Targeted resequencing

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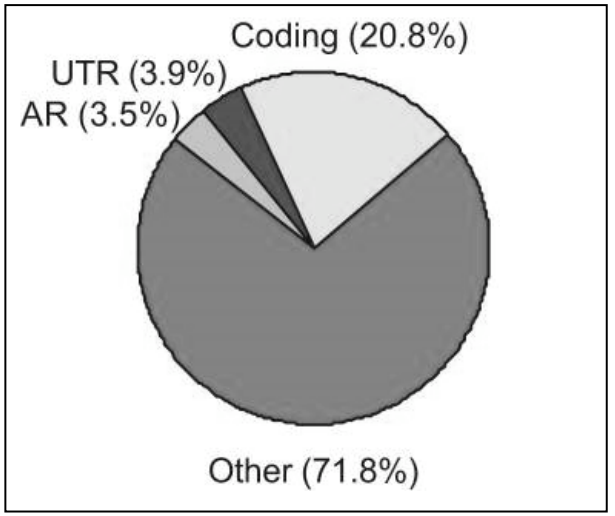
Snapshots of Genome Wide Analysis in Human Disease (MPG), 4/20/2010

Vamsi Mootha,
PI

How can I assess a small genomic target across many samples?

Limitations of whole exome sequencing

- expensive (1 Illumina lane/sample)
- coding only (~20% of all functional elements)



Multi-Species Conserved Seqs

Margulies et al. 2003

How can I assess a small genomic target across many samples?

Limitations of whole exome sequencing

- expensive (¥ 1 Illumina lane/sample)
- coding only (~20% of all functional elements)

Goal: sequence arbitrary small genomic regions, across many samples

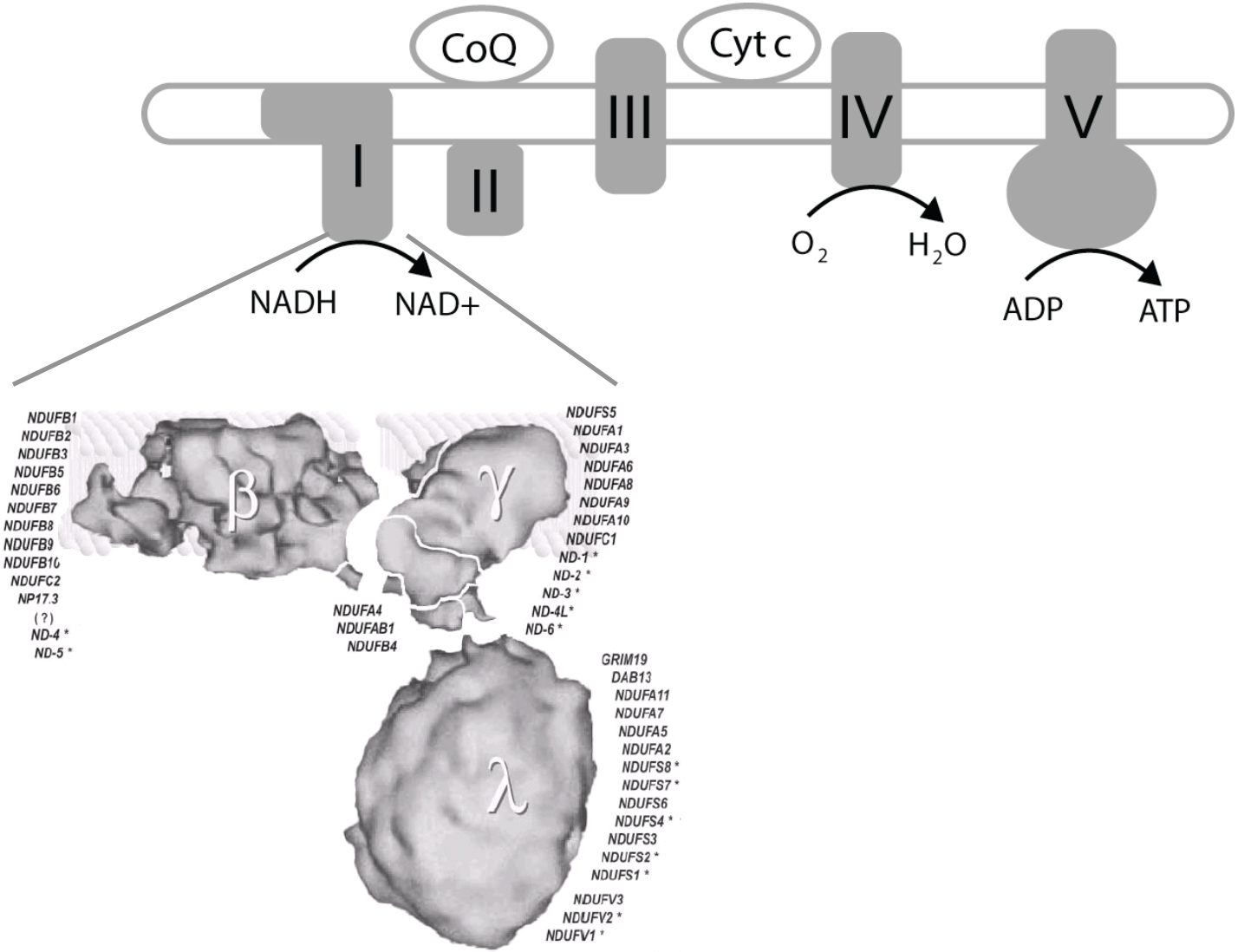
- genomic regions, eg GWAS
- non-coding genic, eg UTRs, regulatory, conserved seqs
- small number of candidate genes (100s)

