as mutation or VLP
Analysis Algorithm: Postnatal Cases

Analysis of Characterized Genes and mtDNA

- Positive findings

<table>
<thead>
<tr>
<th>No positive findings</th>
</tr>
</thead>
</table>

Analysis of Novel Genetic Etiologies

- Positive findings

| No positive findings |

Concurrent:

- Sanger Sequencing Confirmation
- Familial co-segregation Analysis

<table>
<thead>
<tr>
<th>Alteration confirmed &amp; co-segregated</th>
</tr>
</thead>
</table>

| Alteration not confirmed and/or failed to co-segregate |

FINAL REPORT

Ambrigenetics
## Results Categories

### Characterized Genetic Etiologies

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Clinically relevant alteration(s) detected</td>
</tr>
<tr>
<td>Likely Positive</td>
<td>Alteration(s) with likely clinical relevance detected</td>
</tr>
<tr>
<td>Uncertain</td>
<td>Alteration(s) of uncertain clinical relevance detected</td>
</tr>
<tr>
<td>Negative</td>
<td>No clinically relevant alteration(s) detected</td>
</tr>
</tbody>
</table>

### Novel Genetic Etiologies

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncertain, Strong Evidence</td>
<td>Alteration(s) of potential clinical relevance detected</td>
</tr>
<tr>
<td>Uncertain, Moderate Evidence</td>
<td>Alteration(s) of potential clinical relevance detected</td>
</tr>
<tr>
<td>Negative</td>
<td>No alteration(s) with potential clinical relevance detected</td>
</tr>
</tbody>
</table>

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 Approach for Mendelian Genetic Diagnosis

<table>
<thead>
<tr>
<th>Disease Category</th>
<th>Total No. of Patients</th>
<th>Diagnostic Rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected Clinical Cohorts:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Ambry Genetics</em></td>
<td>500</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td><em>Baylor College of Medicine</em></td>
<td>250</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td><em>Baylor College of Medicine</em></td>
<td>2000</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td><em>UCLA</em></td>
<td>814</td>
<td>26%</td>
</tr>
<tr>
<td>Single Clinic Cohorts:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Kennedy Kreiger Institute (Neurogenetics Clinic)</em></td>
<td>78</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td><em>Columbia University Medical Center</em></td>
<td>115</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td><em>Duke University Medical Center</em></td>
<td>119</td>
<td>24%</td>
</tr>
</tbody>
</table>

*Diagnostic rate is 38% including novel genes
Diagnostic Exome Sequencing in the Neonatal Setting

- Negative: 36%
- Uncertain: 14%
- Positive / likely positive: 50%
- Novel genes: 21%
- Characterized genes: 79%
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 —
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%)
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 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 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

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 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

Chart 1: Results and Inheritance

- Negative (3/7)
- AR (2/7)
- AD (1/7)
- XLR (1/7)
### Alamillo et al. (2015): Alterations Identified

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Diagnosis</th>
<th>Inheritance</th>
<th>Alteration</th>
<th>Classification</th>
<th>Origin</th>
<th>ESP/ExAC/1000Genomes</th>
<th>Previously Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COL1A2</td>
<td>Osteogenesis imperfecta II</td>
<td>AD</td>
<td>c.1361G&gt;T (p.G454V)</td>
<td>pathogenic</td>
<td>de novo</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>2</td>
<td>GBE1</td>
<td>Glycogen storage disease IV</td>
<td>AR</td>
<td>c.1064G&gt;A (p.R355H)</td>
<td>likely pathogenic</td>
<td>inherited</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.1543C&gt;T (p.R515C)</td>
<td>pathogenic</td>
<td>inherited</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>3</td>
<td>OFD1</td>
<td>Oral-facial-digital syndrome 1</td>
<td>XLR</td>
<td>c.929T&gt;C (p.F310S)</td>
<td>likely pathogenic</td>
<td>maternally inherited</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>4</td>
<td>RAPSN</td>
<td>RAPSN-associated Fetal Akinesia Deformation Sequence</td>
<td>AR</td>
<td>c.484G&gt;A (p.E162K)</td>
<td>pathogenic</td>
<td>inherited</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>
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 \textit{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 \textit{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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical Details</th>
<th>Differential Diagnosis</th>
<th>Sample Type</th>
<th>&gt;1 Affected Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>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.</td>
<td>Autosomal recessive or X-linked syndrome</td>
<td>Extracted DNA</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>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.</td>
<td>Patterning defect or cardiac malformation sequence</td>
<td>Extracted DNA</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Male fetus with renal anomalies. Previous affected pregnancy had similar features. Parents both unaffected.</td>
<td>Hereditary renal dysplasia spectrum</td>
<td>Extracted DNA</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Alamillo et al. (2015): Organ Systems Involved

<table>
<thead>
<tr>
<th></th>
<th>Cardiovascular</th>
<th>Craniofacial</th>
<th>Dysmorphic Features</th>
<th>Gastrointestinal</th>
<th>Genitourinary</th>
<th>Musculoskeletal</th>
<th>Neurologic</th>
<th>Ophthalmologic</th>
<th>Pulmonary</th>
<th>Renal</th>
<th>Intrauterine Growth Retardation</th>
<th>Increased NT/Cystic Hygroma</th>
<th>Edema/Hydrops</th>
<th>Ultrasound Soft Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive Result</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Negative Result</strong></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
</tbody>
</table>
• 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.
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

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2. Analysis of characterized genes
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6. Secondary findings report
Diagnostic Exome Sequencing: Candidates

Results revealed compound heterozygous \textit{ASPM} alterations in the fetal sample:

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

\textbf{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.

Diagnostic Exome Sequencing: ASPM

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

Results Summary

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Inheritance</th>
<th>Characterized/Novel Gene*</th>
<th>Protein Change</th>
<th>Nucleotide Change</th>
<th>Genotype</th>
<th>Alteration Type</th>
<th>Alteration Classification</th>
<th>Gene Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPM</td>
<td>Autosomal recessive</td>
<td>Characterized</td>
<td>p.Y462*</td>
<td>c.1386C&gt;G</td>
<td>Heterozygous, maternal</td>
<td>Nonsense</td>
<td>Pathogenic</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.8988-1G&gt;C</td>
<td>Heterozygous, paternal</td>
<td>Splice</td>
<td>Pathogenic</td>
<td></td>
</tr>
</tbody>
</table>

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.
QUESTIONS

Let’s Find the Answer.