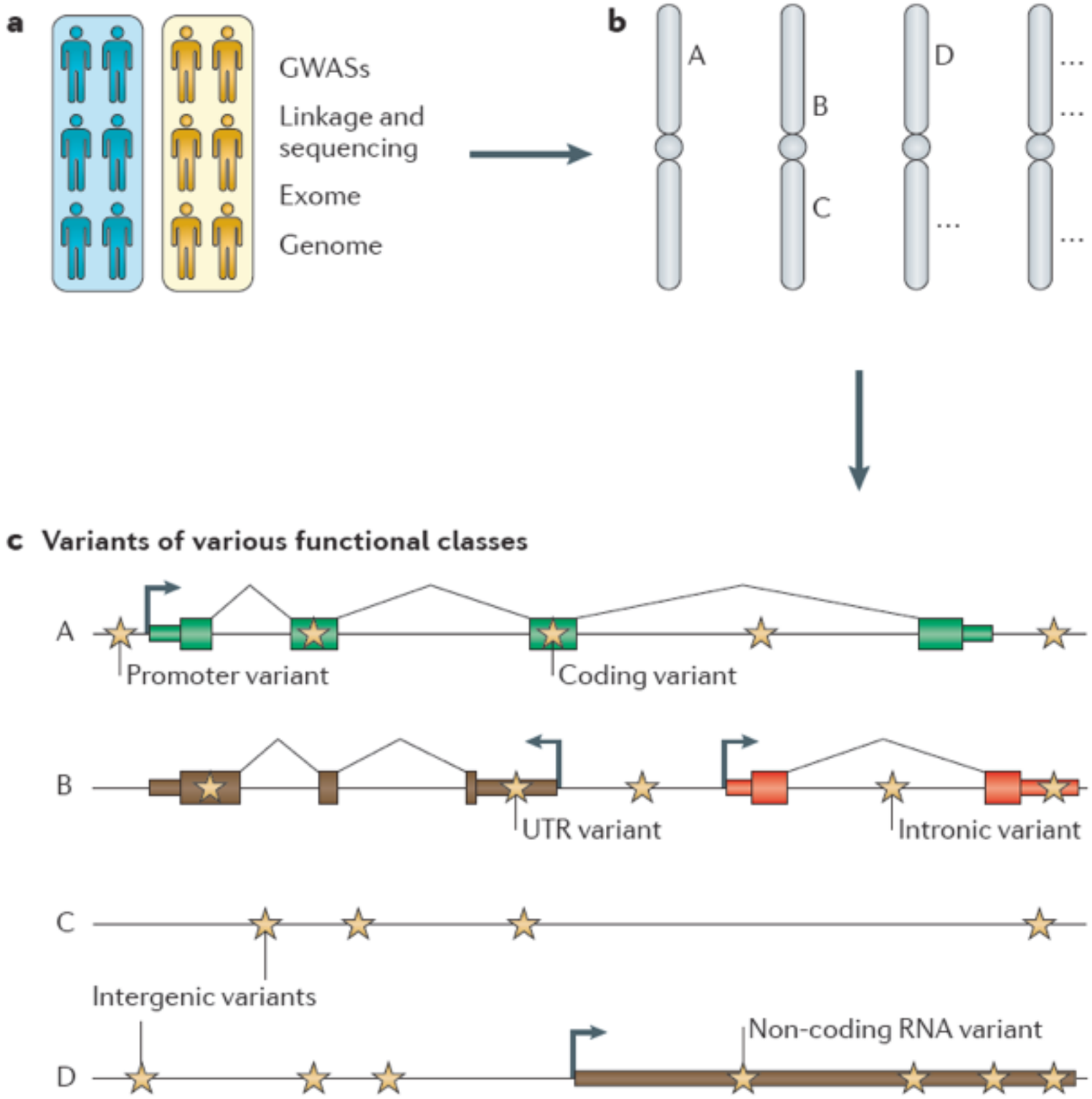
Identification of Causal Variants in Individual Human Genomes

Greg Cooper
HudsonAlpha Institute for Biotechnology
UAB Genomics Immersion Course
October 2015

Human Genetics



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Cooper and Shendure *NRG* 2011

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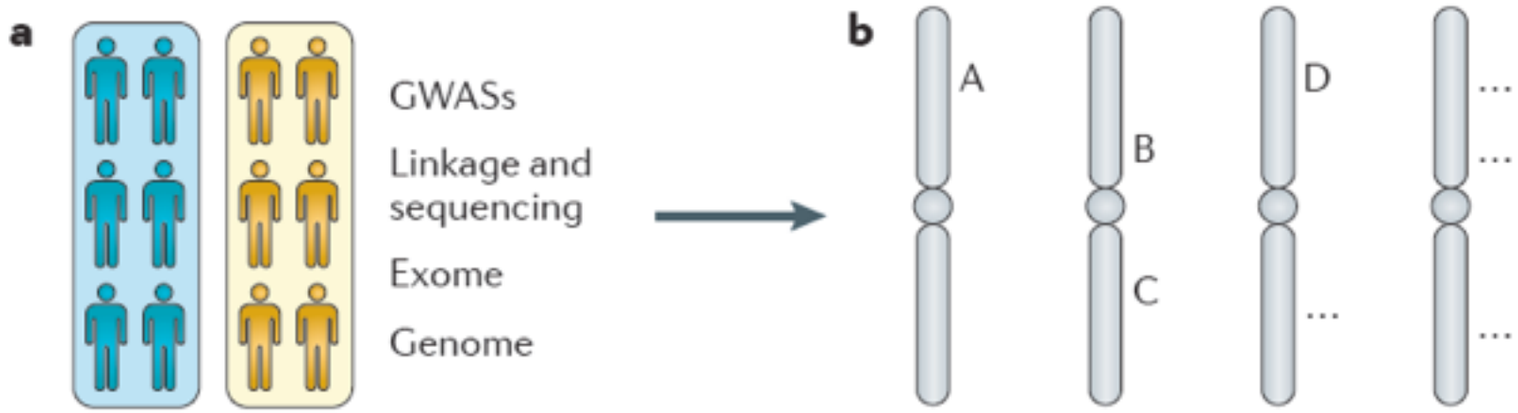
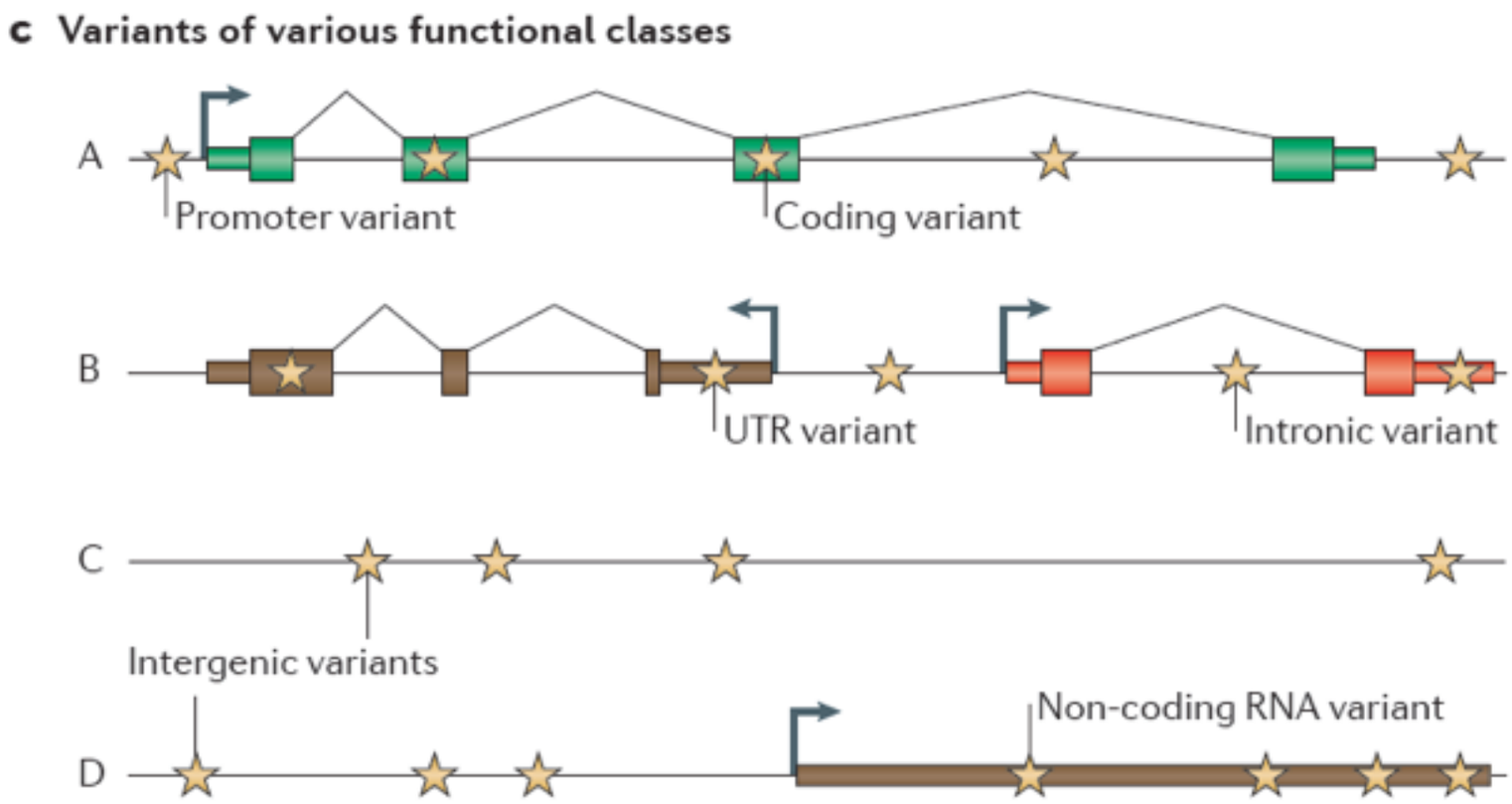


Statistical Power



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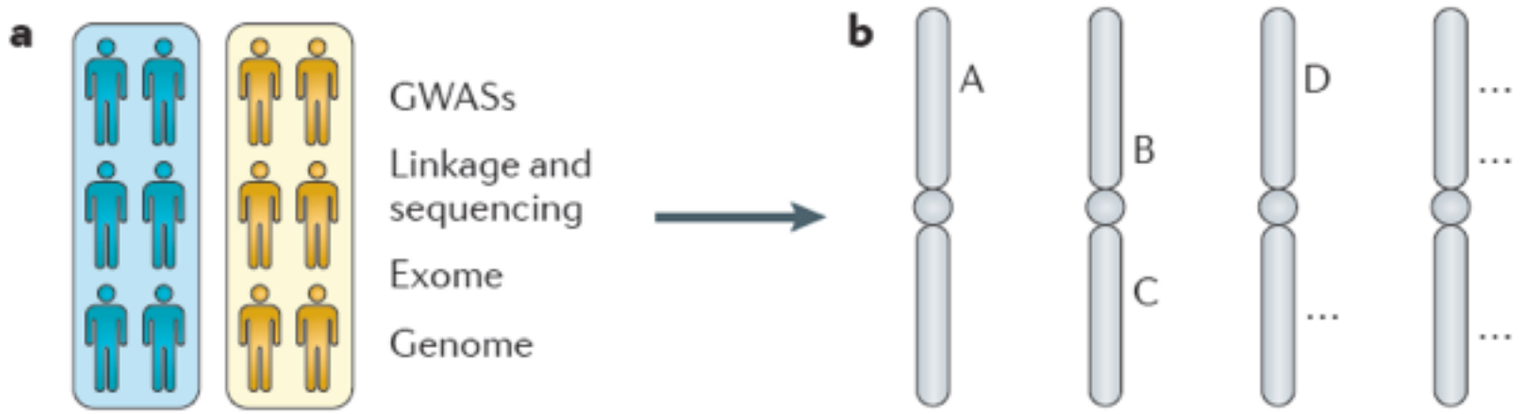
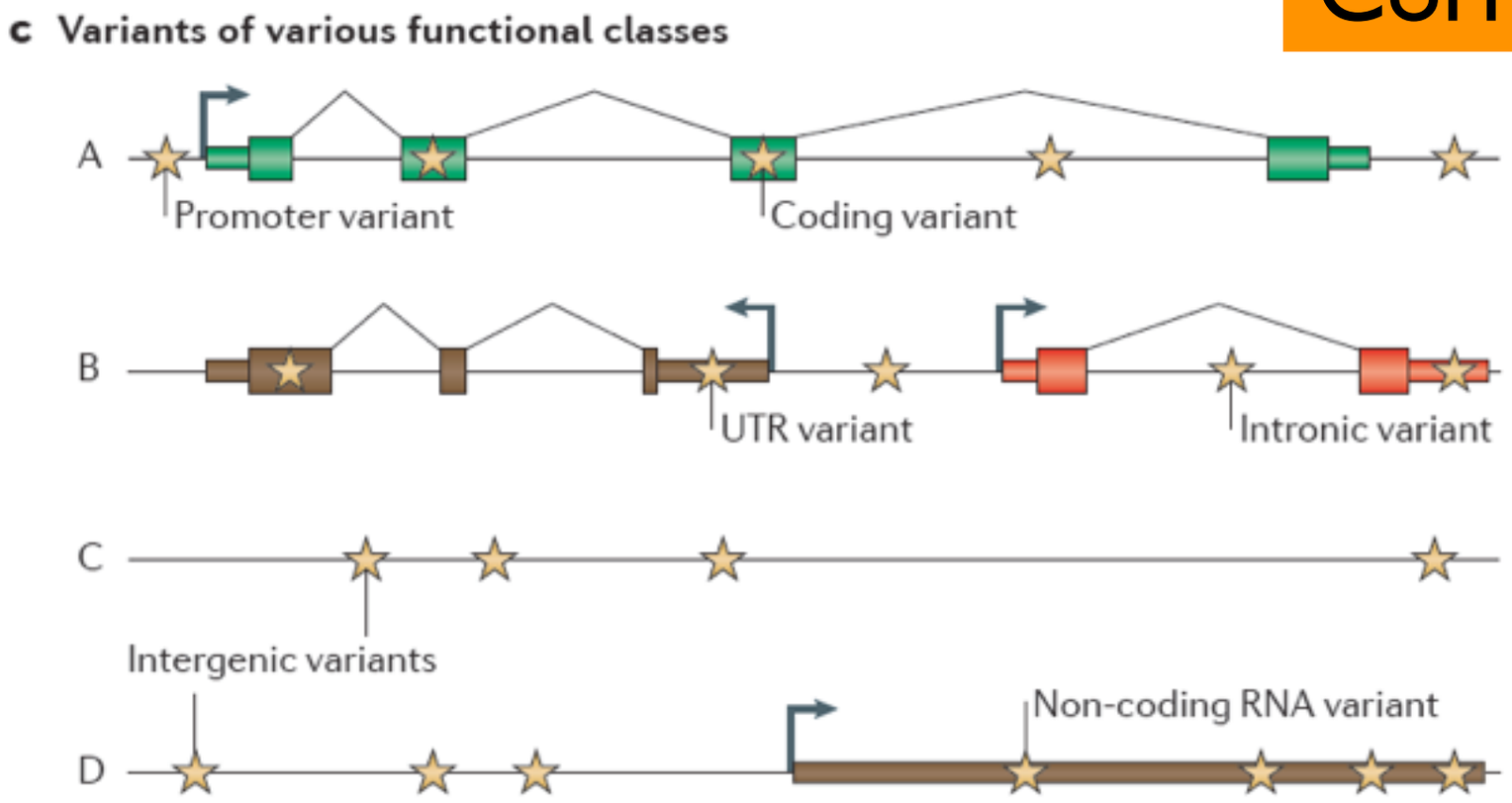


Statistical Power

Haplotypic Correlations



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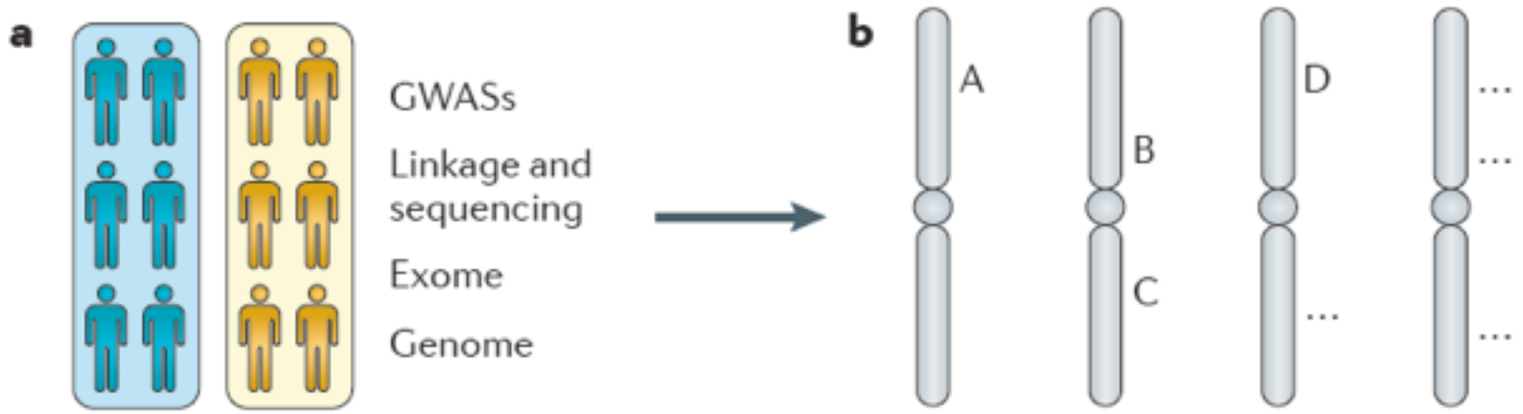