Outline

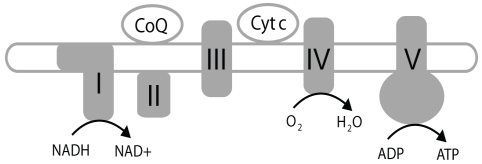
Snapshot of Mito10k (100 candidate genes x 100 patients)

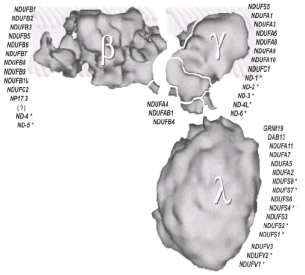
Lessons to share

Complex I of the mitochondrial respiratory chain



Complex I deficiency





Structure

- 46 subunits, 10 known assembly factors

Complex I Deficiency

- Most common OXPHOS disease
- Phenotypes
  - range from neonatal lethal, to adult-onset neurodegeneration
  - leukodystrophy, cardiomyopathy, seizures, Leigh syndrome
- 26 disease-related genes (19 subunits + 7 assembly factors)

Diagnosis difficult

No effective treatment

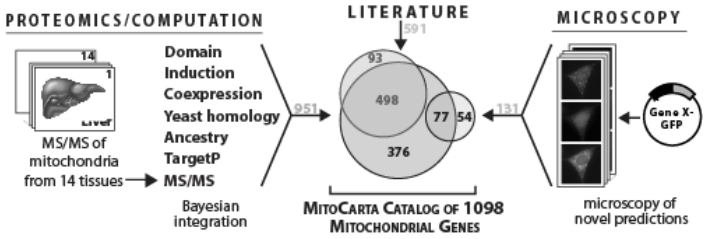
Goal: identify molecular basis for complex I deficiency

Method: sequence candidate genes in patient cohort

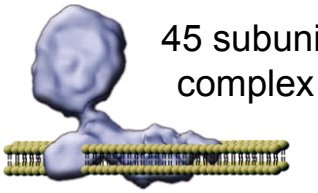
New candidates for complex I deficiency

Catalog of 1100 mitochondrial genes

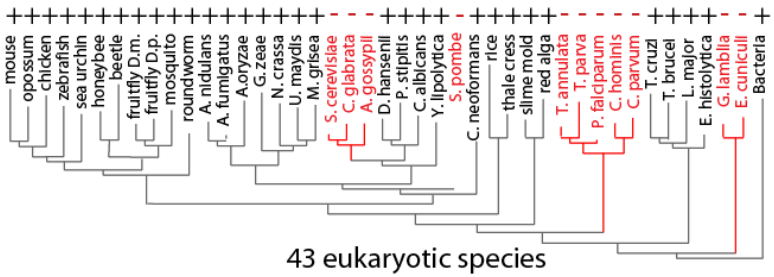
(Pagliarini, Calvo et al. Cell 2008)



