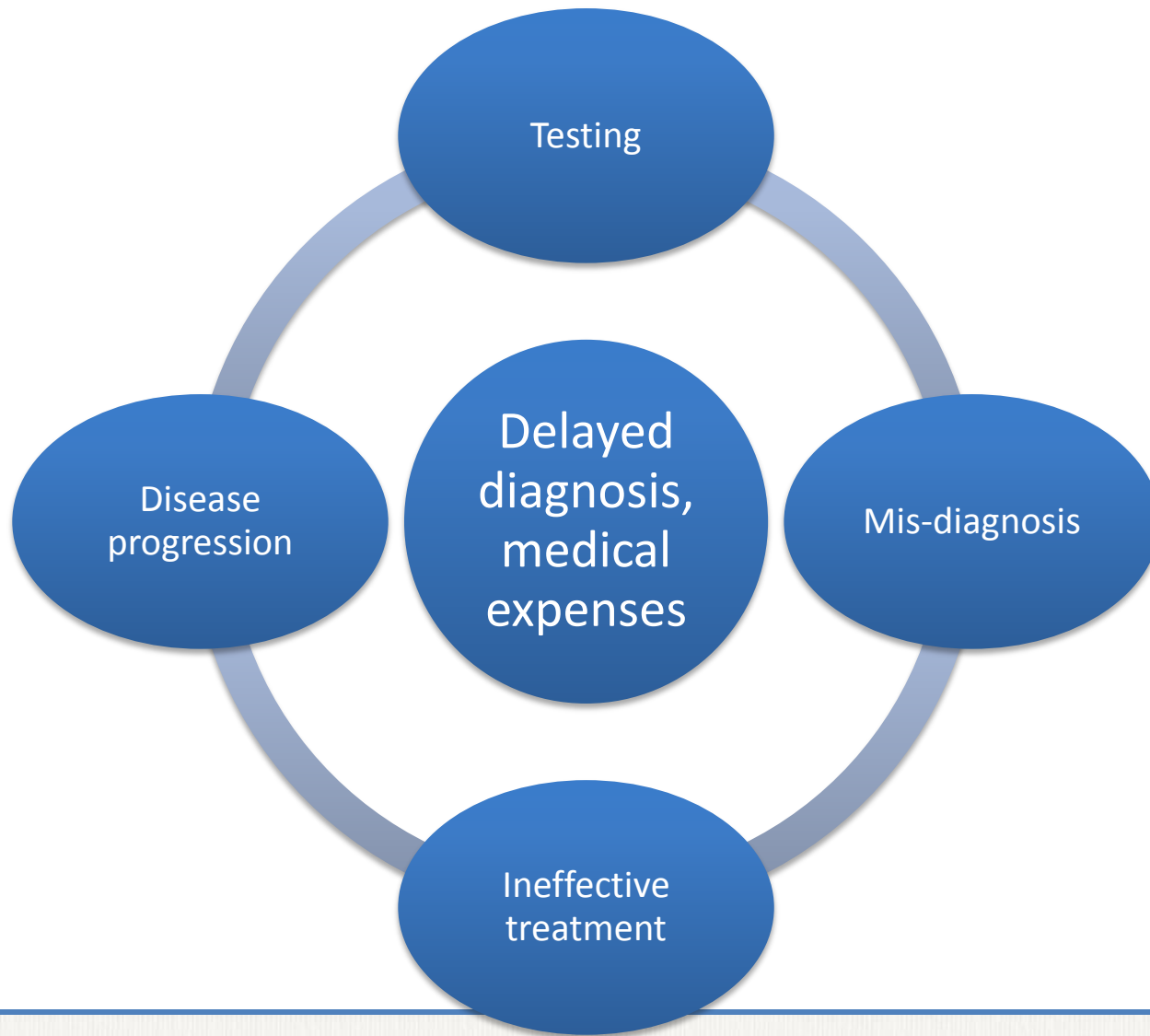


Module 3: Using NGS for diagnostic dilemmas

A typical diagnostic odyssey



Ending a “Diagnostic Odyssey”

Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease

Elizabeth A. Worthey et al. *Genet Med* 2011;13(3):255–262.
Medical College of Wisconsin

Intractable inflammatory bowel disease

- Whole Exome Sequencing of 15 month old male infant
- ~16,000 total variants within coding portion of genes (SNVs, small duplications or deletions. ~1500 were “novel”
- ~7,100 were non-synonymous substitutions, premature stops, or small insertions or deletions in coding exons of which , ~ 1,100 were novel, not present in databases of normal variants
- 136 variants fit an autosomal recessive (AR) or X-linked (XL) inheritance model
- 1 variant among the 136 that fit AR or XL inheritance, altered a conserved amino acid, was predicted to be damaging, was not present in reference genome, and was not in a gene in which deleterious mutations causing a different phenotype was known.

Mode of inheritance

○ Dominant

One copy of gene affected

Heterozygote



XIAP
Indication
for bone
marrow
transplantati
on

~7,000 potentially damaging variants
in genes, 1,100 of them novel

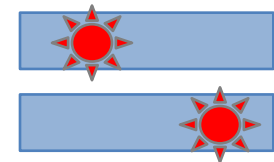
○ Recessive

Both copies of gene affected

Homozygote



Compound
heterozygote



X-linked

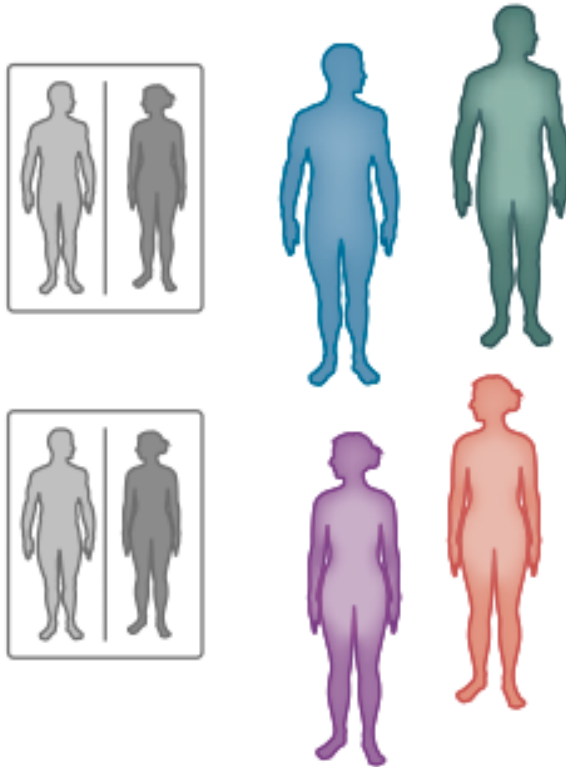


136 fit these inheritance patterns

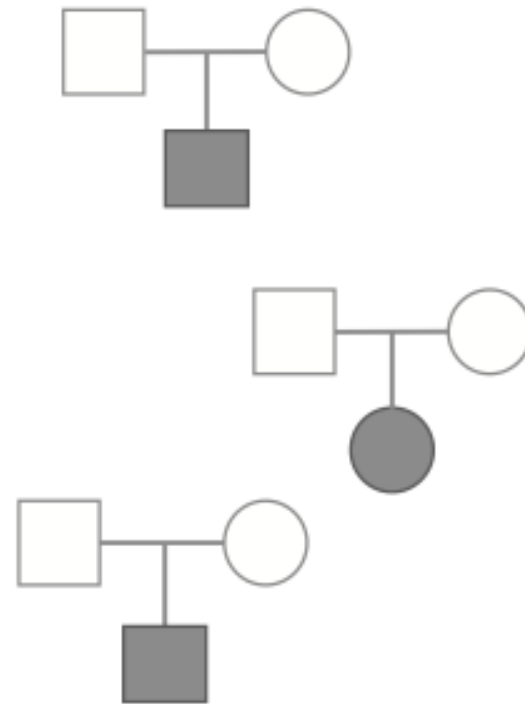
Using unrelated patients

Boycott et al. Ann Rev Med 2014

a Strategy 1: Multiple unrelated patients with the same disease



Unrelated patients with the same disease



Unrelated trios with the same disease

The Trio in Whole Genome Analysis

Original Article

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