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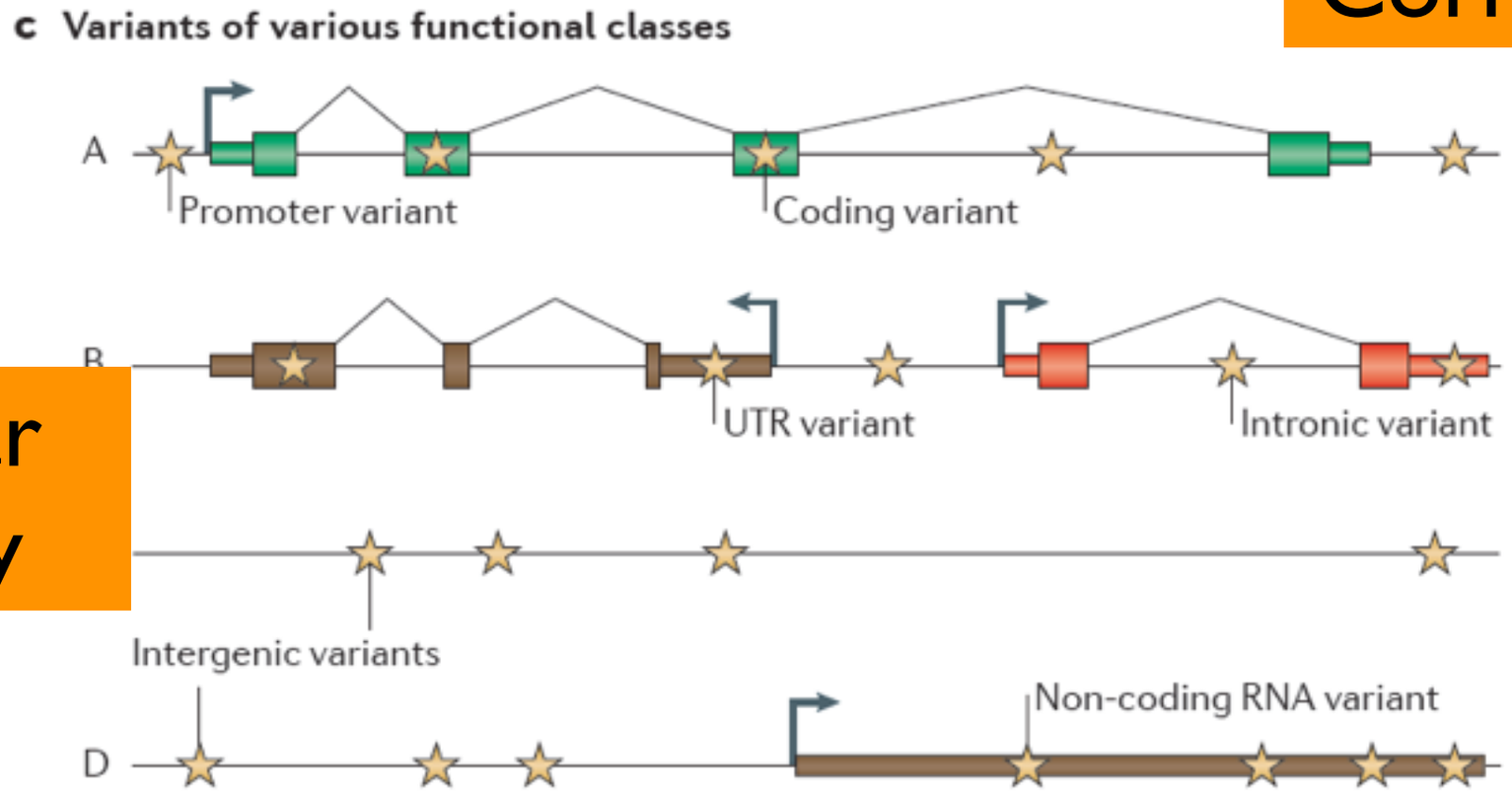
Haplotypic Correlations

Molecular Diversity



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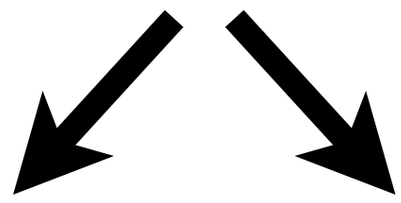

Function Evolution

Some Terminology

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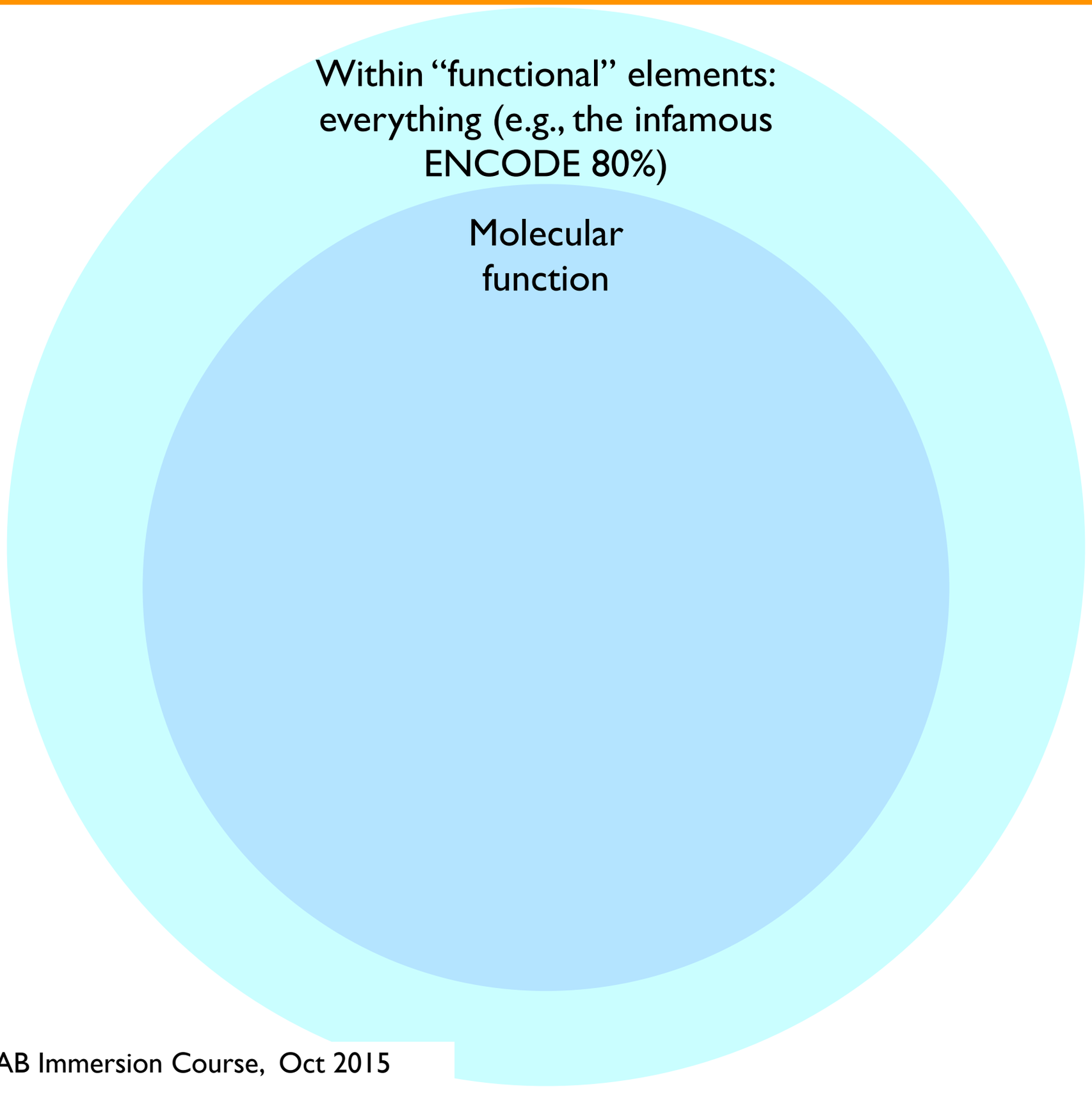
  - Deleterious, i.e., reduces survival or reproductive success and imposes fitness costs

Property Domains

Within "functional" elements:
everything (e.g., the infamous
ENCODE 80%)

Property Domains



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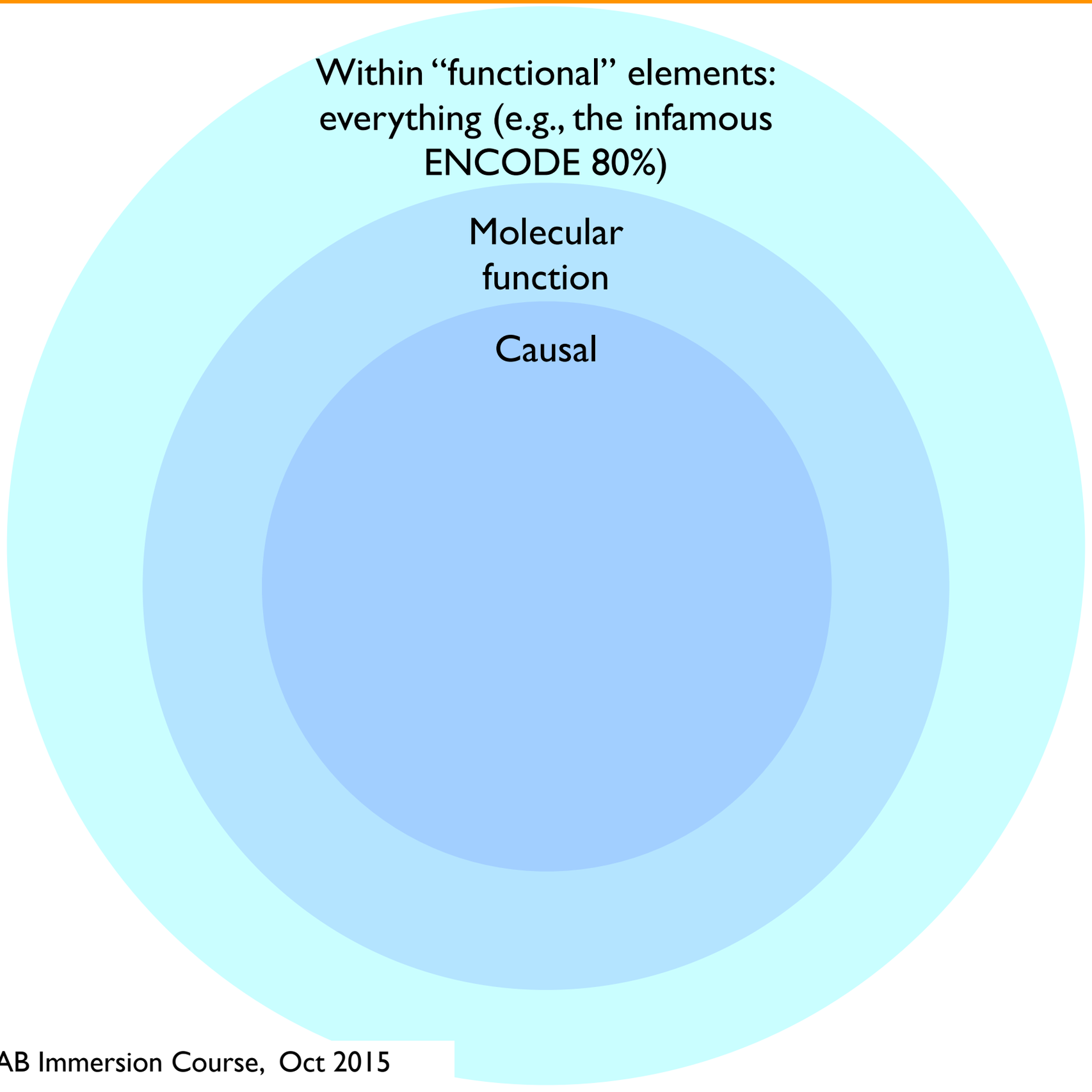

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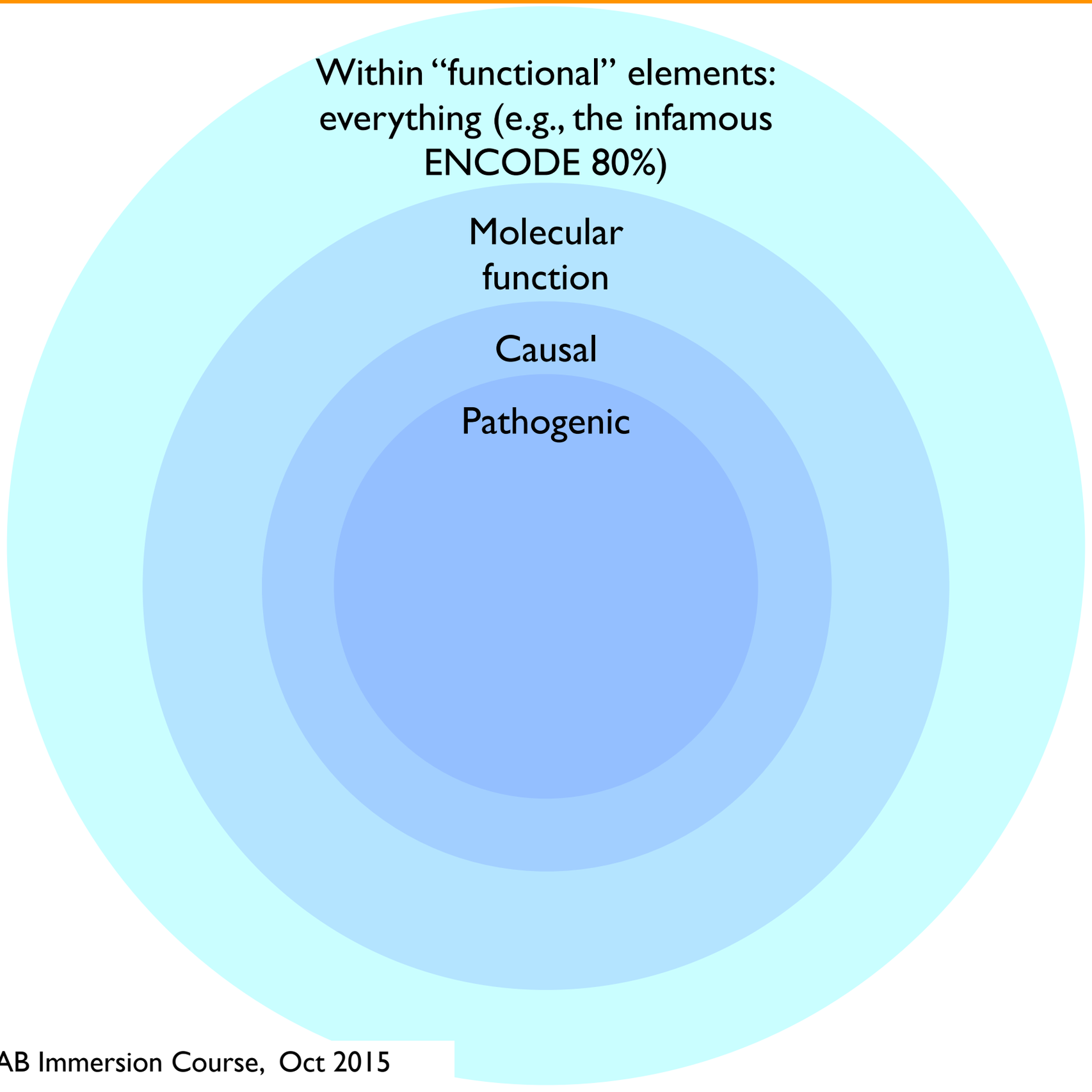

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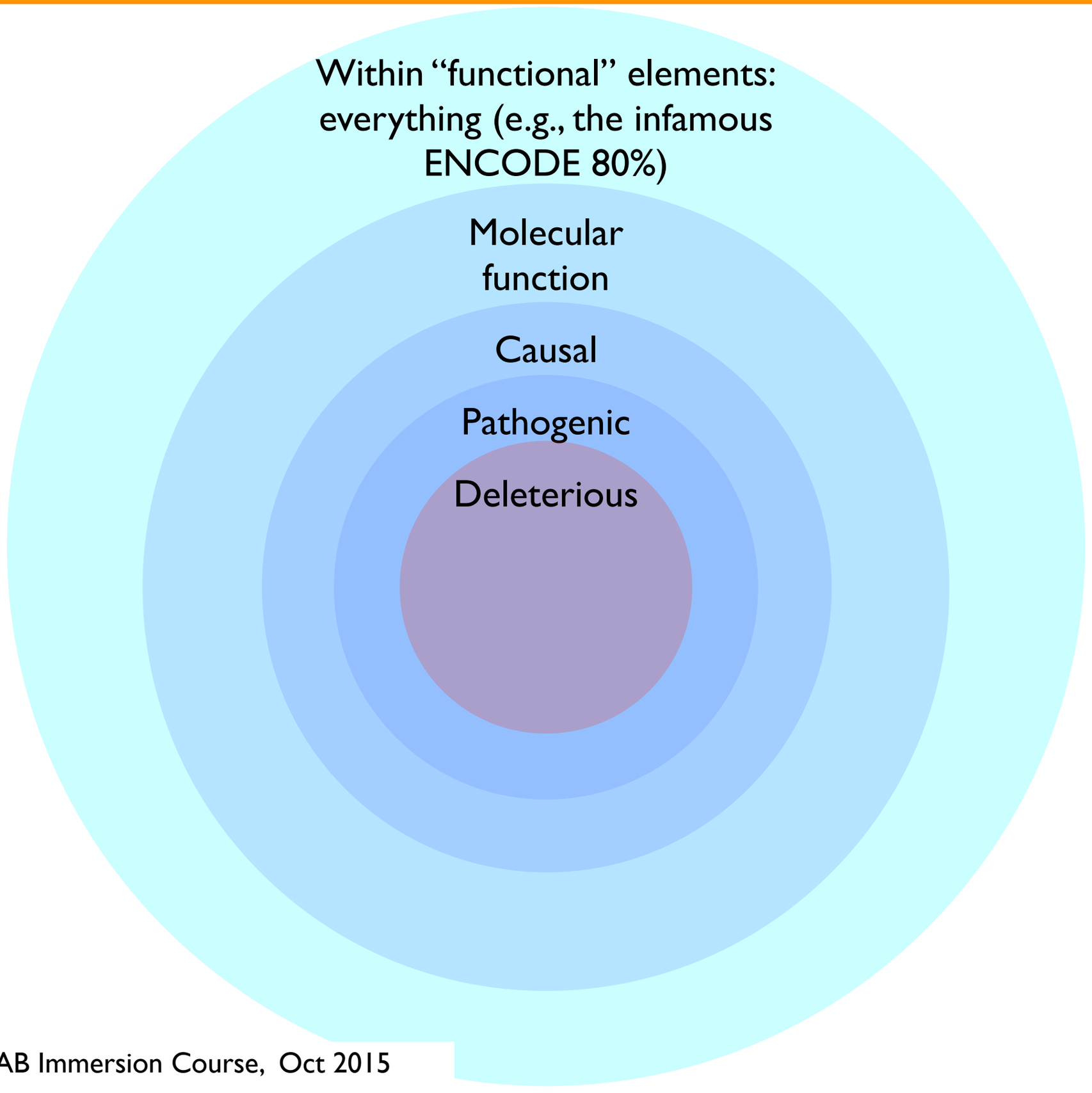