Phylogenetic profiling



45 subunits complex I



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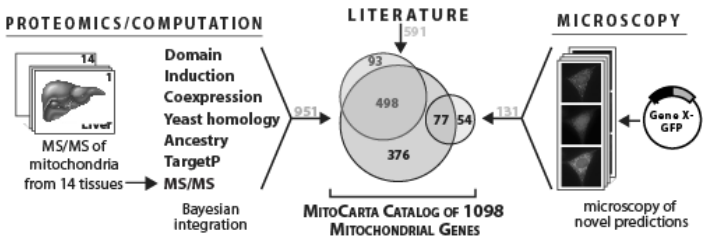
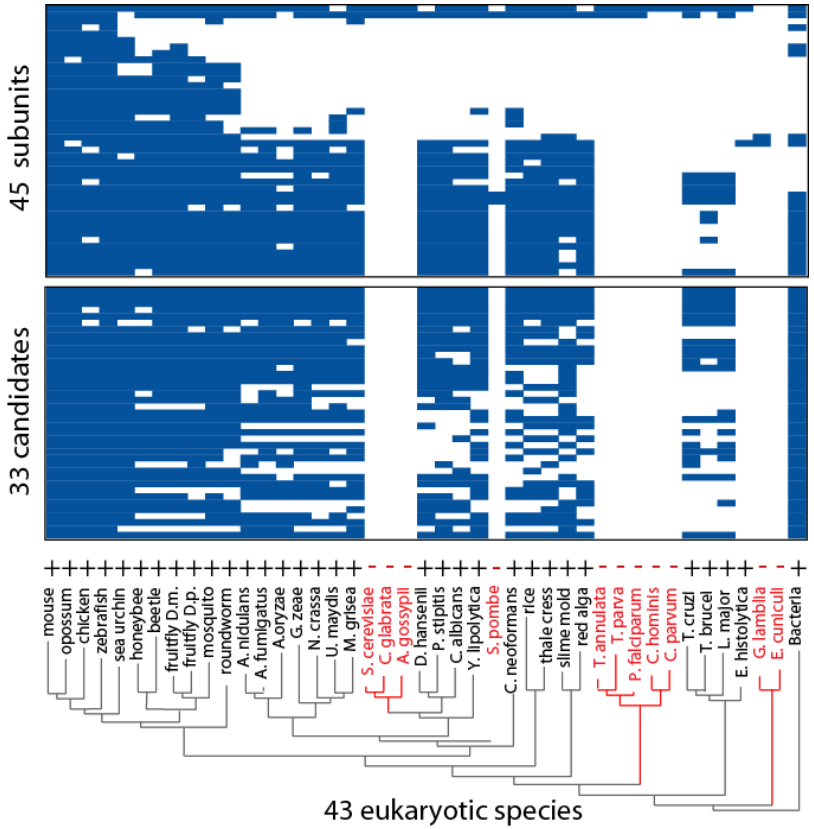


103 candidate genes

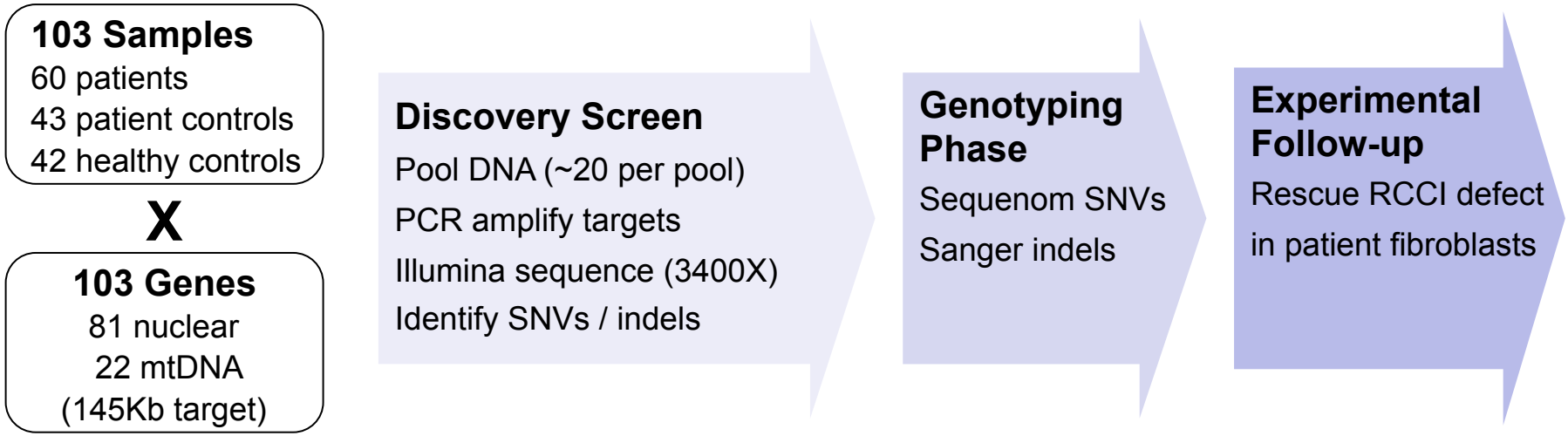
22 mtDNA

81 nuclear

Phylogenetic profiling



Mito10K: sequence 100 genes X 100 patients



Mito10K: sequence 100 genes X 100 patients

103 Samples
60 patients
43 patient controls
42 healthy controls

X

103 Genes
81 nuclear
22 mtDNA
(145Kb target)

Discovery Screen
Pool DNA (~20 per pool)
PCR amplify targets
Illumina sequence (3400X)
Identify SNVs / indels