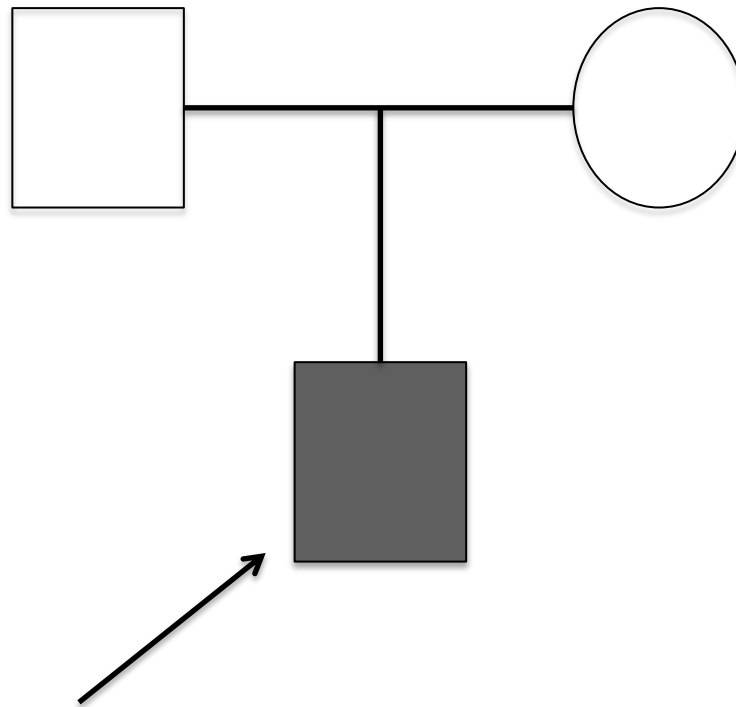
Yaping Yang, Ph.D. et al. .

250 consecutive patients with undiagnosed
diseases undergoing trio analysis

N Engl J Med
Volume 369(16):1502-1511
October 17, 2013



The Trio in Whole Genome Analysis



Mode of inheritance

- Dominant

One copy of gene affected

- New Mutation

New variant in the patient not present in either parent

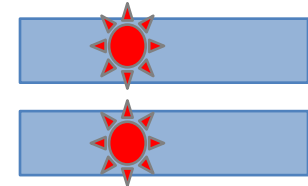
Heterozygote



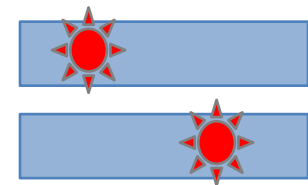
- Recessive

Both copies of gene affected

Homozygote



Compound heterozygote



X-linked



Representative Filtering Scheme

Whole Genome Sequencing

4,000,000 variants

Not located within or near an exon

Whole Exome Sequencing

~40,000 variants

Too frequent in public databases

~1,500 variants

Synonymous change with
no effect on mRNA splicing

~200 variants

Fits AR
Inheritance pattern

~ 4 variants

Which Genes
Make
Biological
Sense?

Same Gene
Mutated in
Other
Affected
Individuals?

Fits new mutation model

~ 2 variants

Which patients are getting WES

Disease Category	Age at Time of Testing				
	Fetus	< 5 yr	5-18 Yr	>18 yr	Total
Neurological (+/- other disorders)	1	110	86	16	213
Non-Neurological	3	14	8	12	37
Total	4	124	94	28	250

Diagnostic yield in 250 WES patients

- The underlying genetic defect was found in 62 (25%) of 250 consecutive patients
 - 33 were dominant (with 29 new mutations)
 - 16 recessive
 - 9 X-linked
 - 4 had two diseases

WES to identify a gene for MFDM

- Tested 4 unrelated individuals with mandibulofacial dysostosis
- Assumptions: All individuals would have a mutation in the same gene (not necessarily the same mutation). Condition is rare