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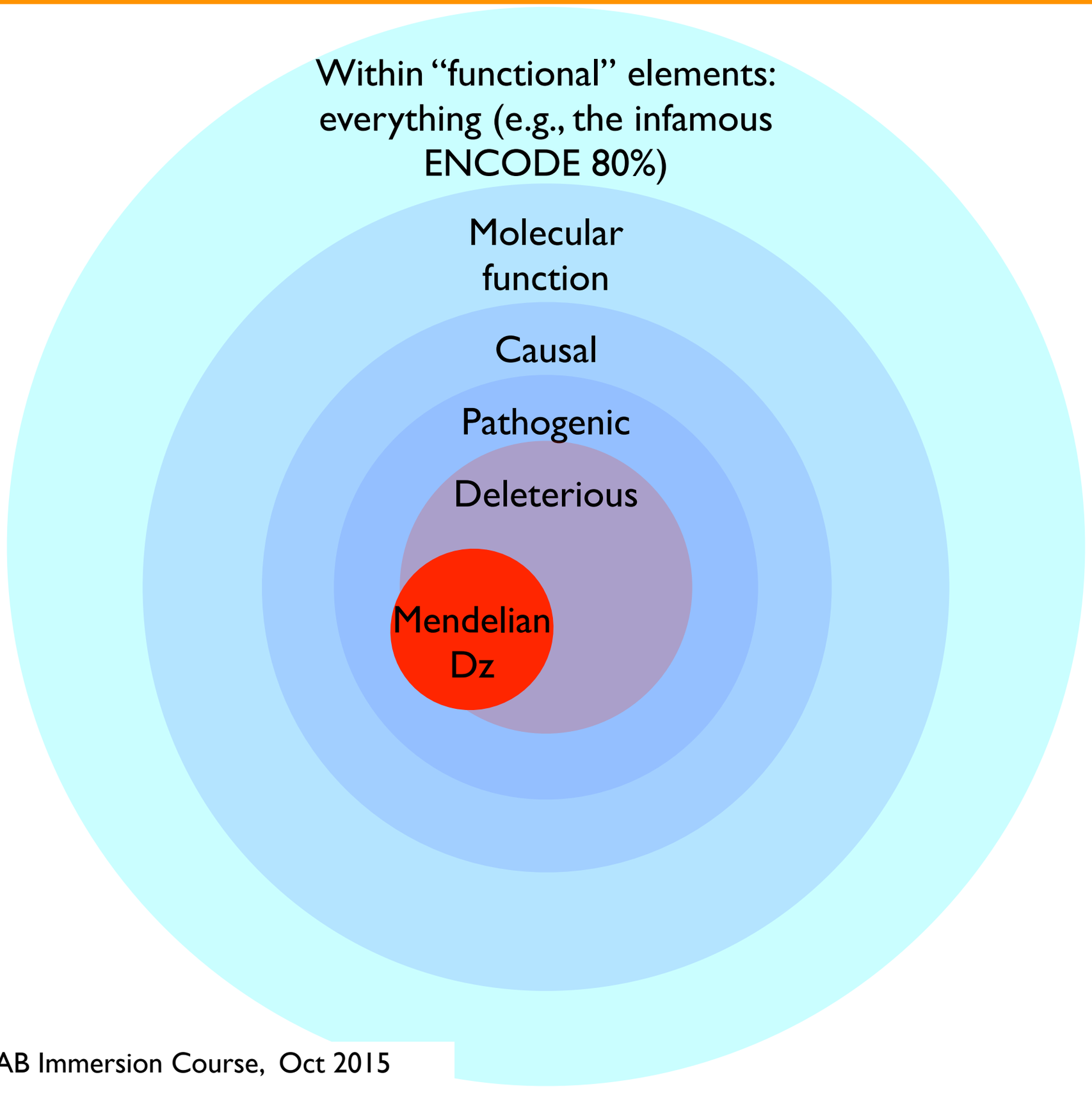

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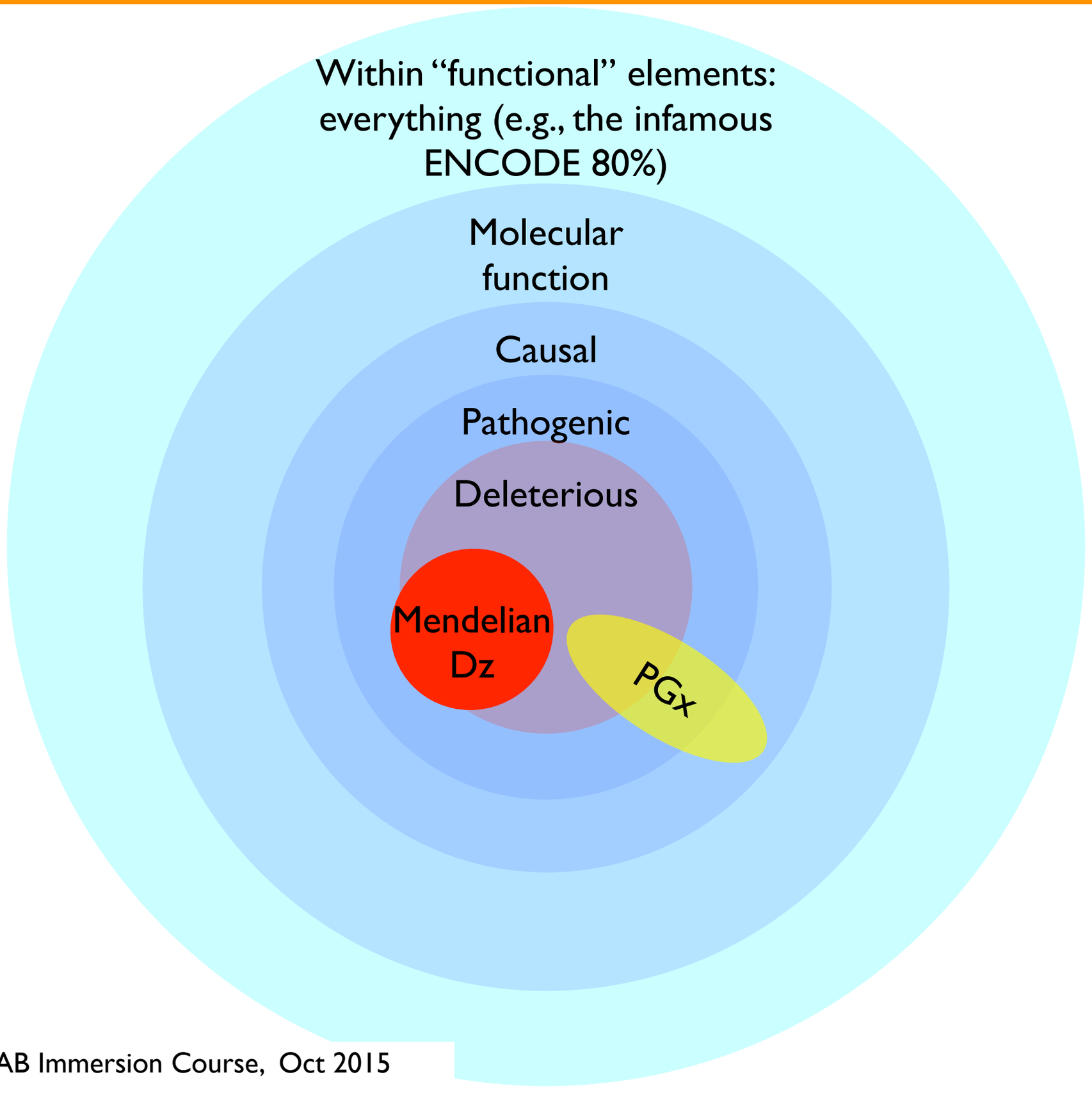

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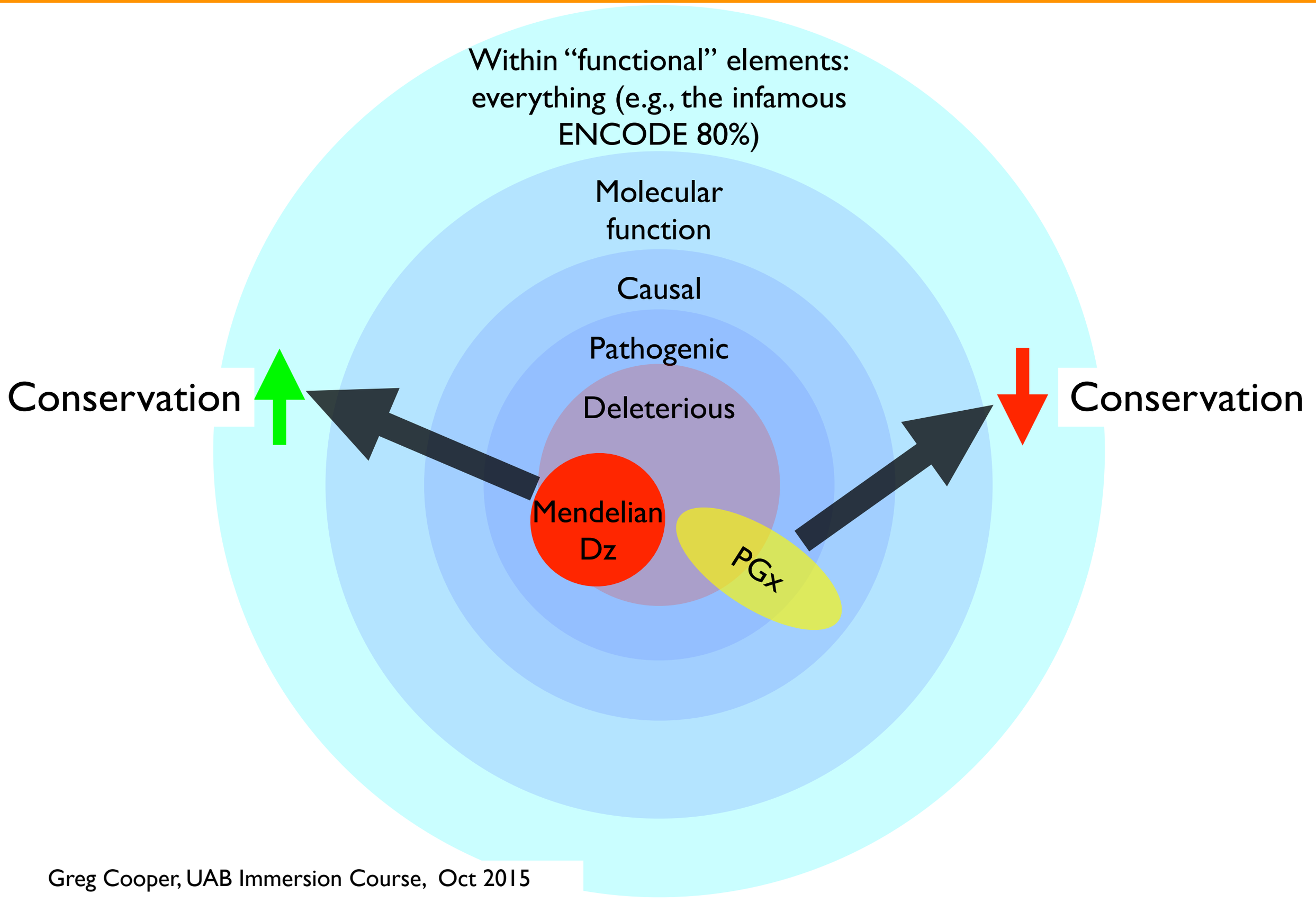

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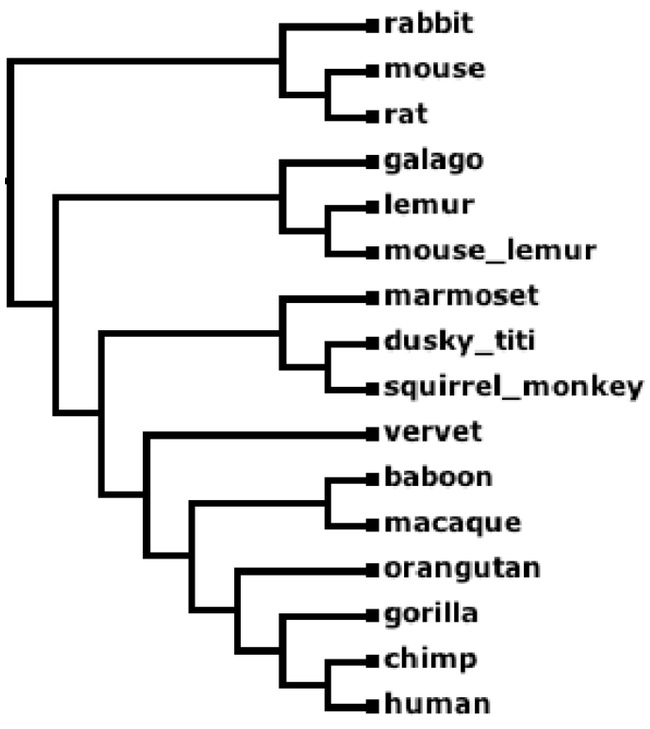
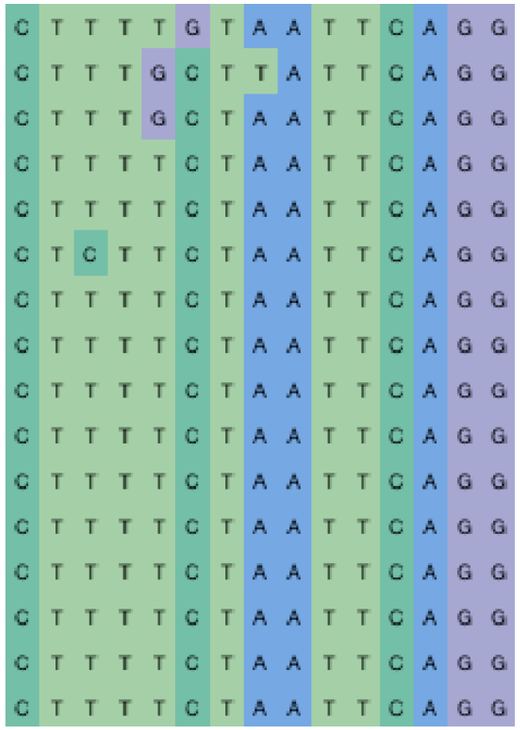
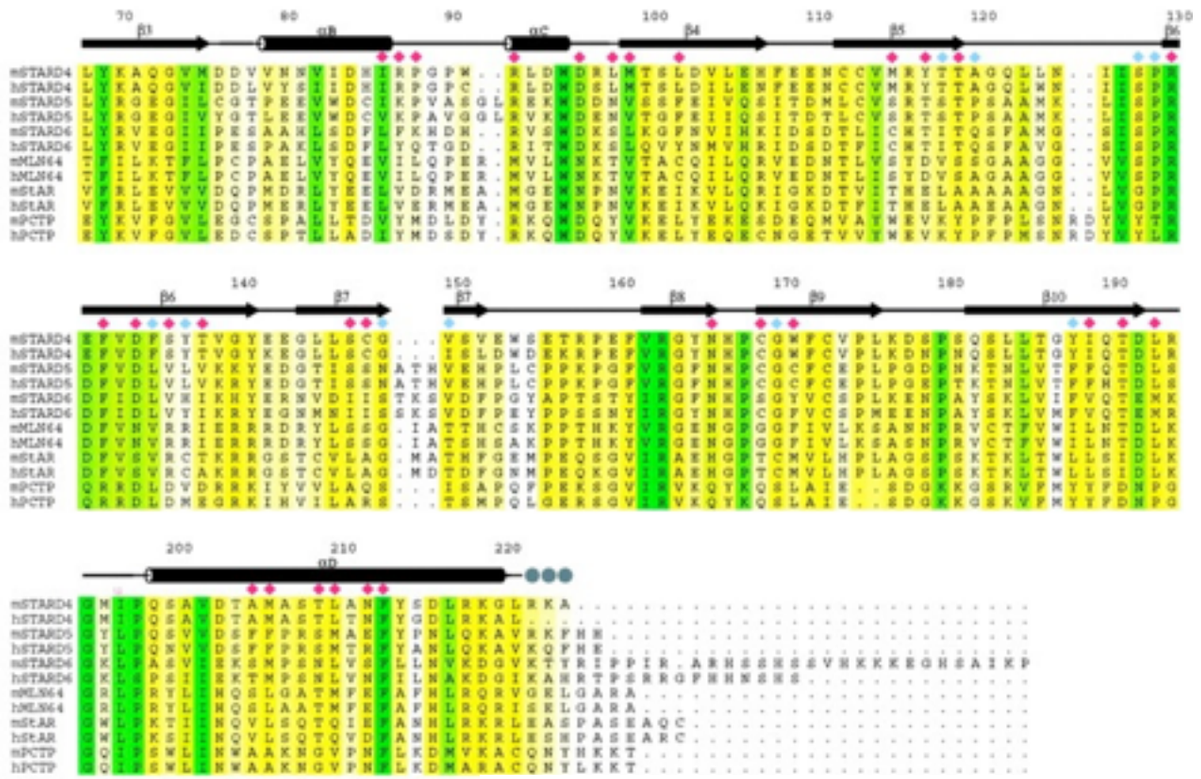
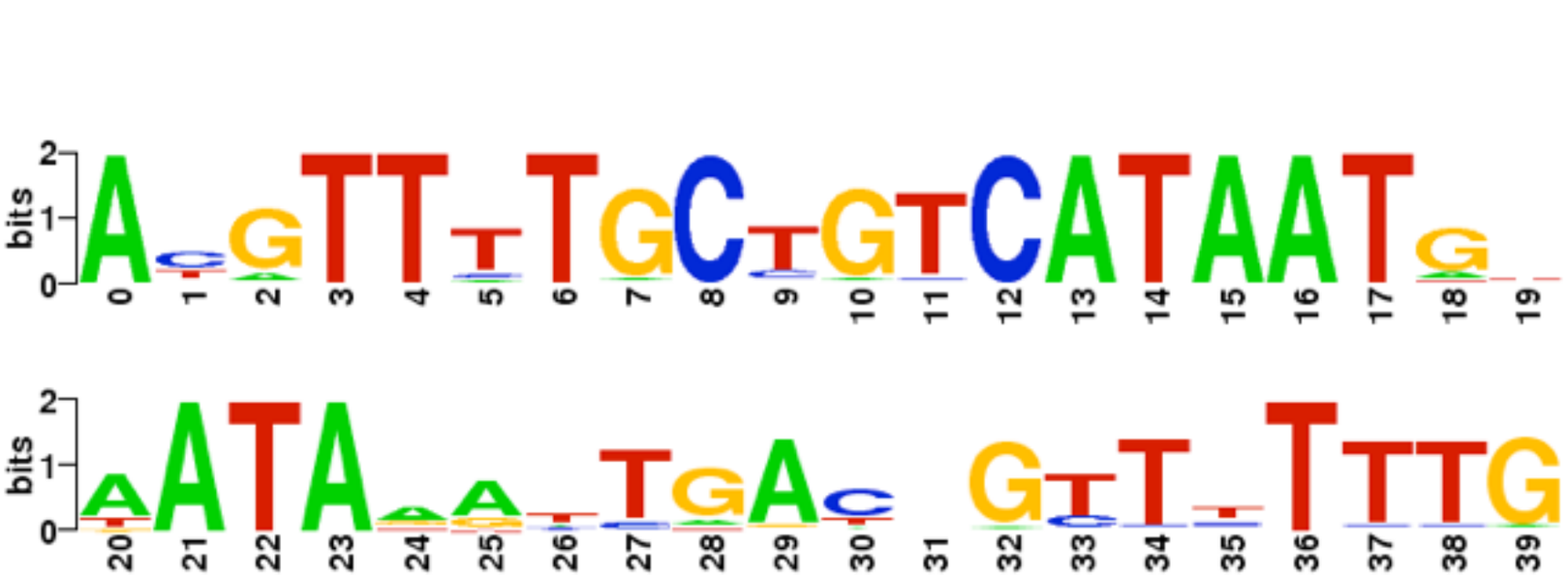
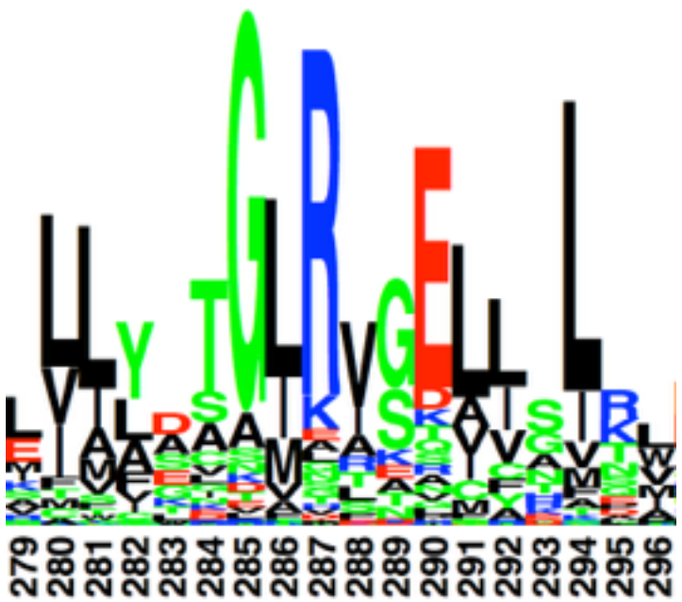
Sequence Comparisons