Genotyping Phase
Sequenom SNVs
Sanger indels

Experimental Follow-up
Rescue RCCI defect in patient fibroblasts

Challenge: detect singleton SNVs, given high error rates

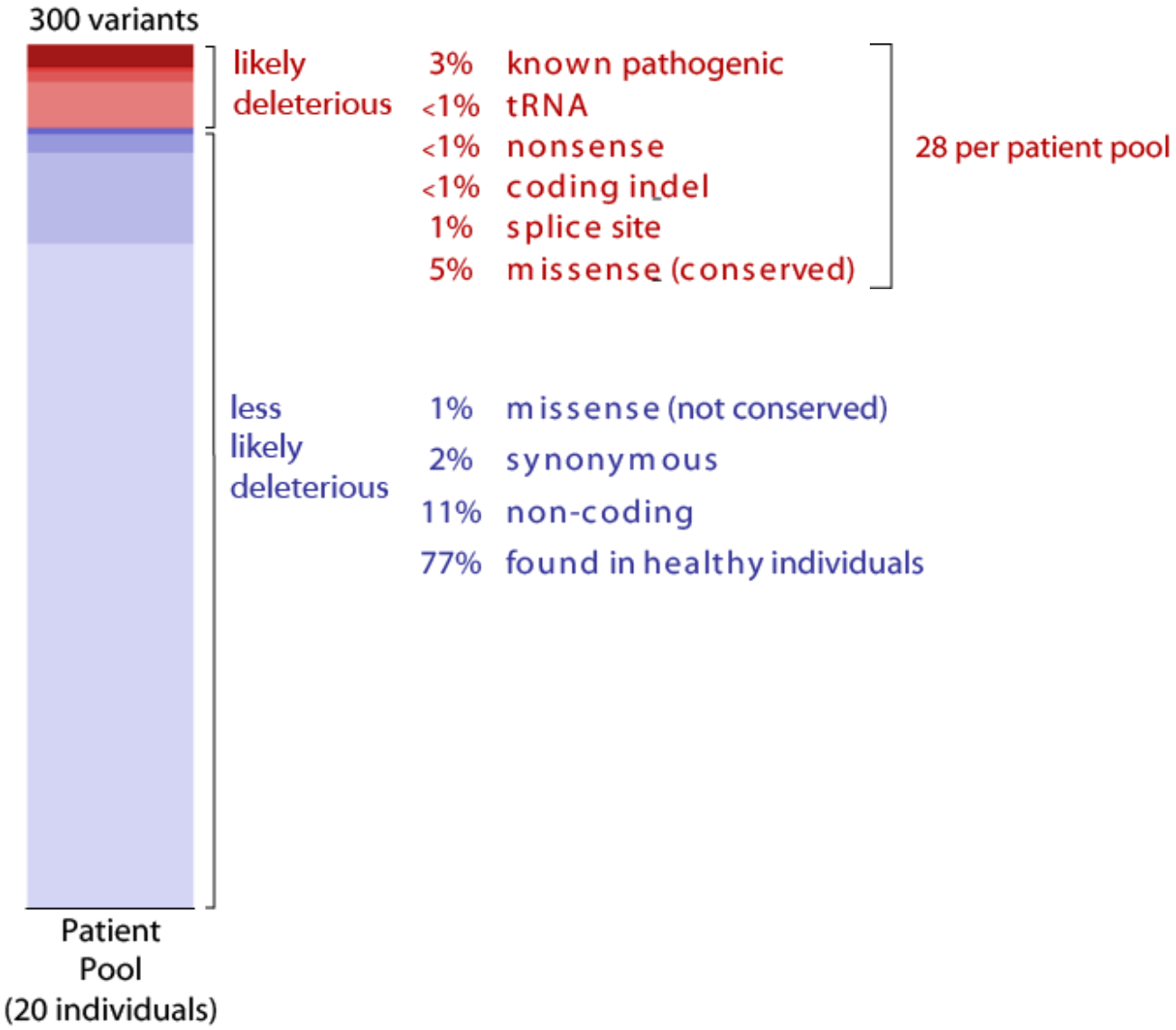
Method: Syzygy

- 92% sensitivity (76% singletons)
- 9% false positives

~300 SNVs detected per pool

Manuel Rivas

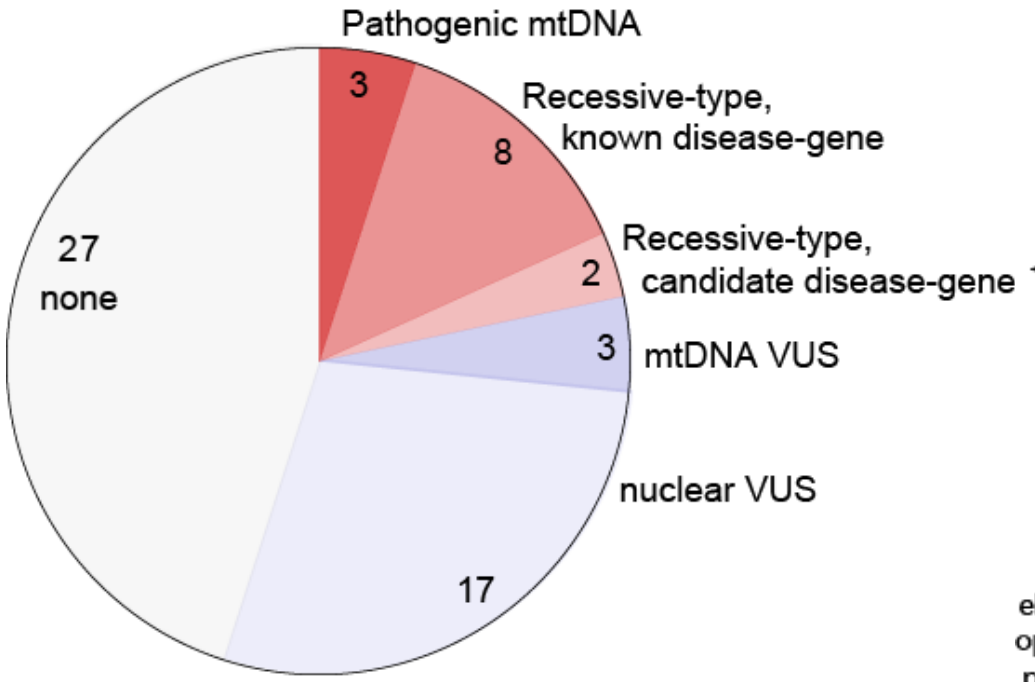
Can we identify likely deleterious variants?



