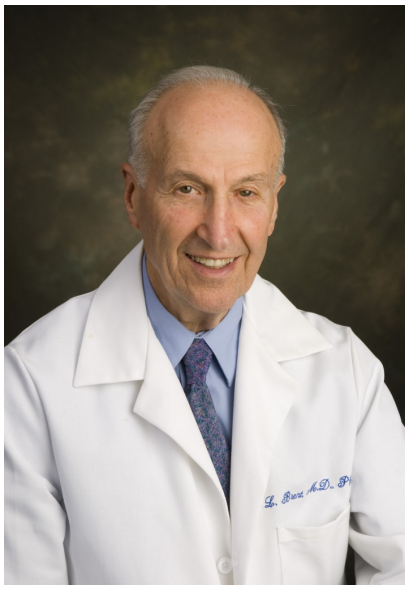
Genetic Mutations Cause Many Birth Defects: What We Learned from the FORGE Canada Project

Jan M. Friedman, MD, PhD
University of British Columbia
Vancouver, Canada

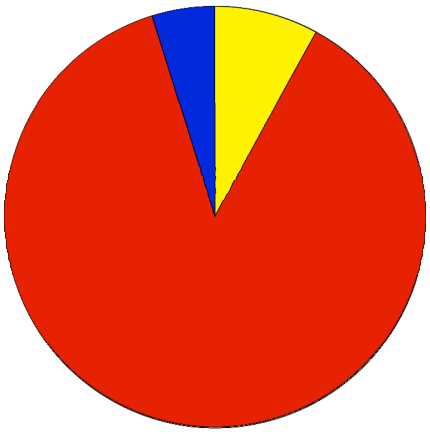
I have no conflicts of interest related to this work.

Robert L. Brent, MD, PhD



Causes of Birth Defects

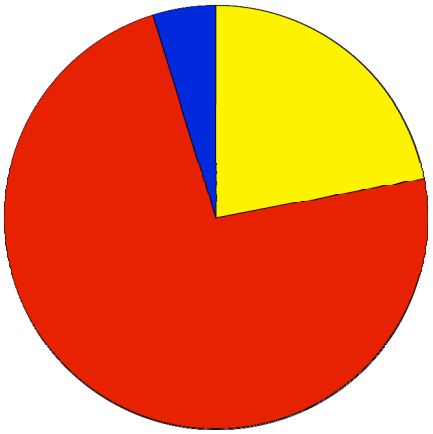
1980



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

Causes of Birth Defects

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

FORGE CANADA CONSORTIUM

CANADIAN PEDIATRIC GENETIC DISORDERS SEQUENCING CONSORTIUM
CONSORTIUM CANADIEN DE SÉQUENÇAGE DES MALADIES GÉNÉTIQUES PÉDIATRIQUES

Finding
Of
Rare
disease
GEnes
in
Canada

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

GenomeCanada

GenomeBritishColumbia
GenomeQuébec
McLaughlin Centre for Molecular Medicine

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
- Summarized in *Beaulieu CL, et al. Am J Hum Genet 94:809-17, 2014*

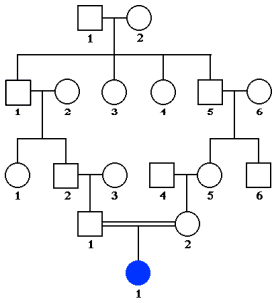
FORGE Canada

- 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%)