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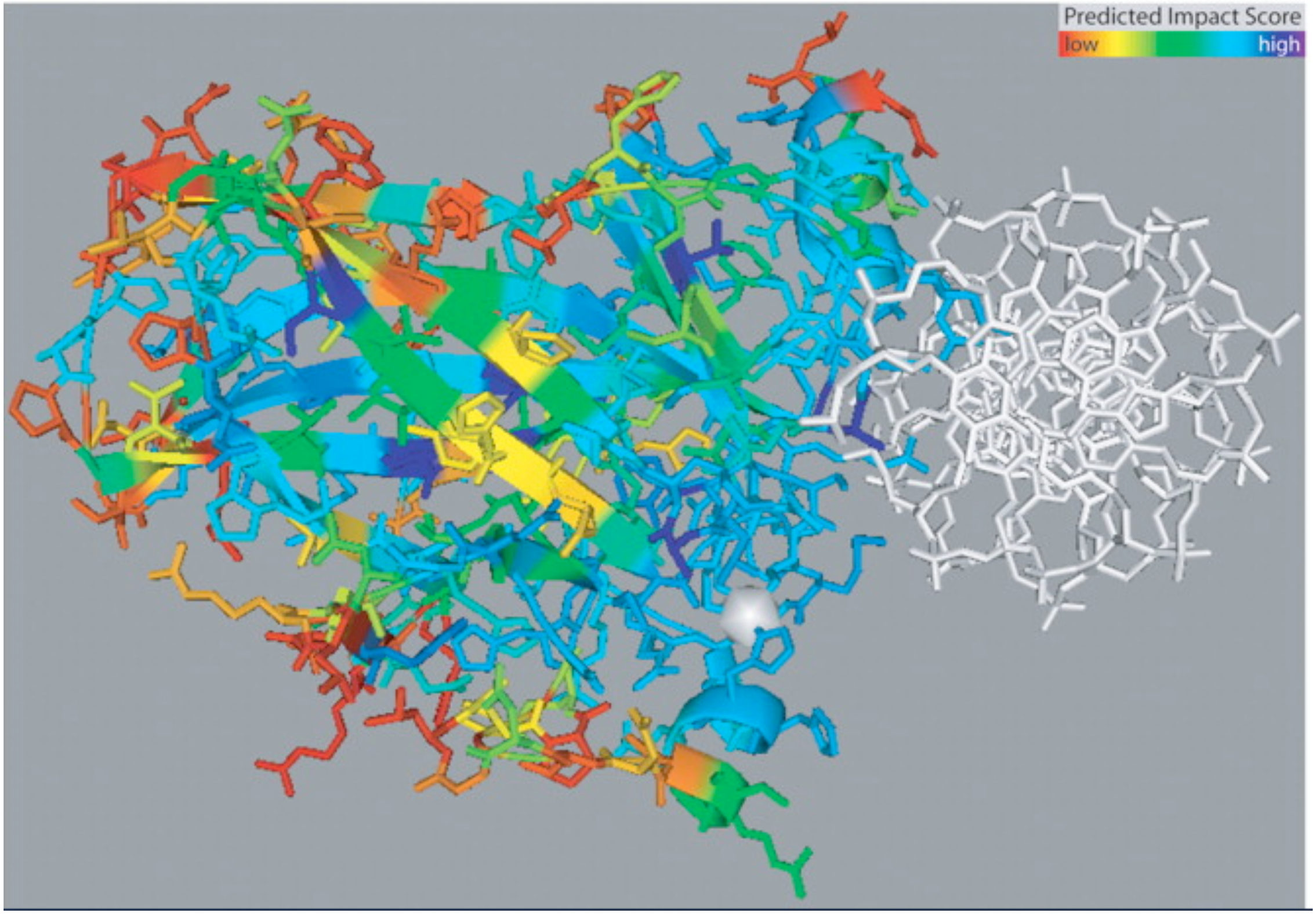
- Sequence similarity measures crucial to all methods of variant annotation and to both coding and non-coding variants

Constraint and Structure



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Stone and Sidow 2005 *Genome Research*

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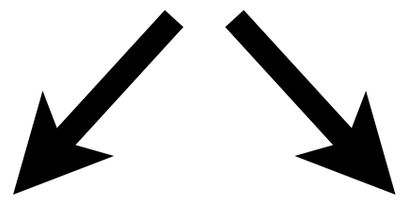

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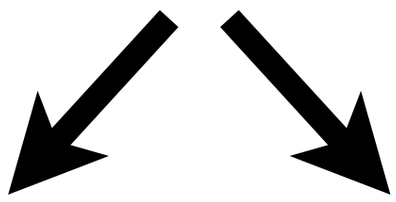
Non-Synonymous Variants



Function Evolution

Annotations for Non-Synonymous Variants

Focus on non-synonymous variants because:

- Enriched for functional effects
- Enriched for disease effects, especially severe and Mendelian
- Stop codons and splice-site disruptions in special category
- However, most observed non-synonymous variants likely have little or no effect on phenotype

Non-Synonymous SNP Annotation

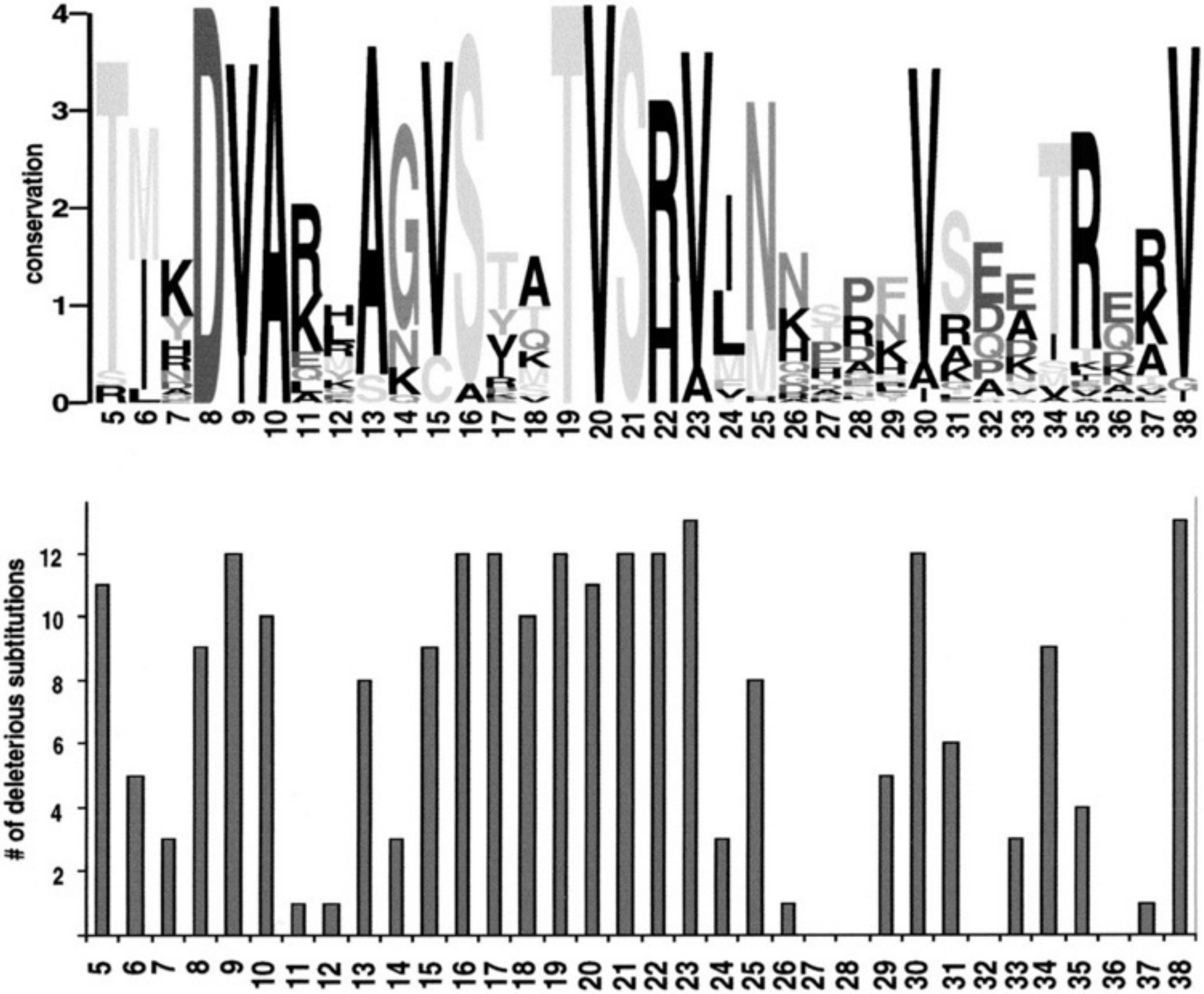
Table 1 | **Tools for protein-sequence-based prediction of deleteriousness**

Name	Type	Information	URL	Refs
MAPP	Constraint-based predictor	Evolutionary and biochemical	http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html	27
SIFT	Constraint-based predictor	Evolutionary and biochemical (indirect)	http://sift.bii.a-star.edu.sg/	39
PANTHER	Constraint-based predictor	Evolutionary and biochemical (indirect)	http://www.pantherdb.org/	41
MutationTaster*	Trained classifier	Evolutionary, biochemical and structural	http://www.mutationtaster.org/	40
nsSNP Analyzer	Trained classifier	Evolutionary, biochemical and structural	http://snpanalyzer.uthsc.edu/	44
PMUT	Trained classifier	Evolutionary, biochemical and structural	http://mmb2.pcb.ub.es:8080/PMut/	38
polyPhen	Trained classifier	Evolutionary, biochemical and structural	http://genetics.bwh.harvard.edu/pph2/	35
SAPRED	Trained classifier	Evolutionary, biochemical and structural	http://sapred.cbi.pku.edu.cn/	42
SNAP	Trained classifier	Evolutionary, biochemical and structural	http://www.rostlab.org/services/SNAP/	36
SNPs3D	Trained classifier	Evolutionary, biochemical and structural	http://www.snps3d.org/	51
PhD-SNP	Trained classifier	Evolutionary and biochemical (indirect)	http://gpcr2.biocomp.unibo.it/~emidio/PhD-SNP/PhD-SNP_Help.html	37

*Also makes predictions for synonymous and non-coding variant effects: for example, splicing. MAPP, Multivariate Analysis of Protein Polymorphism; polyPhen, polymorphism phenotyping.

Cooper and Shendure 2011 *NRG*

SIFT -- Sequence Conservation



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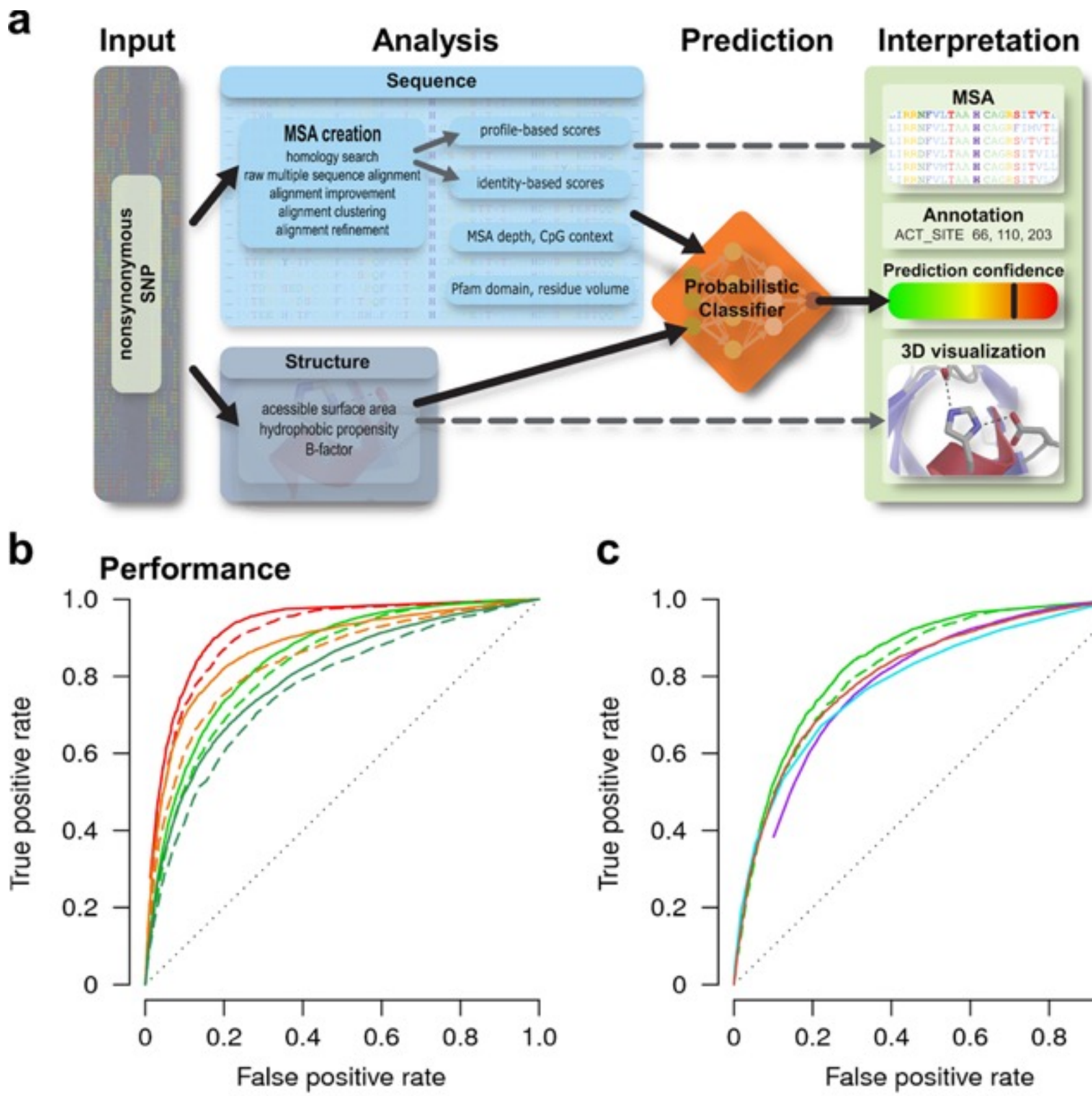
Ng and Henikoff 2001 *Genome Research*

Polyphen -- Trained Classifier on Many Features



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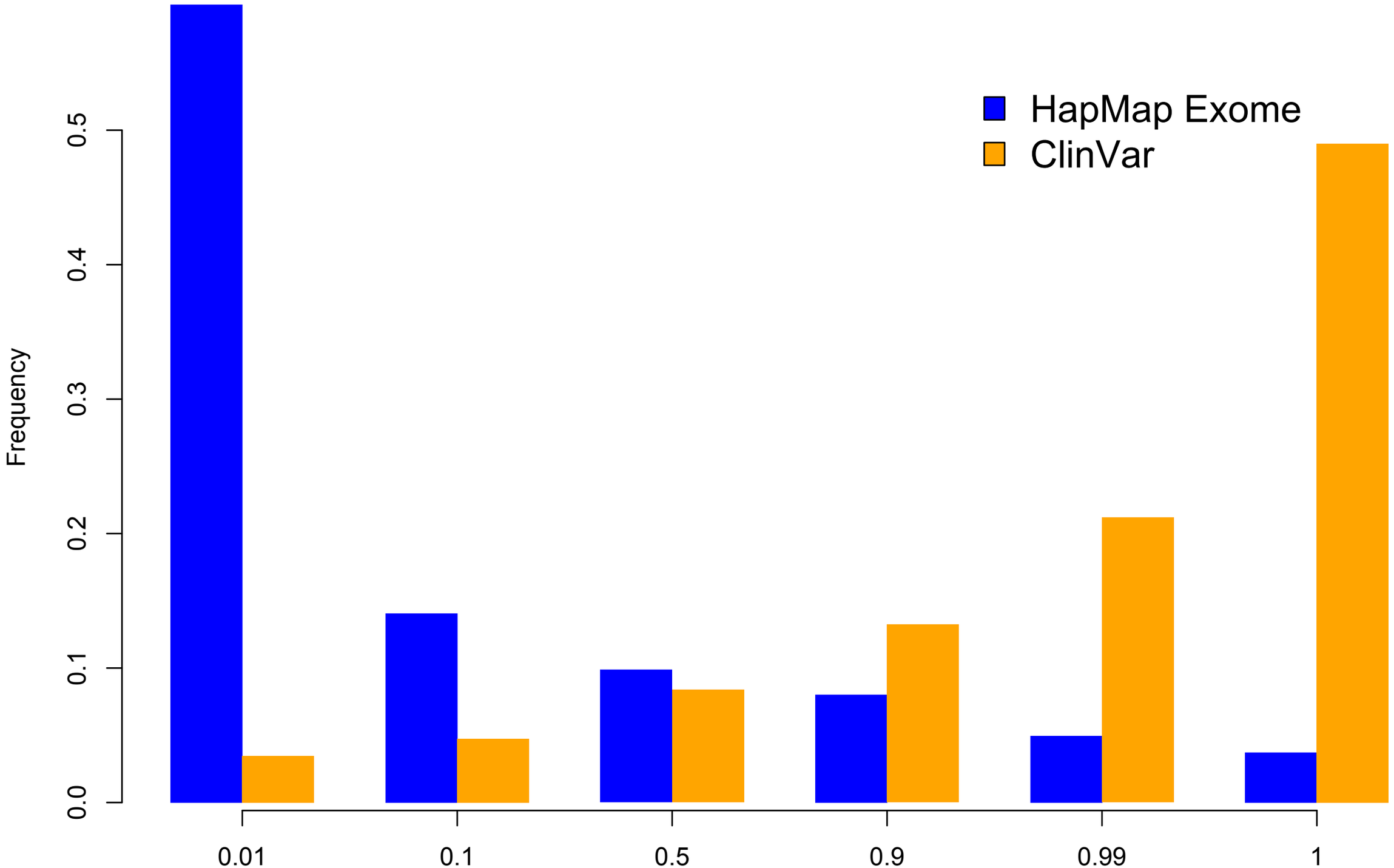
Adzhubei *et al* 2010 *Nature Methods*

PolyPhen Enrichment for Pathogenic Mutations



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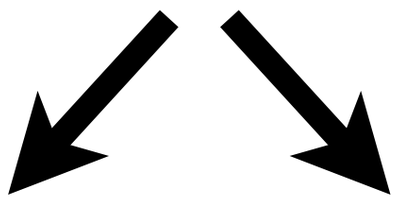

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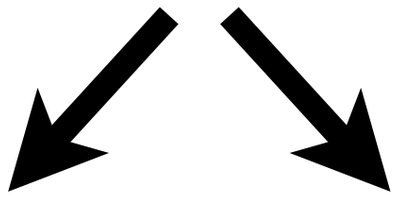

Function Evolution

What about the other 99%?