Can we find causal mutations in patient cohort?

Expect: known pathogenic mtDNA variants

recessive-type variants in known nuclear genes

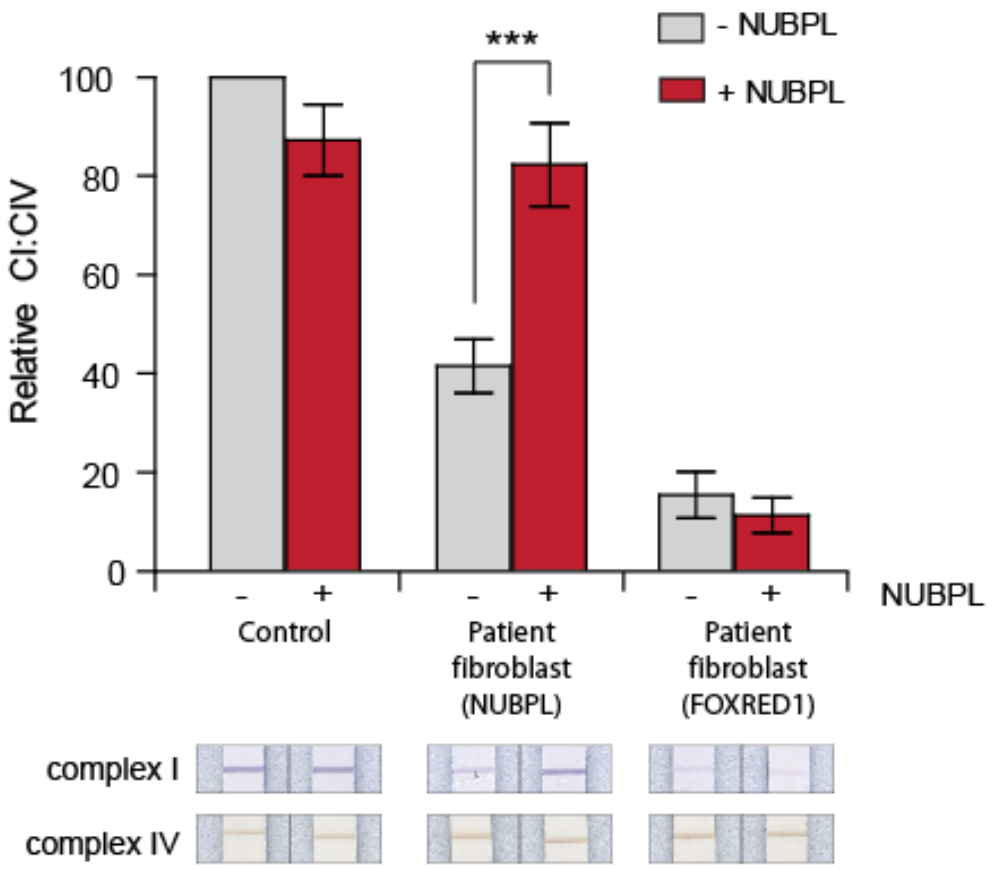


60 patients with complex I deficiency

FOXRED1 (compound het)
NUBPL (homozygous)

How can we establish pathogenicity of novel genes?

