Genetic Mutations Cause Many Birth Defects: What We Learned from the FORGE Canada Project

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I have no conflicts of interest related to this work.

Robert L. Brent, MD, PhD
Causes of Birth Defects

1980

- Teratogens: 5%
- Genetic: 8%
- Multifactorial/Unknown: 87%

2010

- Teratogens: 5%
- Genetic: 22%
- Multifactorial/Unknown: 73%
Next Generation Sequencing

Advantages of Next Generation Technologies

In comparison to sequencers used for Human Genome Project:

- 8,000,000 times more sequence produced per run
- 2400 times faster
- 3,000,000 times cheaper
Genome-Wide Sequencing

- Exome or whole genome sequencing
- Offers the promise of finding the mutation that causes any genetic disease in any patient

Finding Of Rare disease GEnes in Canada
FORGE Canada

- **Purpose:** To use next generation sequencing to identify genes that cause rare diseases in Canadian children
- Project launched April 2011, completed June 2013

FORGE Canada

- Led by Kym Boycott, Jacques Michaud and Jan Friedman
- Participants included
  - >150 scientists and clinicians
  - All 21 clinical genetics services in Canada
  - 3 Genome Canada Science and Technology Innovation Sequencing Centres
FORGE Canada Success

- 264 disorders studied
- Exome sequencing of 783 samples
- Molecular diagnosis in 146 disorders (55.3%)

Finding The Causative Gene

Consanguineous families
Finding The Causative Gene

- 60 studied, 42 (70%) found
  - 20 novel
  - 22 known

Consanguineous Families

Consanguineous Families

- 4 y/o
- Microcephaly
- Profound ID
Consanguineous Families

- 18 y/o
- Microcephaly
- Profound ID

- 23 y/o
- Microcephaly
- Profound ID

Exome sequencing in older sister showed 9 genes with homozygous rare variants. Of these, one gene was known to be associated with the phenotype: *NSUN2* (truncating mutation)

Patients of Dr. Anna Lehman
Finding The Causative Gene

≥2 Affected sibs, nonconsanguineous

Finding The Causative Gene

• 62 studied, 28 (45%) found
  - 13 novel
  - 25 known

≥2 Affected sibs, nonconsanguineous
≥2 Affected Sibs, Nonconsanguineous

- Healthy, non-consanguineous couple
- Referred in second pregnancy for genetic evaluation of recurrent multiple fetal anomalies


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≥2 Affected Sibs, Nonconsanguineous

First pregnancy
- 21 4/7 weeks: fetal growth retardation, severe microcephaly, cerebellar hypoplasia and bilateral renal agenesis
- Pregnancy terminated, female fetus, findings confirmed

≥2 Affected Sibs, Nonconsanguineous

Second pregnancy
- 18 5/7 weeks: FGR, microcephaly, arhinencephaly, cerebellar hypoplasia and bilateral renal cystic dysplasia and hypoplasia
- Pregnancy terminated, female fetus, findings confirmed


≥2 Affected Sibs, Nonconsanguineous

- Exome sequencing performed on frozen CVS from second pregnancy and blood from both parents
- Postulated compound heterozygote for inactivating mutations of one of 1644 genes known or suspected to be involved in structure or function of cilia

≥2 Affected Sibs, Nonconsanguineous

- 35 loci in the fetus showed compound heterozygosity for rare non-synonymous variants
- 3 loci on list of “ciliopathy genes”
- One locus: KIF14, both variants truncating, showed expected segregation pattern in family


≥2 Affected Sibs, Nonconsanguineous

- Spontaneous mutation of locus in mouse: growth restriction, microcephaly, cerebellar hypoplasia, and motor impairment in homozygote
- Mouse KO: same phenotype
- Zebrafish morpholino: ciliopathy

Finding The Causative Gene

Unrelated patients, same disorder (overlap strategy)

Finding The Causative Gene

- 32 disorders studied
- Causative genes found in 30 (94%)
  - 15 novel genes
  - 7 known genes
(overlap strategy)
**Overlap Strategy**

- **Brother and Sister**
  - DD, truncal hypotonia, involuntary movements, myopathic facies, seizures and neurological regression
  - Extensive workup negative (metabolic, mitochondrial, muscle biopsy, CMA)


**Overlap Strategy**

- **Boy died age 5 years** (autopsy: acute hypoxic encephalopathy)
- **Girl died in her sleep at 9 months of age** (autopsy: hypoxic/ischemic changes of brain)

Overlap Strategy

- Exome sequencing performed on both children and mother
- Rare, conserved, deleterious homozygous or compound heterozygous variants
  - Brother: 22, Sister: 26
  - Shared: 2
  - 1 segregated properly: NGLY1


Overlap Strategy

- 3 y/o boy with compound heterozygous mutations of same locus described previously as “variant of interest” in 2012
- Through social media, parents collected 7 additional cases identified by exome sequencing, published in 2014

Family History in Birth Defects

• Not genetic
  • Autosomal recessive
  • X-linked recessive

Family History in Birth Defects

• Not genetic
  • Autosomal recessive
  • X-linked recessive
  • Dominant (new mutation)
Causes of Intellectual Disability

2010

- Teratogens: 5%
- Genetic: 22%
- Multifactorial/Unknown: 73%

Causes of Intellectual Disability

2015

- Teratogens: 5%
- Genetic: 63%
- Multifactorial/Unknown: 32%
### Other Birth Defects

- Bilateral anophthalmia/severe microphthalmia: ≥80% genetic, most new mutations
- Congenital diaphragmatic hernia: ≥35% genetic, most new mutations
- Congenital heart defects: ≥40% genetic, most new mutations

### Causes of Birth Defects

- The proportion of birth defects that are caused by genetic factors is much greater than Bob Brent thought in 1980
- Most cases are sporadic and result from *de novo* mutations