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Non-Coding Variant Annotation

Two main sources of data for annotating non-coding variants:

- Comparative genomics
  - sites under strong selection more likely to result in a deleterious effect when mutated
  - typically based on mammalian genomic comparisons, in contrast with protein-level annotations
- Functional genomics:
  - transcription factor binding, open chromatin, etc
  - chromatin modifications
  - proximity to known functional features like promoters, exons

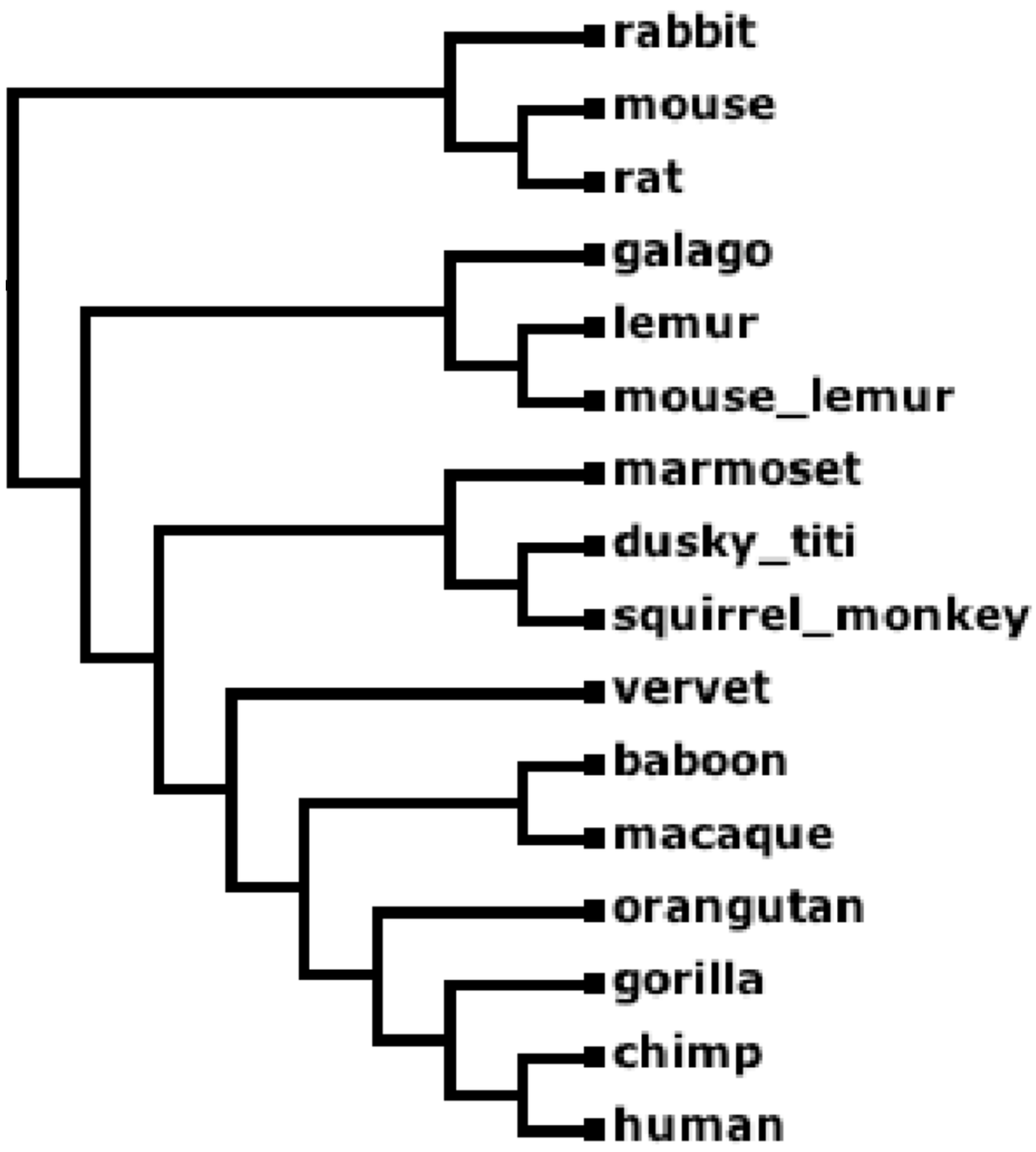
Nucleotide-Level Annotations From Comparative Genomics

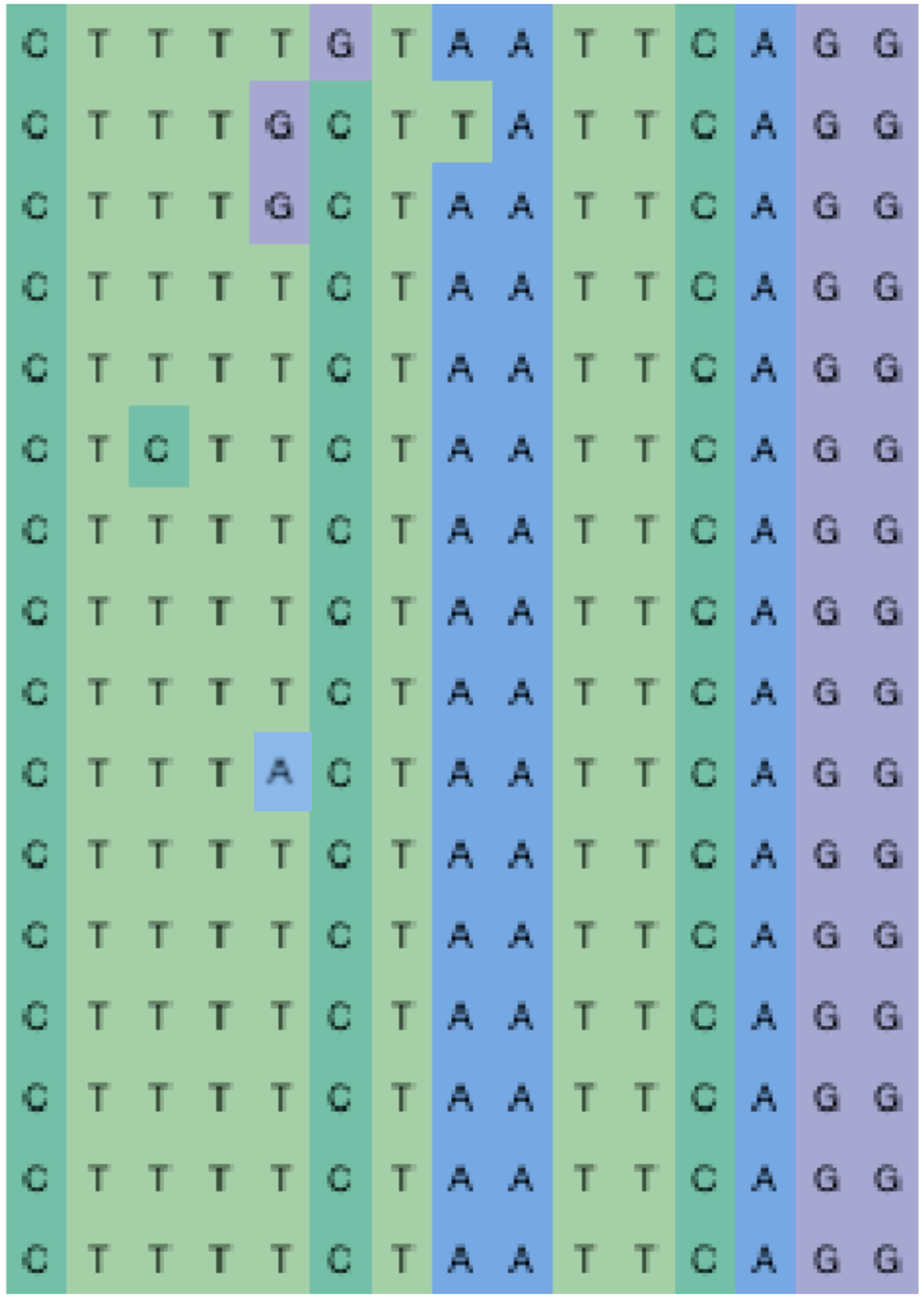
Table 2 | **Tools for nucleotide-sequence-based prediction of deleteriousness**

Name	Type	Information	URL	Refs
phastCons	Phylogenetic HMM	Evolutionary	http://compgen.bscb.cornell.edu/phast/	60
GERP	Single-site scoring	Evolutionary	http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html	67
Gumby	Single-site scoring	Evolutionary	http://pga.jgi-psf.org/gumby/	21
phyloP	Single-site scoring	Evolutionary	http://compgen.bscb.cornell.edu/phast/	66
SCONE	Single-site scoring	Evolutionary	http://genetics.bwh.harvard.edu/scone/	68
binCons	Sliding-window scoring	Evolutionary	http://zoo.nhgri.nih.gov/binCons/index.cgi	69
Chai Cons	Sliding-window scoring	Evolutionary and structural	http://research.nhgri.nih.gov/software/chai	71
VISTA	Visualization tool (various scores)	Evolutionary	http://genome.lbl.gov/vista/index.shtml	70

GERP, Genomic Evolutionary Rate Profiling; HMM, hidden Markov model; SCONE, Sequence Conservation Evaluation.

Genome Sequence Comparisons





Cooper *et al* 2005 *Genome Research*

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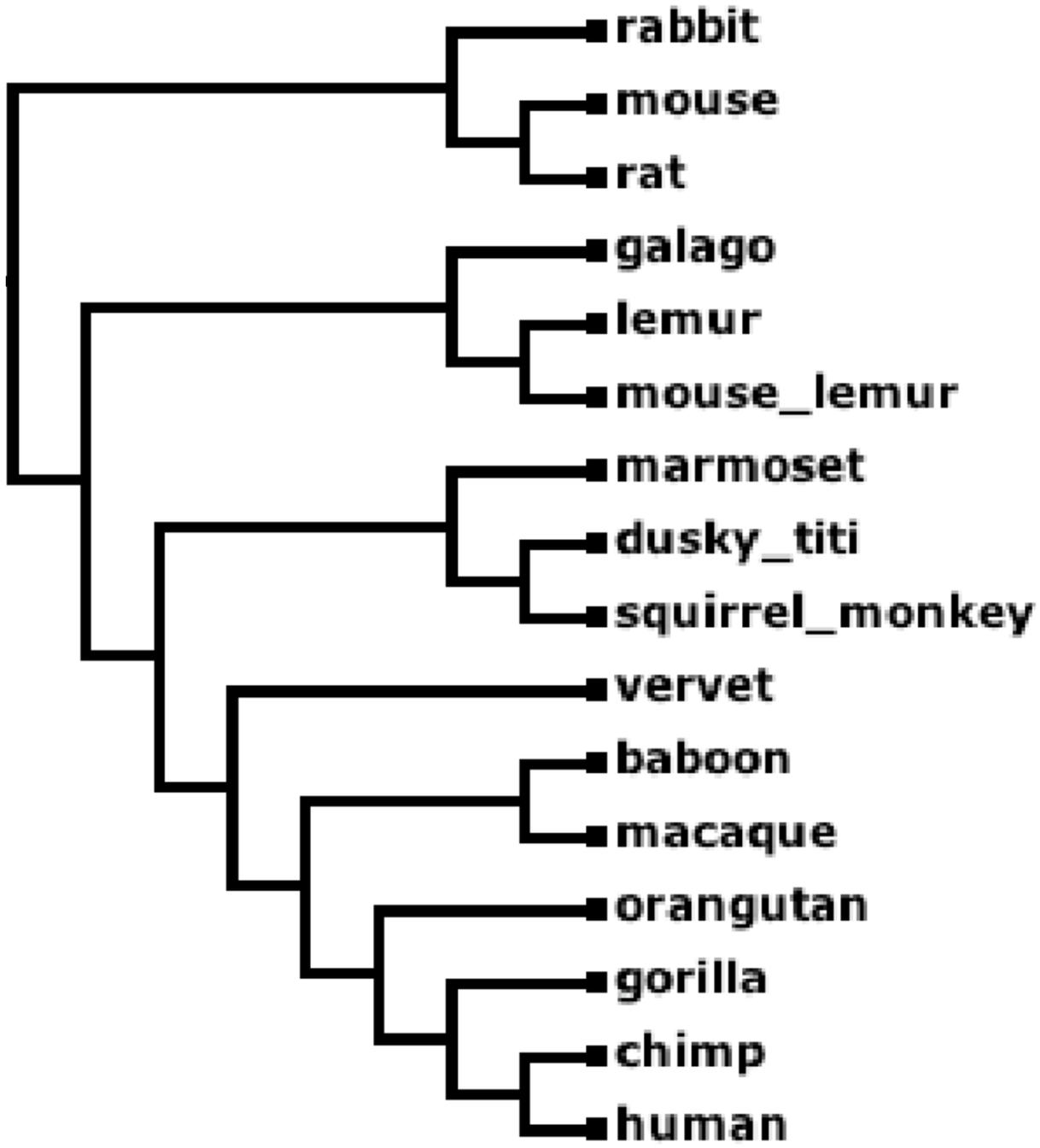

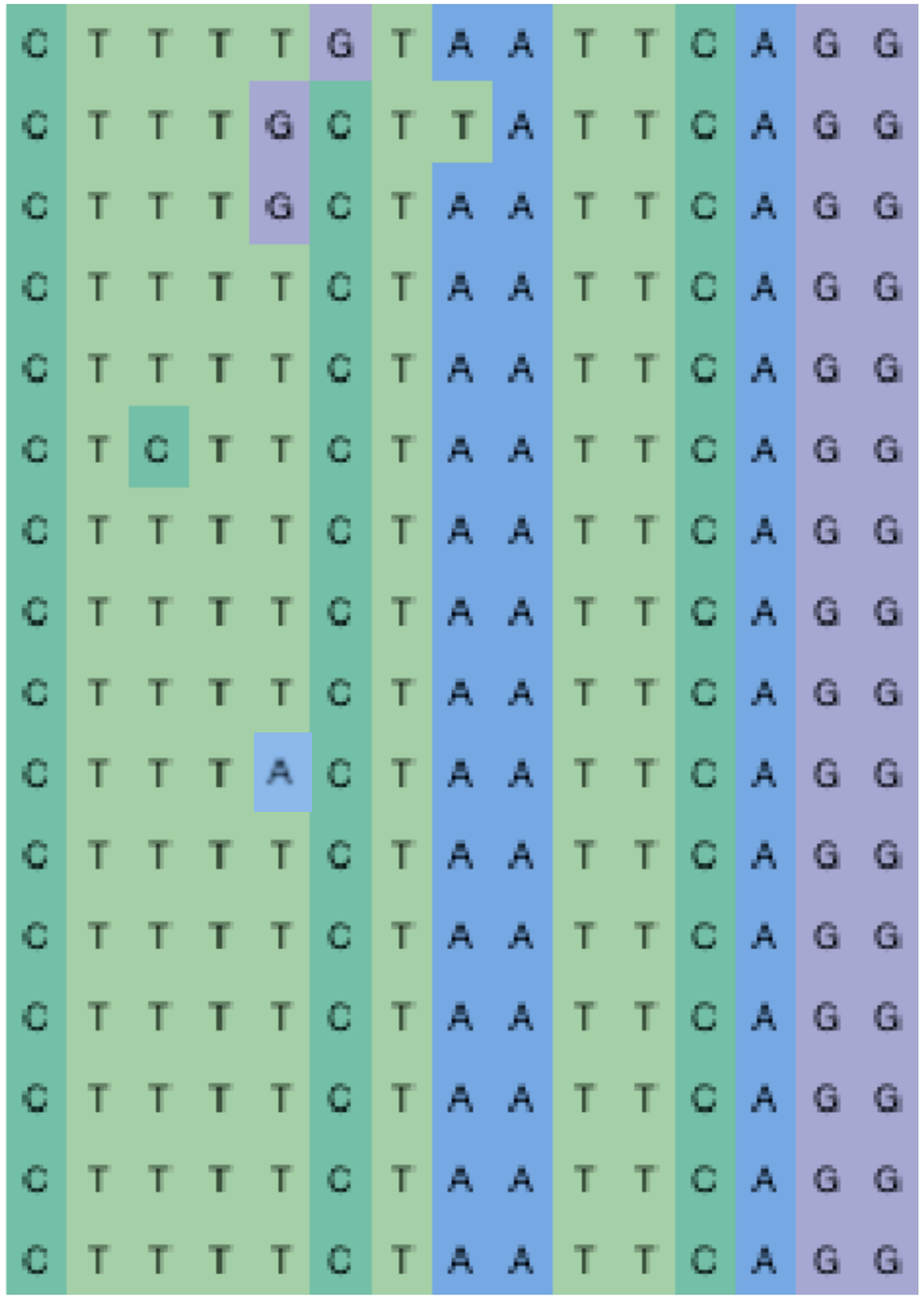

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Cooper *et al* 2005 *Genome Research*

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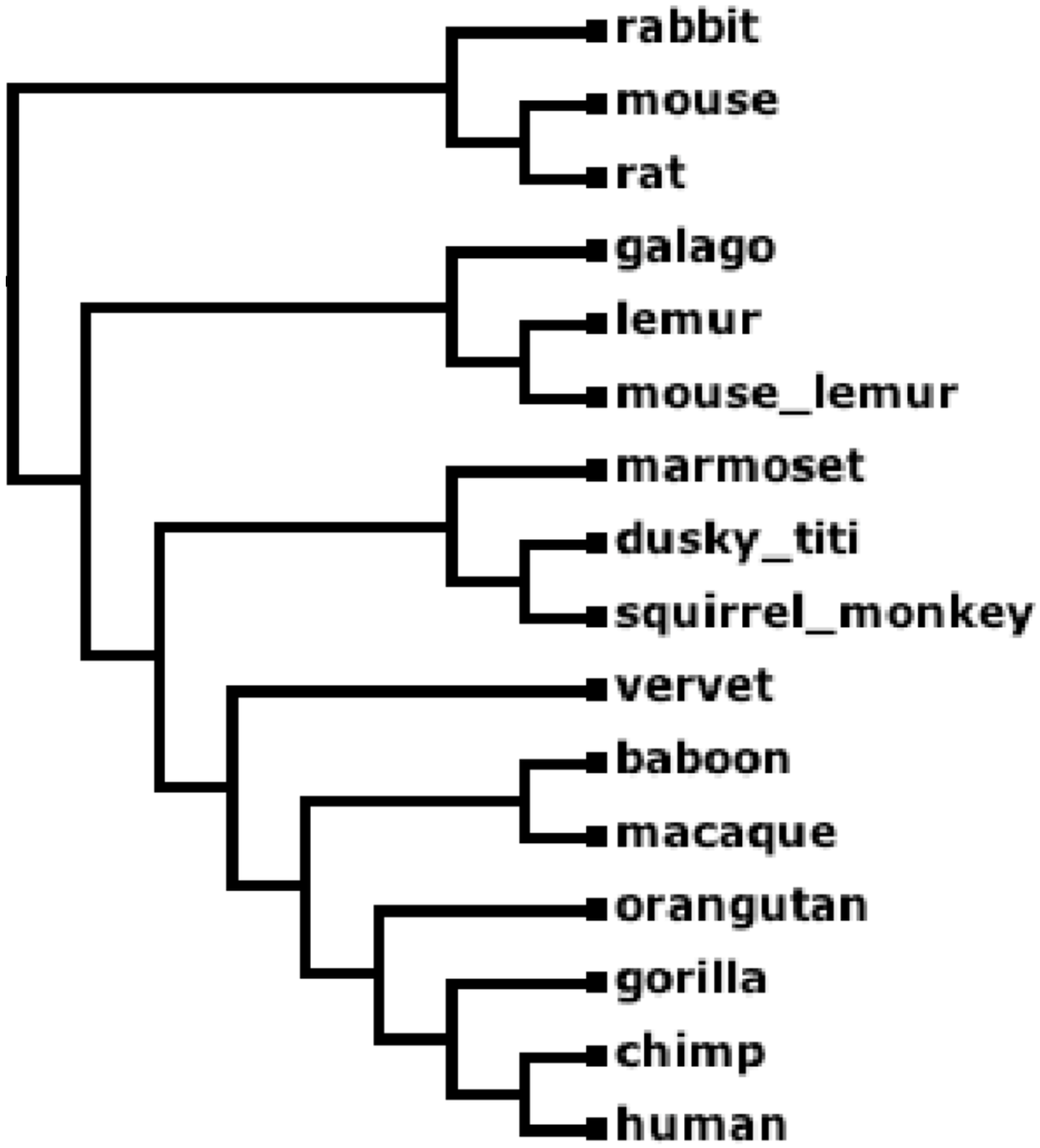