Patient fibroblasts show complex I defect



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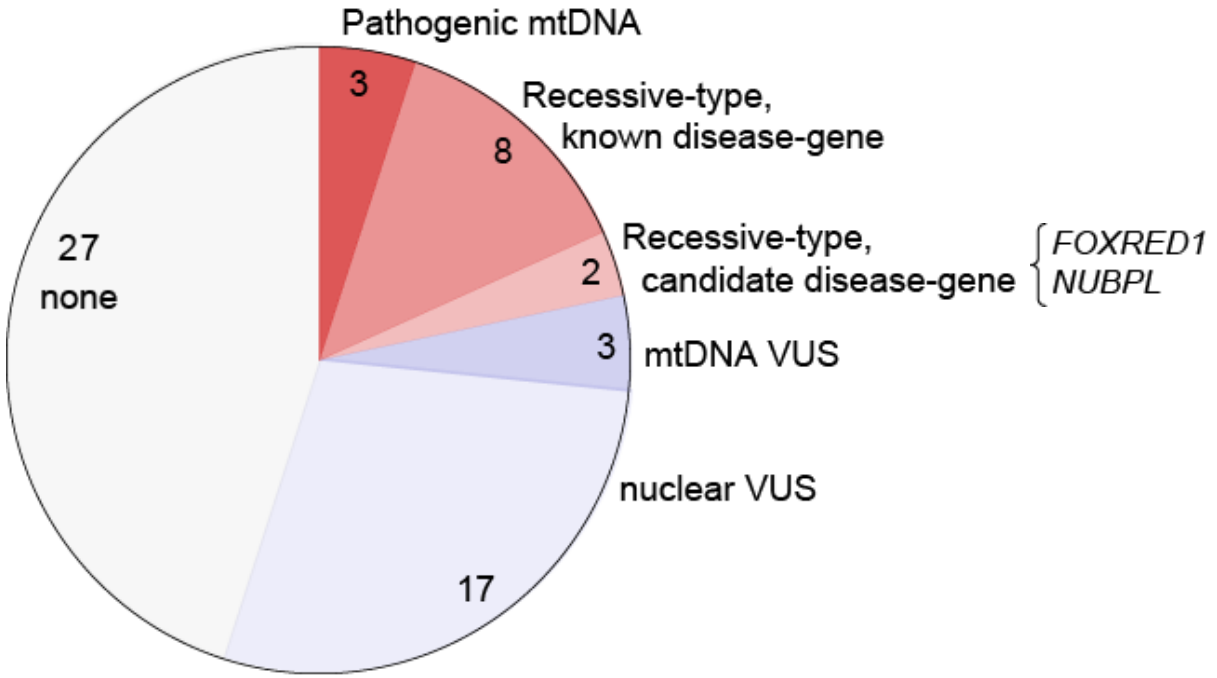


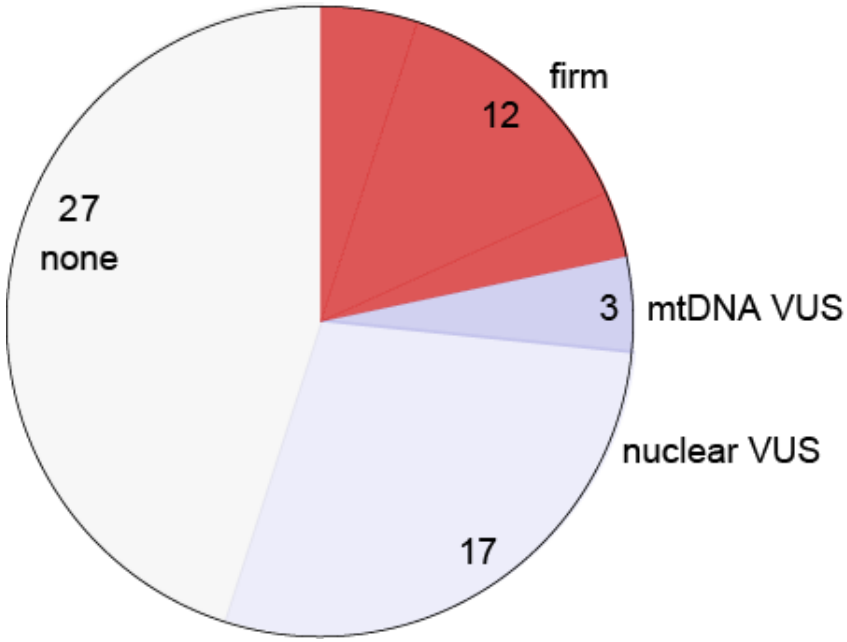
60 patients with complex I deficiency

Can we find causal mutations in patient cohort?

Diagnosis of 22% of patient cohort
2 novel disease-related genes
5 novel mutations in known disease genes

What about rest?

- non-targeted gene?
- non-coding region?
- poor coverage?
- less likely deleterious?
- dominant?
- synergistic variants?



60 patients with complex I deficiency

Mito10K summary

Mito10k: targeted resequencing of ~100 candidate genes x 100 patients

- cost-effective
- diagnosis in 22% of undiagnosed patients
- 2 novel disease-related genes
- Key: availability of cellular models

Available methods/tools

- Syzygy (http://www.broadinstitute.org/ftp/pub/mpg/syzygy/syzygy.zip)
- Definition of ‘likely deleterious’ (Appendix A in this presentation)
- IGV (http://www.broadinstitute.org/igv/)

Outline

Snapshot of Mito10k (100 candidate genes x 100 patients)

Lessons to share

1. sample preparation
2. validation of rare variants
3. cost
4. phasing

Sample preparation: what if I don't have enough DNA?