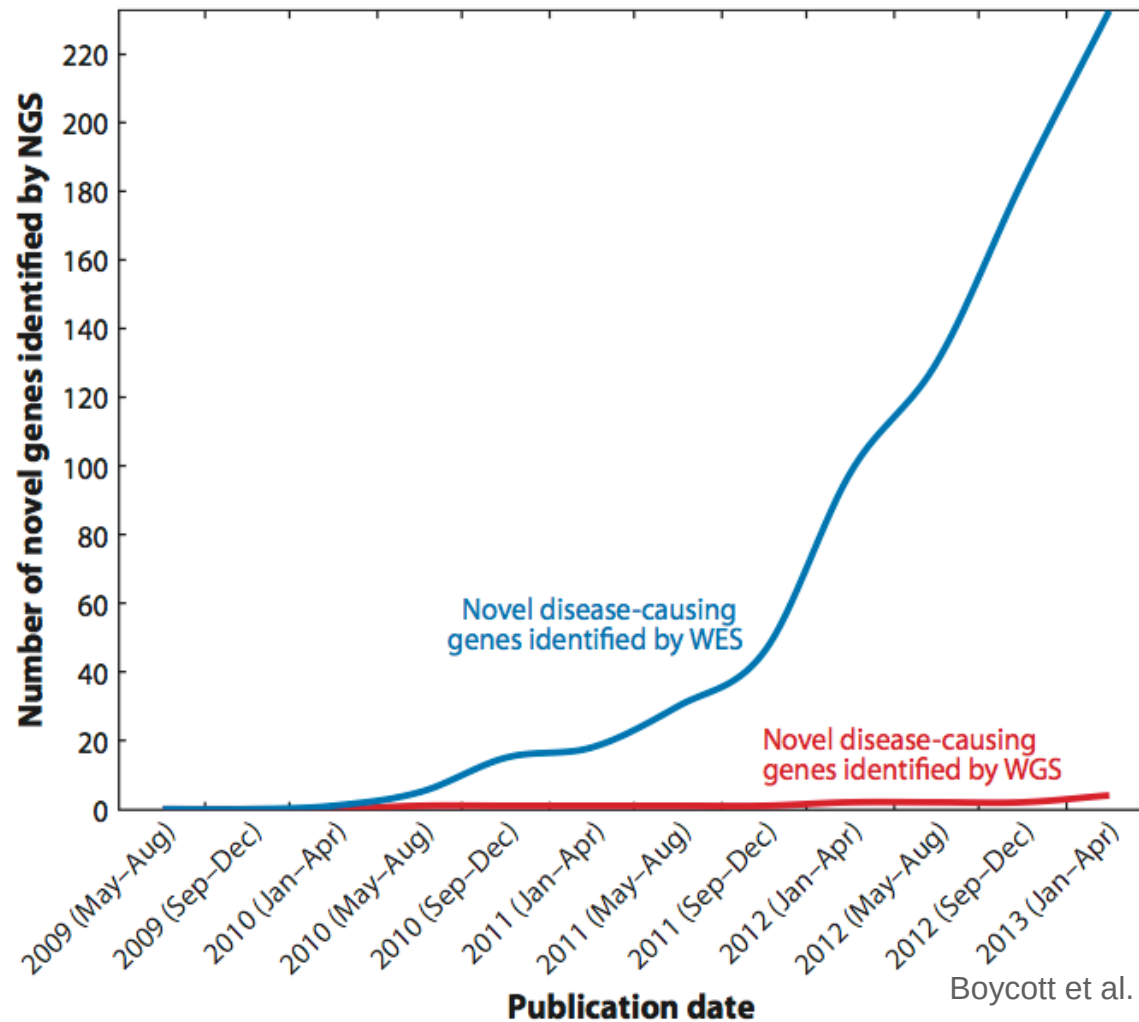
4th patient had probable deletion in *EFTUD2*

Included
EFTUD2

Gene with SNV in N patients, N=1,2,3,4	1	2	3	4
Gene mutated in N patients, N=1,2,3,4	1	2	3	4
Missense, nonsense, in/del, splice	2,500	200	20	2
Allele frequency $\leq 1\%$	1,500	160	8	0

MUC4

Identification of disease genes using NGS



Boycott et al. Ann Rev Med 2014