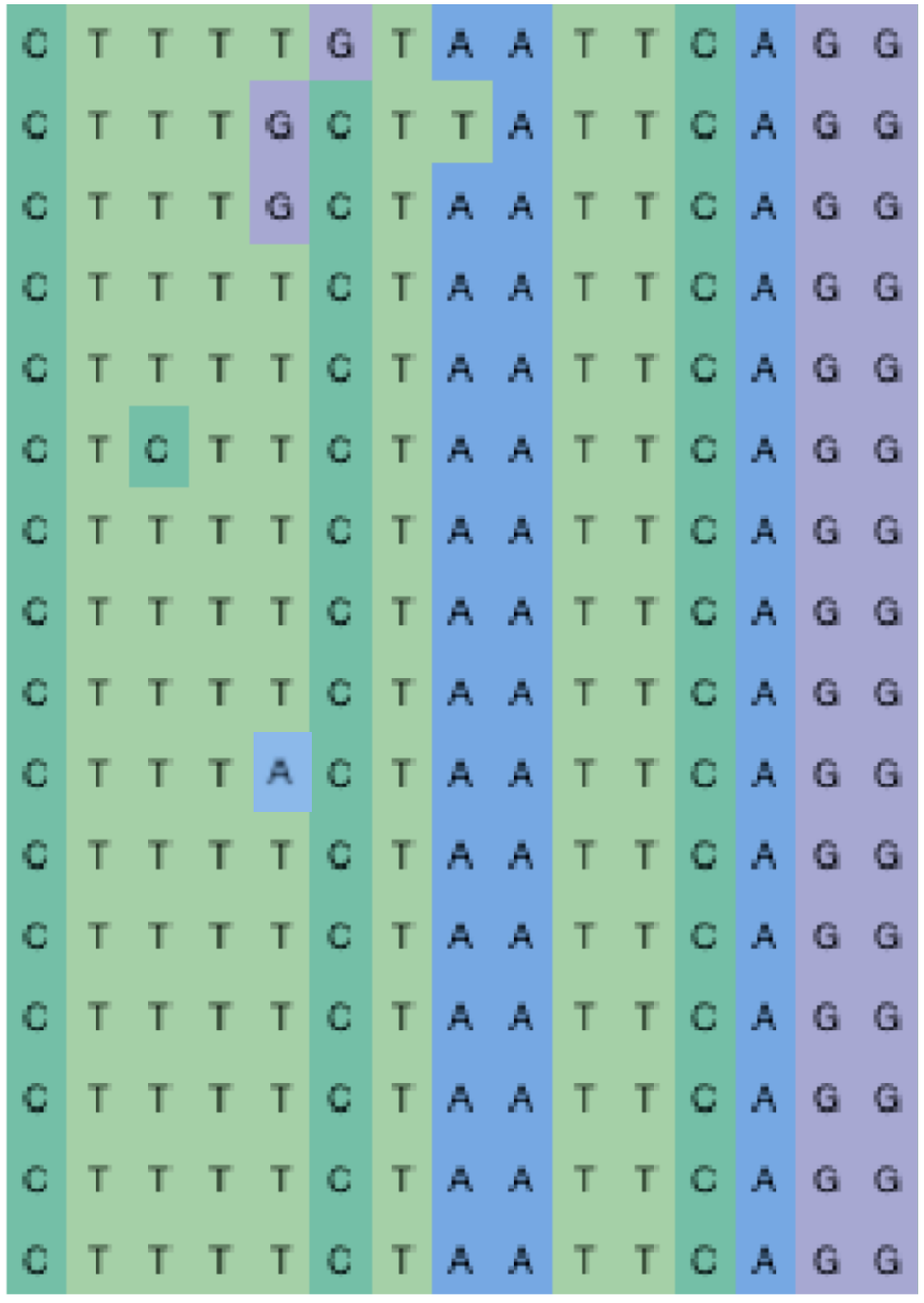

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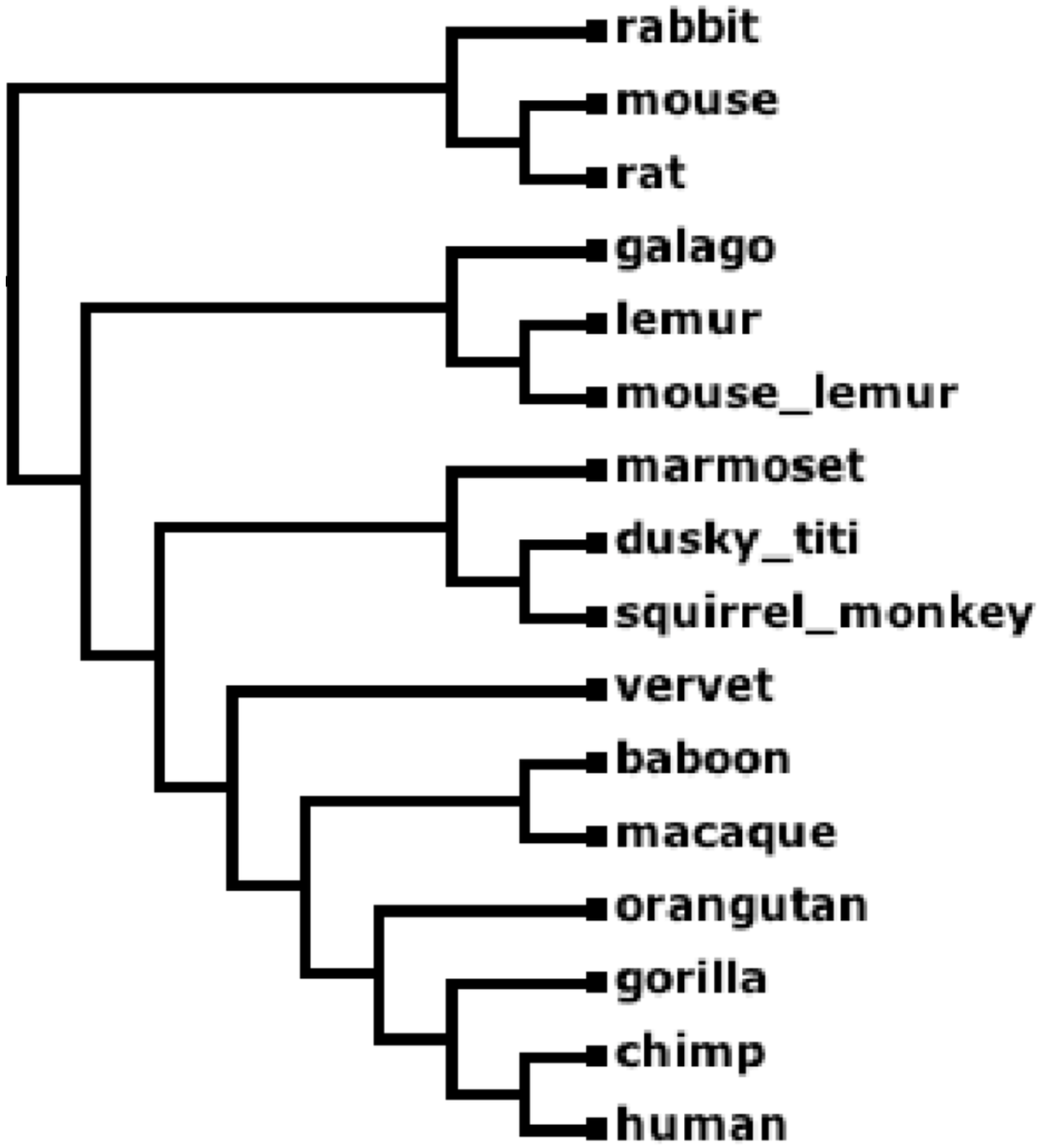

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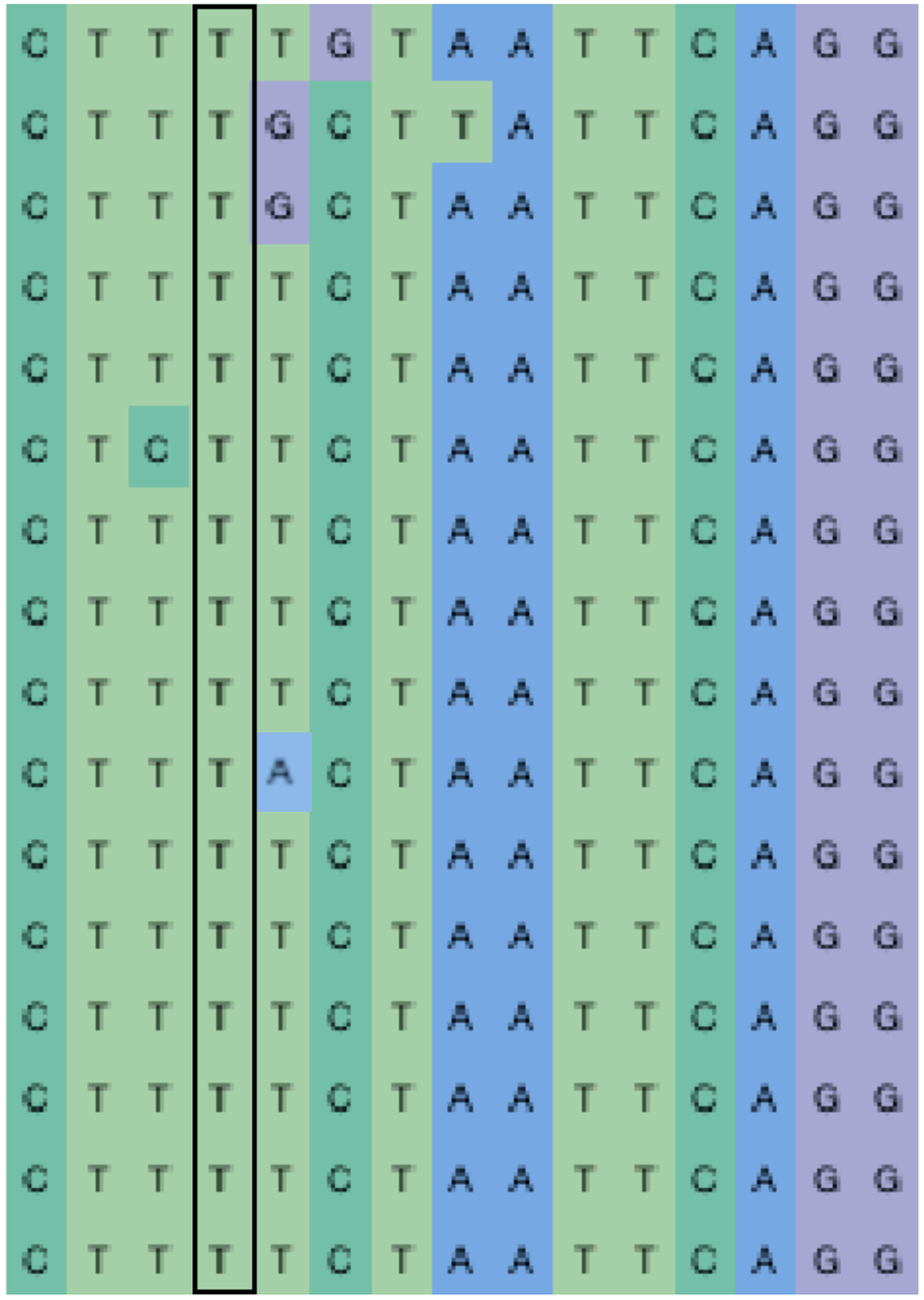

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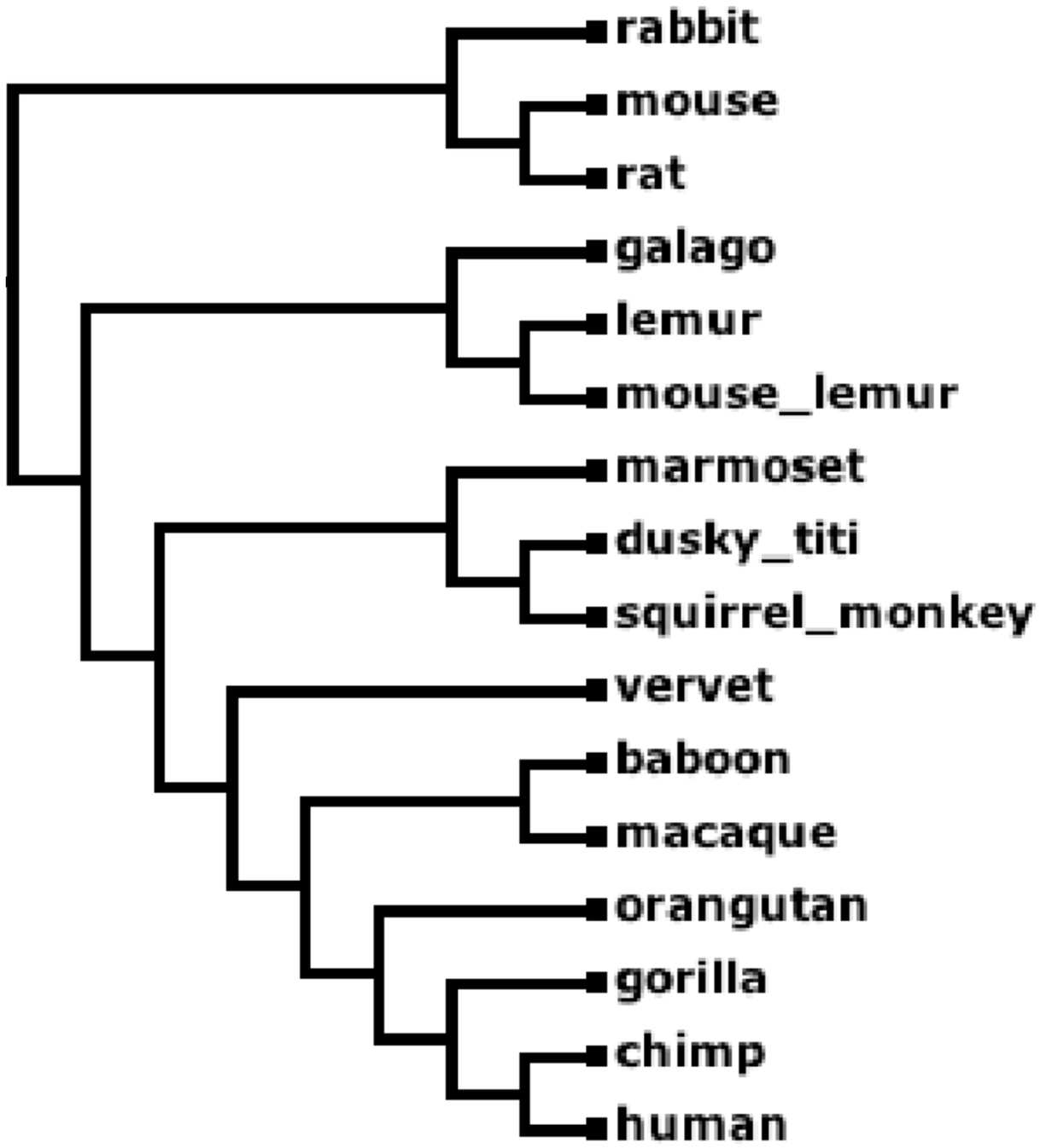