Issue: need 3ug DNA for sequencing

Method: whole genome amplification (WGA)

- pros: works well (straight-forward, inexpensive)
- cons: possible mutations
  - unequal amplification of regions
  - unequal amplification of alleles
  - rolling PCR artifact (if DNA slightly degraded)

Observations:

- Pooling: unequal amplification mtDNA → pools with 96% mtDNA from 1 person
- Single-sample: rolling PCR → exons with excessive coverage, false SNP/indel calls

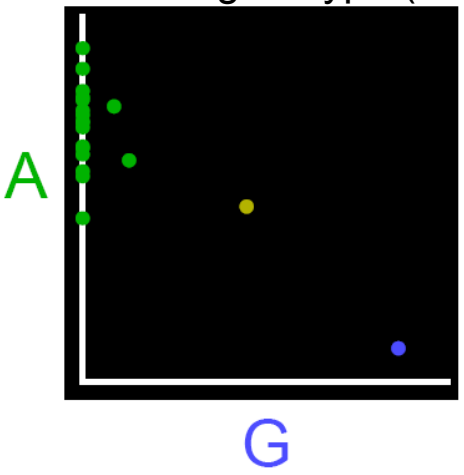
Conclusions: WGA works, however avoid if possible

How can I validate rare variants?

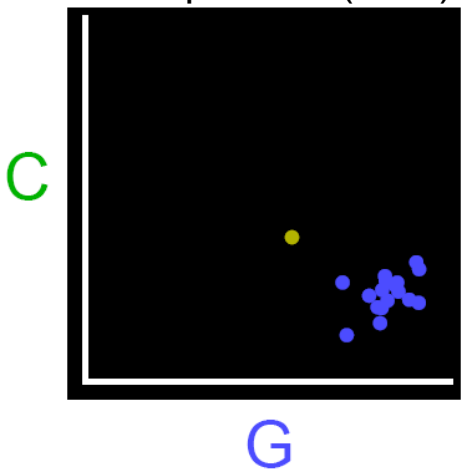
Method: genotype via Sequenom or BeadXPress

Assess Sequenom accuracy: Sanger validation of 82 DNA sites

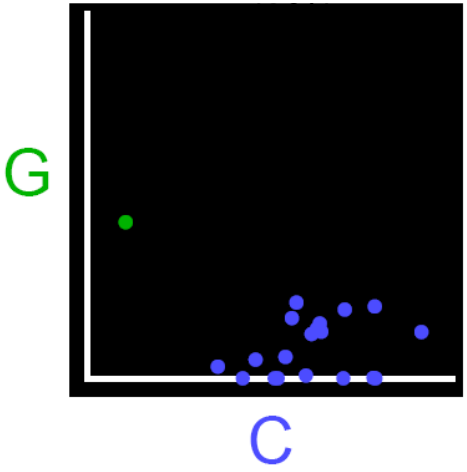
Accurate genotype (88%)



False positive (10%)



Hom/Het Miscall



Caveats (not necessary Sequenom error):

- WGA (mutation or unequal amplification of alleles)
- Sequenom design params (# samples iPLEXed)
- Sample mixups

Conclusions: care with design of rare variant validation

How should I minimize cost in project design?

Observation from Mito10K pilot:

- 7 Illumina lanes (~$10K)
- 40,000 Sequenom genotypes (~20K)
- Months data analysis

Conclusions: to minimize analysis costs: design fewer data tranches, larger # samples

How can I phase rare heterozygous variants?

Issue: If interested in mutations consistent with recessive inheritance,
need to determine if 2 observed rare hets are:
a) in-phase (same parental chromosome), or
b) compound heterozygous (different parental chromosomes)

Method:
common variants: computational methods (eg imputation)
rare variants: genotype familial DNA or experimental method to “phase” variants

Conclusions: obtain parental DNA during sample collection OR
consider experimental methods to phase

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Gabe Crawford
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Entire Genetic Analysis platform

Biological Samples Platform
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Kristin Ardlie
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Entire BSP platform

Sequencing Platform
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Entire Seq platform

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Patients!

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Appendix A: definition of likely deleterious variants

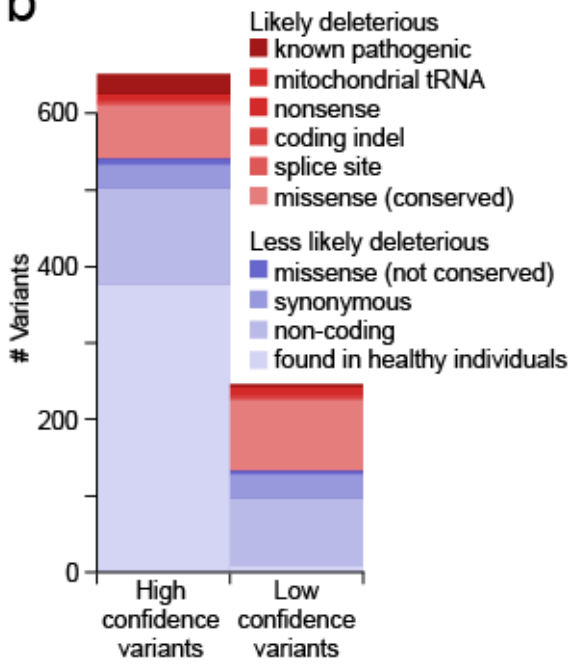
For the Mito10K project, we used the following algorithm to identify variants most likely to underlie a rare, devastating phenotype