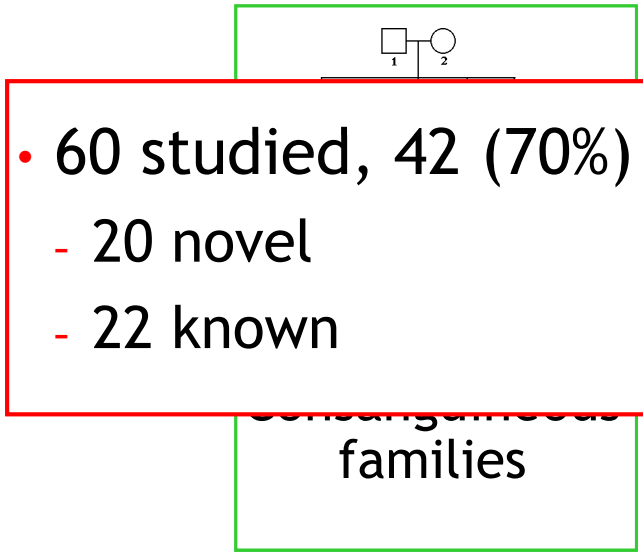
Finding The Causative Gene



Consanguineous families

FORGE CANADA CONSORTIUM

Finding The Causative Gene

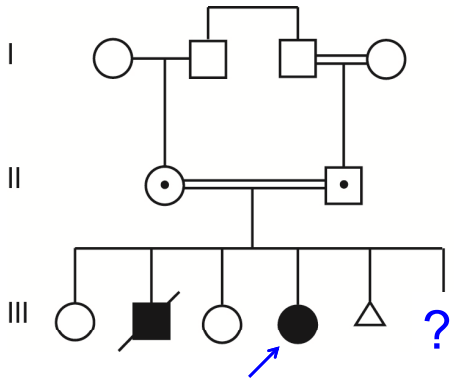


- 60 studied, 42 (70%) found
  - 20 novel
  - 22 known

families

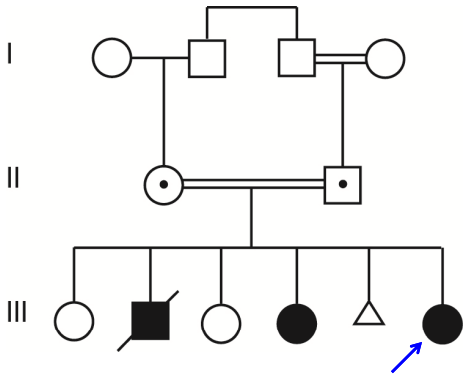
FORGE CANADA CONSORTIUM

Consanguineous Families



- 4 y/o
- Microcephaly
- Profound ID

Consanguineous Families



- 18 y/o
- Microcephaly
- Profound ID

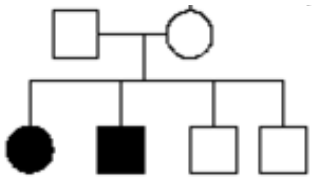
- 23 y/o
- Microcephaly
- Profound ID

Consanguineous Families

- 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

FORGE CANADA CONSORTIUM

Finding The Causative Gene

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

≥2 Affected sibs, nonconsanguineous

FORGE CANADA CONSORTIUM

≥2 Affected Sibs, Nonconsanguineous

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

Filges I, et al. Clin Genet 86:220-8, 2014

≥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

Filges I, et al. Clin Genet 86:220-8, 2014

≥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

Filges I, et al. Clin Genet 86:220-8, 2014

≥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

Filges I, et al. Clin Genet 86:220-8, 2014

≥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

Filges I, et al. Clin Genet 86:220-8, 2014

≥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

Filges I, et al. Clin Genet 86:220-8, 2014

Finding The Causative Gene

Unrelated patients, same disorder (overlap strategy)

FORGE CANADA CONSORTIUM

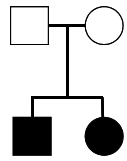
Finding The Causative Gene

- 32 disorders studied
- Causative genes found in 30 (94%)
  - 15 novel genes
  - 7 known genes

(overlap strategy)

FORGE CANADA CONSORTIUM

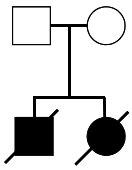
Overlap Strategy



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

Enns GM et al. Genet Med 16:751, 2014

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)

Enns GM et al. Genet Med 16:751, 2014

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*

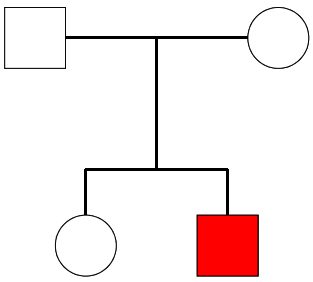
Enns GM et al. Genet Med 16:751, 2014

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

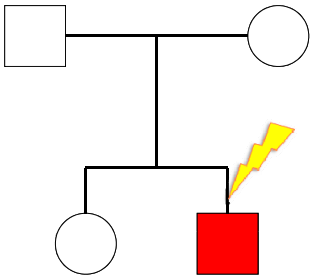
Enns GM et al. Genet Med 16:751, 2014

Family History in Birth Defects



- Not genetic
- Autosomal recessive
- X-linked recessive

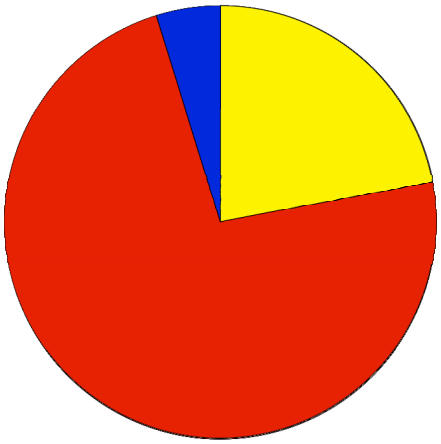
Family History in Birth Defects



- Not genetic
- Autosomal recessive
- X-linked recessive
- Dominant (new mutation)

Causes of Intellectual Disability

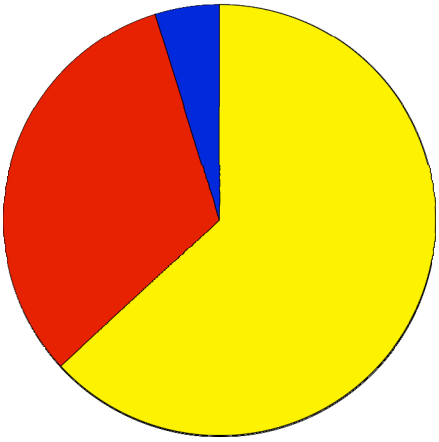
2010



- 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


UBC