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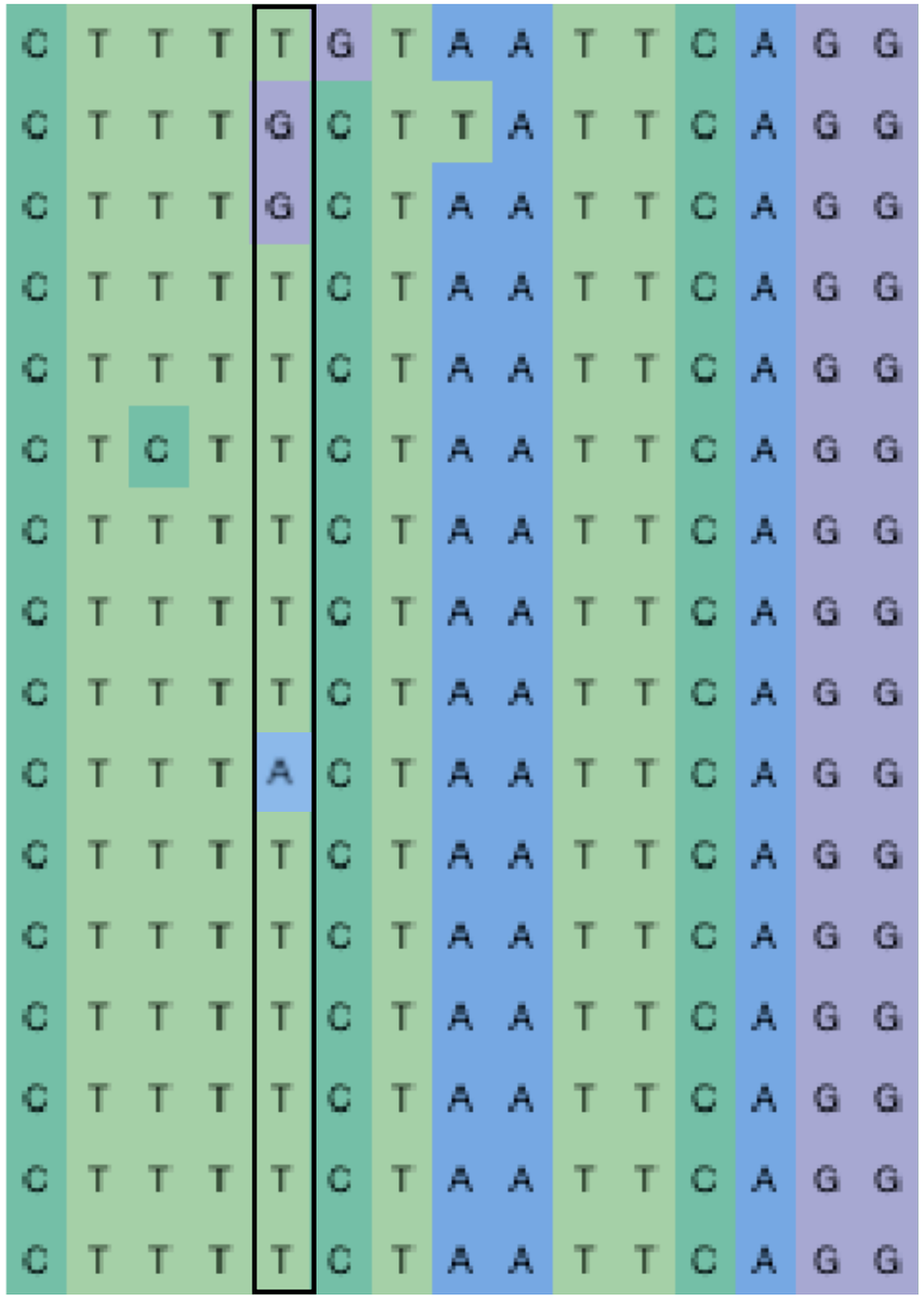
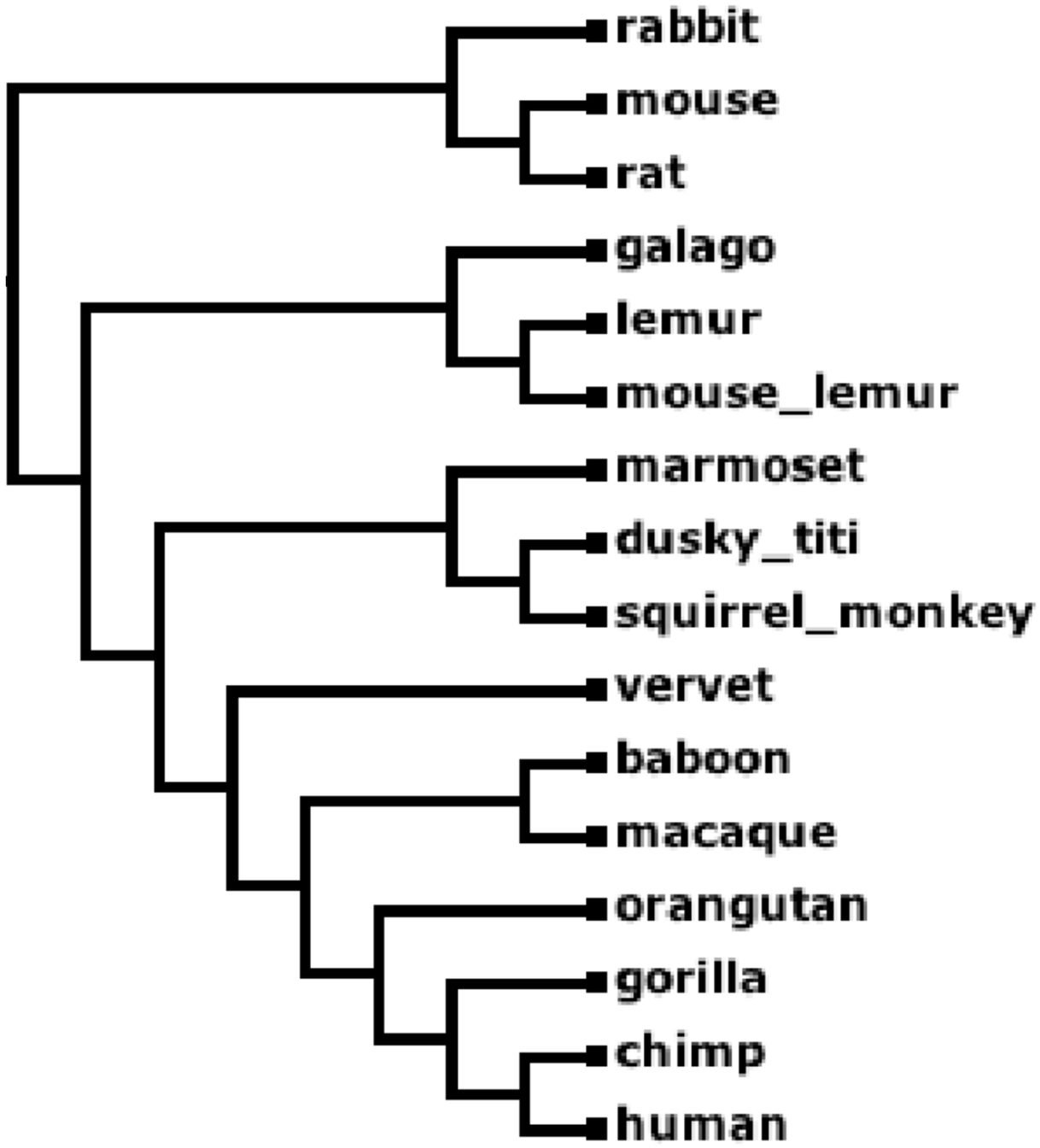


'Rej Subs':

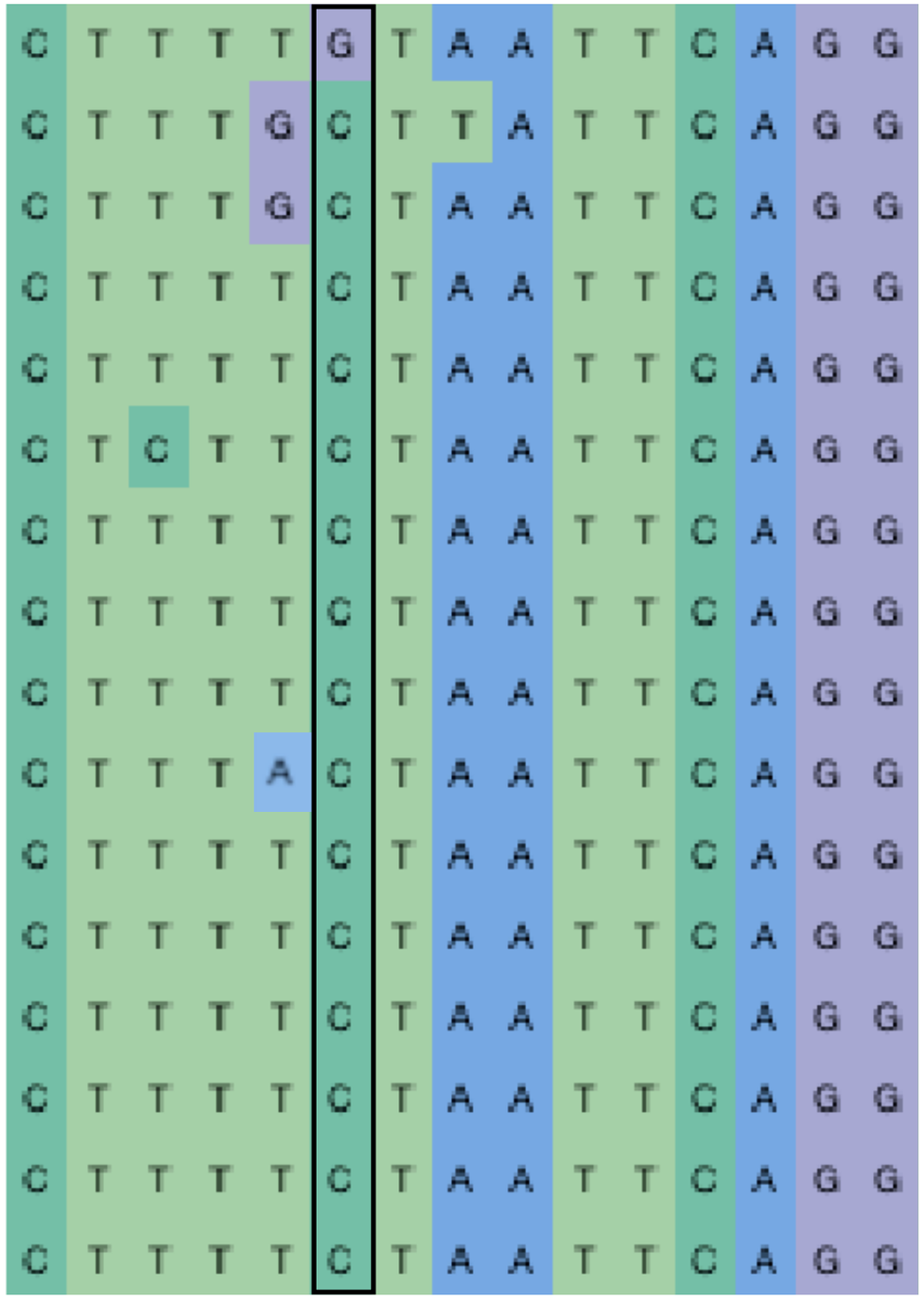
+3

Cooper *et al* 2005 *Genome Research*

Genome Sequence Comparisons



Neutral Average
~5 subs/site



'Rej Subs':

+4

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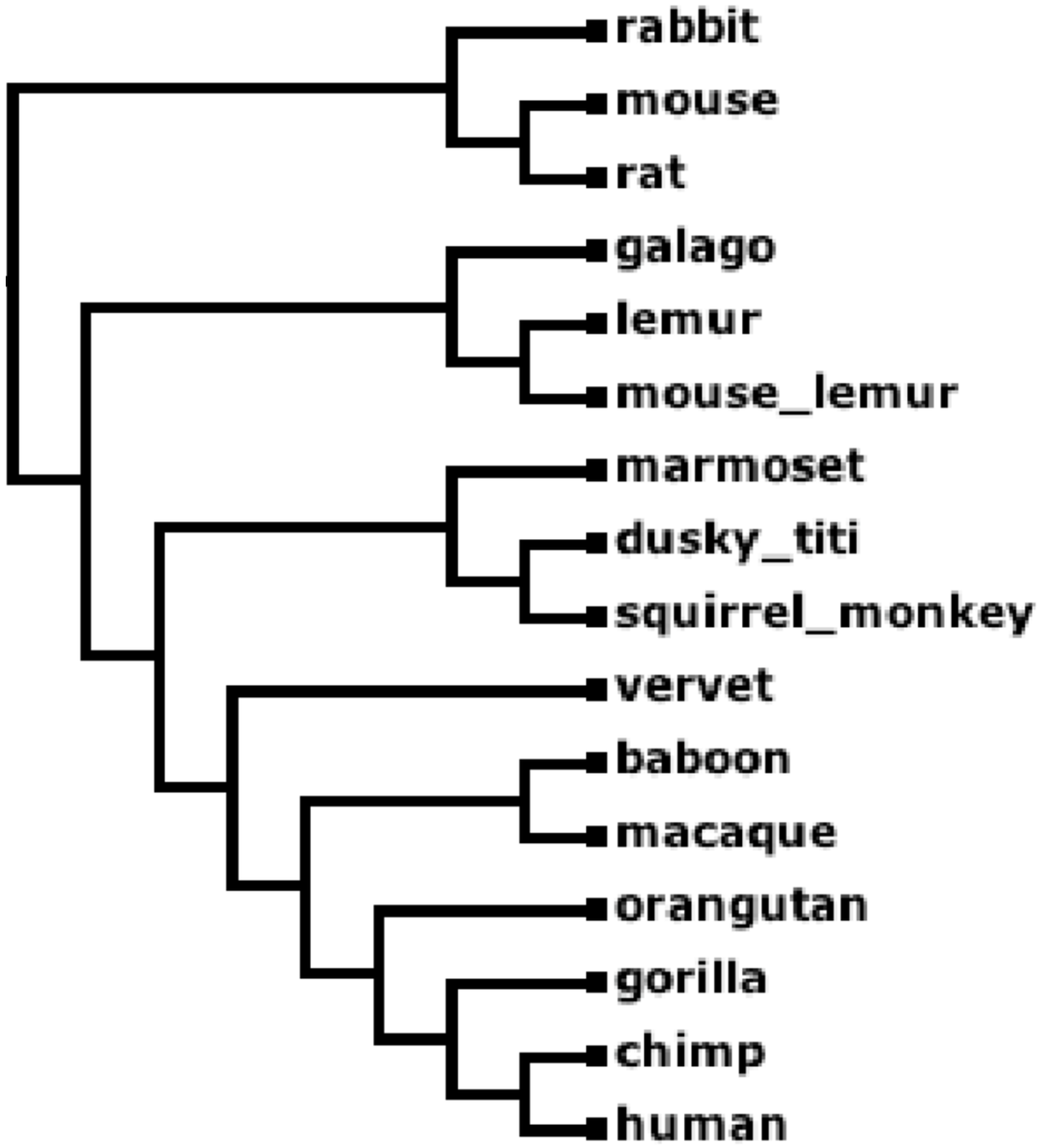

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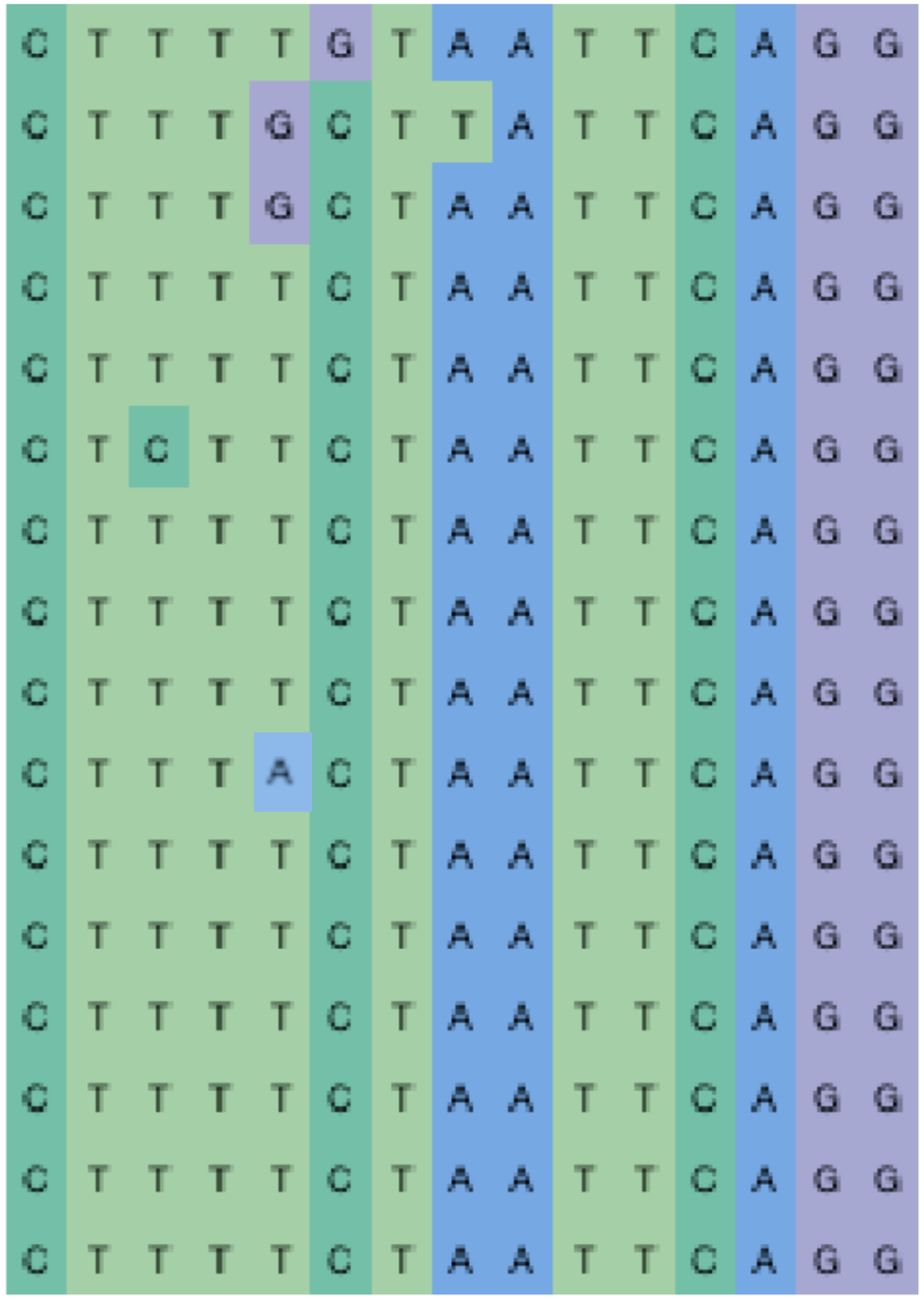
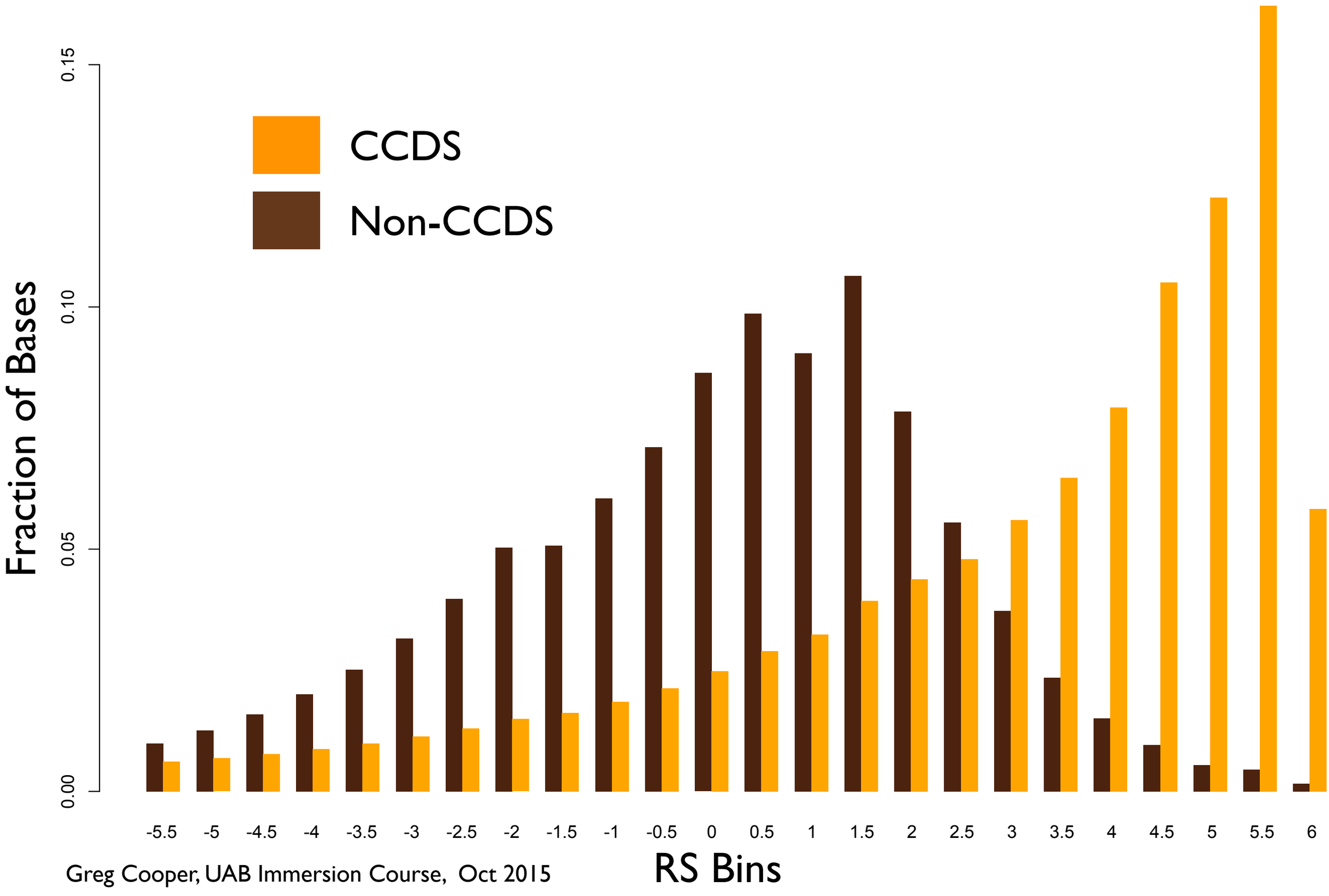


...