1. Include all disease-associated variants (Human Gene Mutation Database & curation)
2. Next, exclude variants present in healthy individuals, based on
    1. dbSNP129
    2. 1000 genomes project, pilot 1
    3. 42 HapMap controls also sequenced
    4. mtDNA variants present in mtDB with minor allele frequency > 0.005
3. Next, include:
    1. coding indel
    2. nonsense variant
    3. missense variant at an amino acid conserved in ≥10 aligned vertebrate species, based on the multiz44way genome alignments (UCSC), or predicted as 'damaging' by PolyPhen-2.0 (HumVar training data)
    4. splice-site (splice acceptor sites -1,-2,-3, and splice donor sites -1,1,2,3,5 selected based on training data consisting of all 8189 HGMD disease-associated splice variants)
    5. tRNA variants in mitochondrial genome
    6. 5' UTR variants which create or disrupt an AUG alternate start codon

a

Variant type	High confidence variant calls			Low confidence variant calls		
	Discovered in patients	Likely deleterious	Validated	Discovered in patients	Likely deleterious	Validated
nDNA						
nonsense	3	2	1	5	5	1
missense	131	60	51	97	86	9
splice	78	28	22	40	16	2
synonymous	92	0	0	33	0	0
UTR	214	0	0	71	0	0
coding indel	3	3	3	0	0	0
mtDNA						
nonsense	0	0	0	0	0	0
missense	37	14	12	0	0	0
synonymous	85	0	0	0	0	0
noncoding	9	2	2	0	0	0
Total	**652**	**109**	**91**	**246**	**107**	**12**

b



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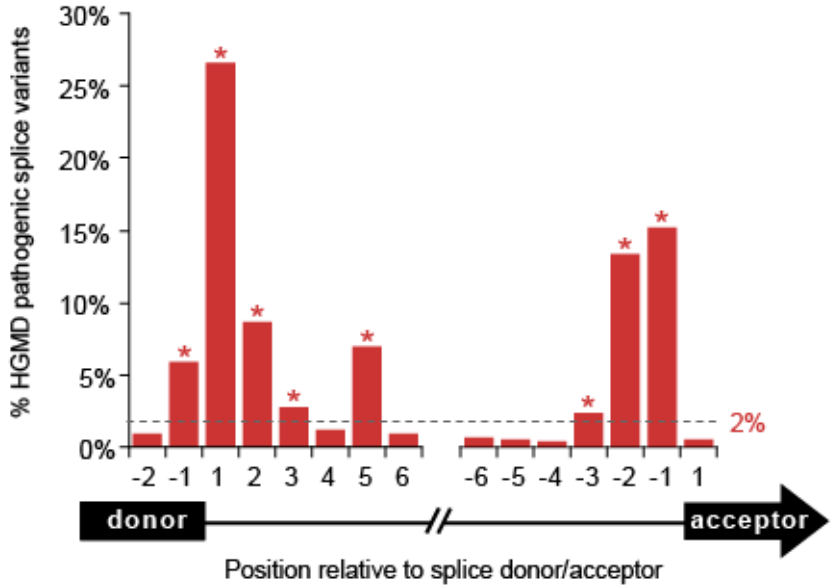

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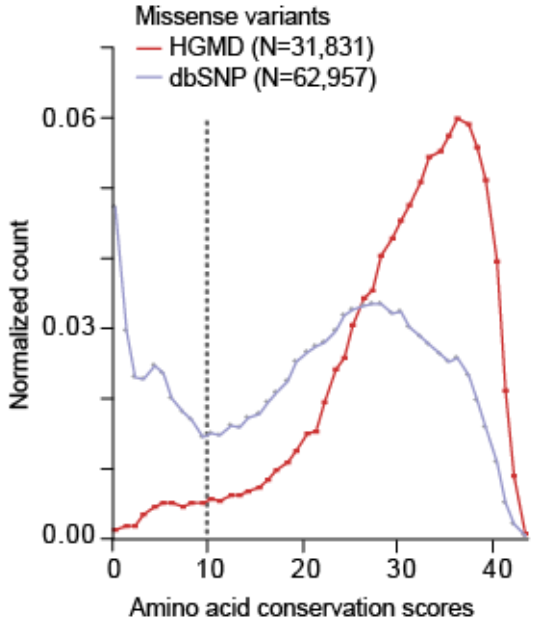

Genetic diagnosis of CI cohort (94 unrelated individuals)