Cooper *et al* 2005 *Genome Research*

Exome vs Genome



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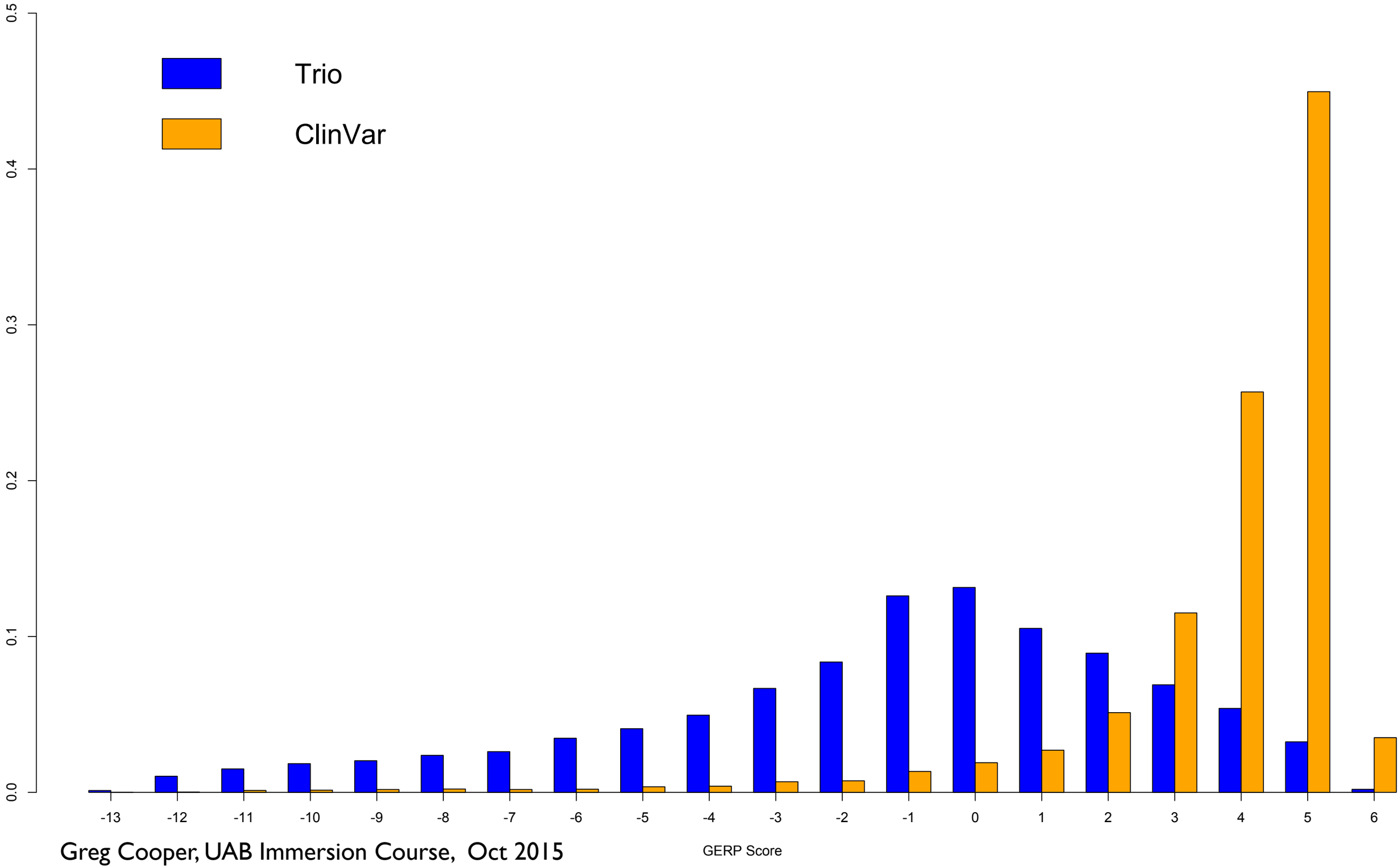
Sequence Conservation of Known Pathogenic Mutations



Nucleotide-Level Functional Annotations

- Functional analysis of non-coding elements has historically received lesser focus than proteins
- Rapidly improving for humans and model organisms
- Key types of available information:
  - Transcription-factor binding events via ChIP-seq
  - Regions of open chromatin via DNase HS/FAIRE/others
  - Chromatin modifications
  - Methylation status
  - Identification of promoters, enhancers, silencers, and insulators via integrative analysis of molecular annotations

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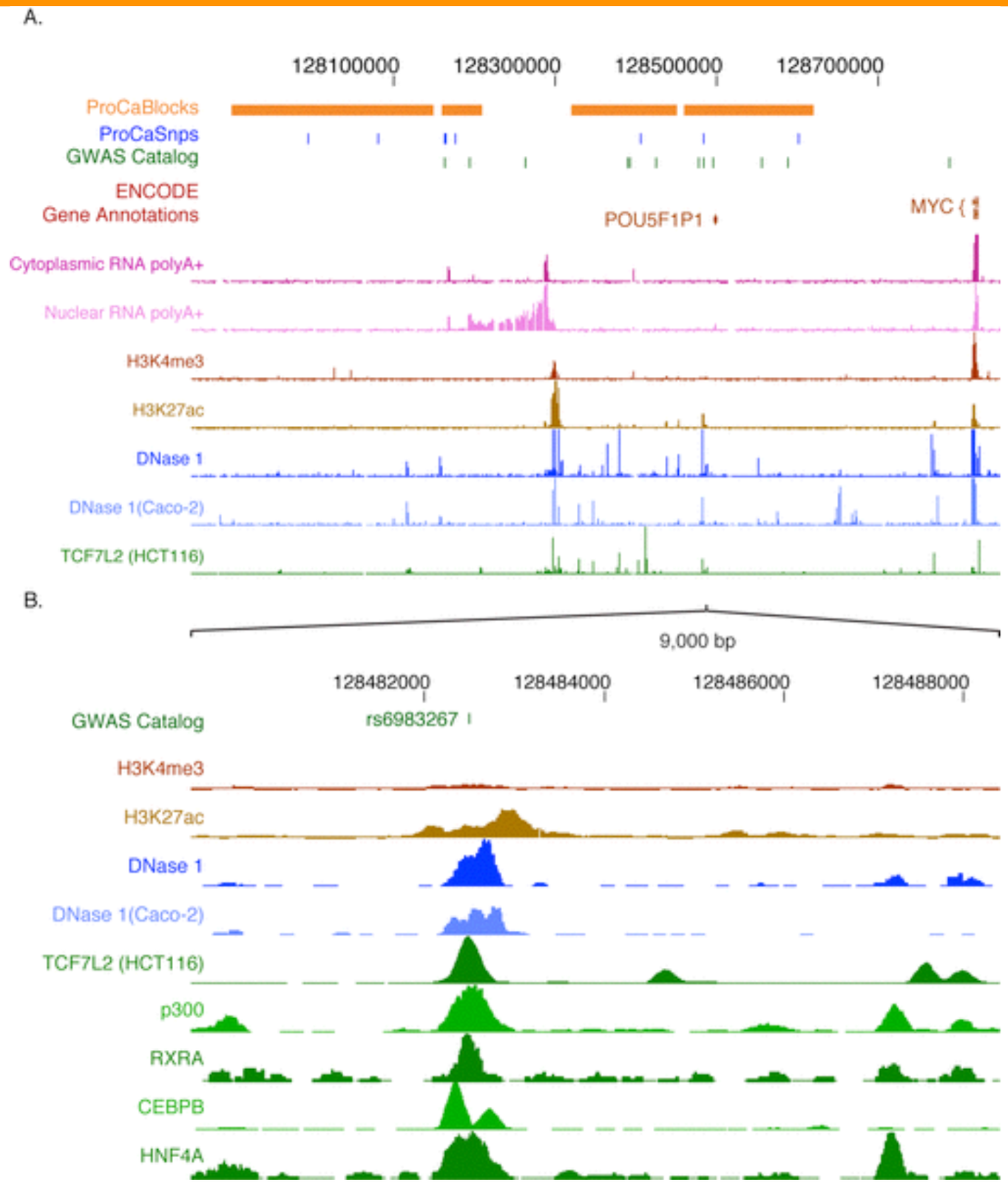

The ENCODE Project Consortium 2011. *PLoS Biology*

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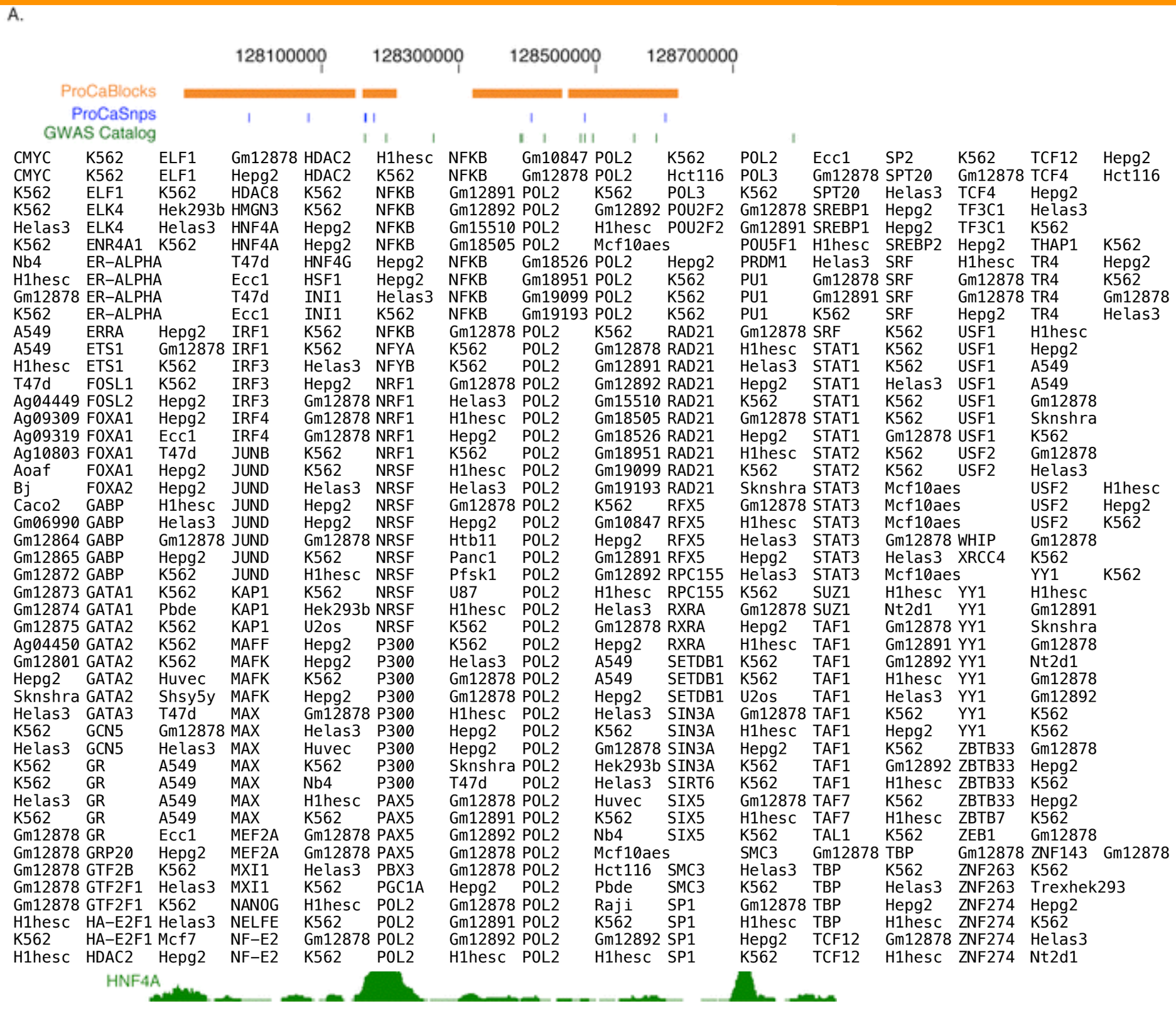

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  - What is the relative value of evolutionary vs functional information?
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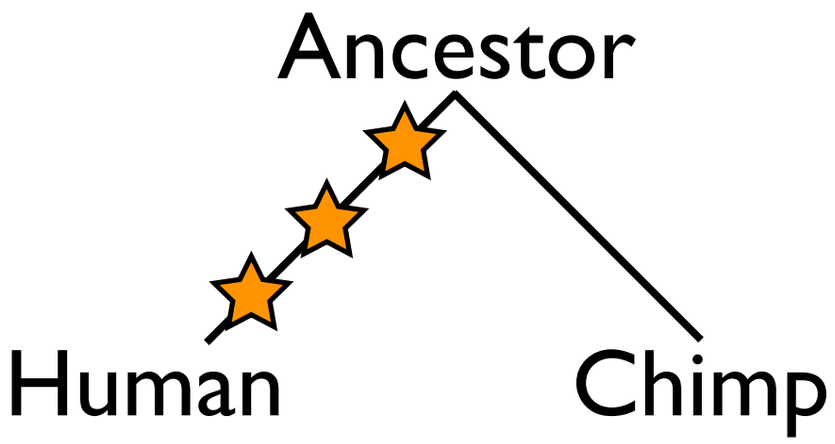
  - What is the value of any specific combination of annotations?
  - How does one cope with the thousands of partially correlated genomic annotations that are available (e.g. ENCODE)?

Annotation Dependent Depletion



Vs Simulated Mutations

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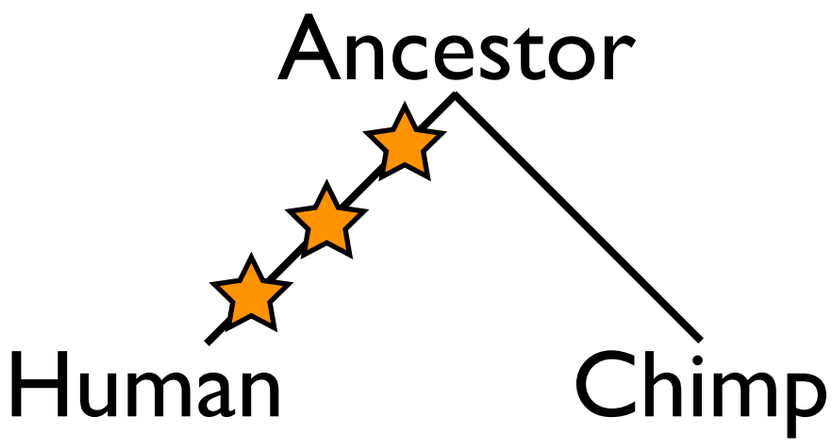

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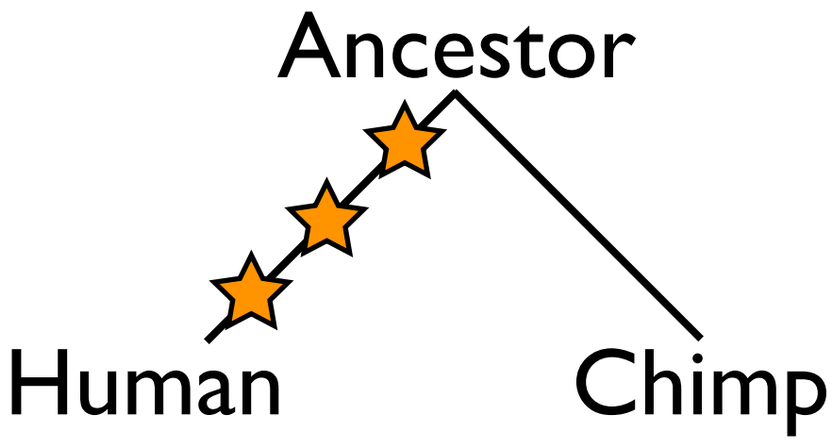

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Annotation	Number Observed	Number Simulated	Observed/ Expected
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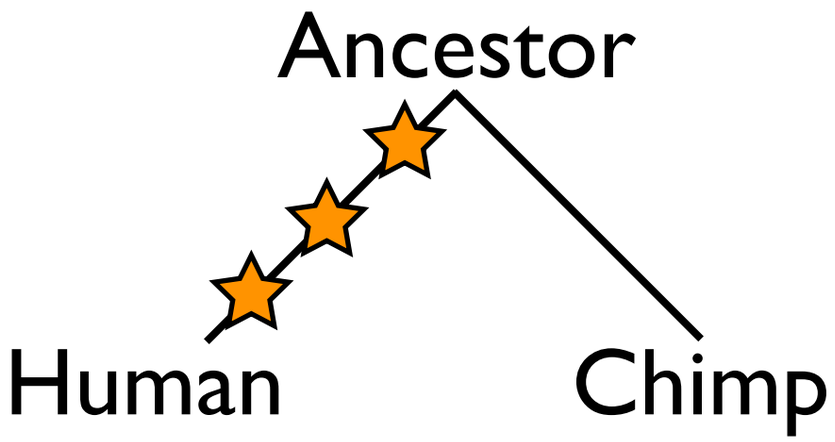

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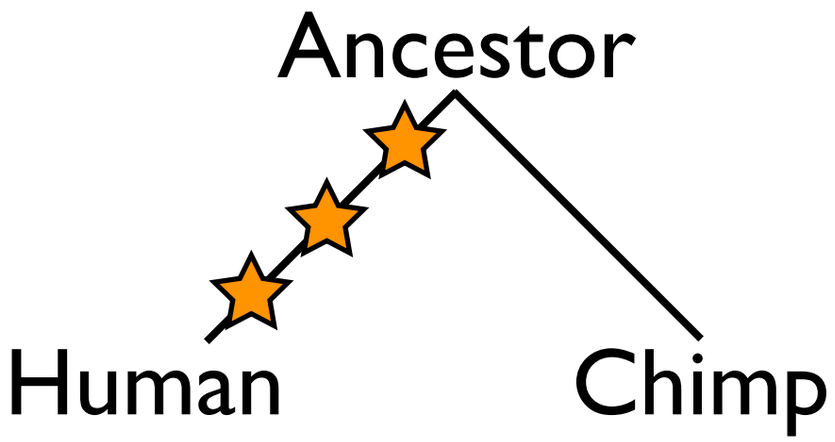

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…	…	…	“ADD"

Combined Annotation Dependent Depletion (CADD)

1. Generate 63 distinct annotations, including conservation scores, ENCODE data (summarized at various levels), gene body annotations, etc.

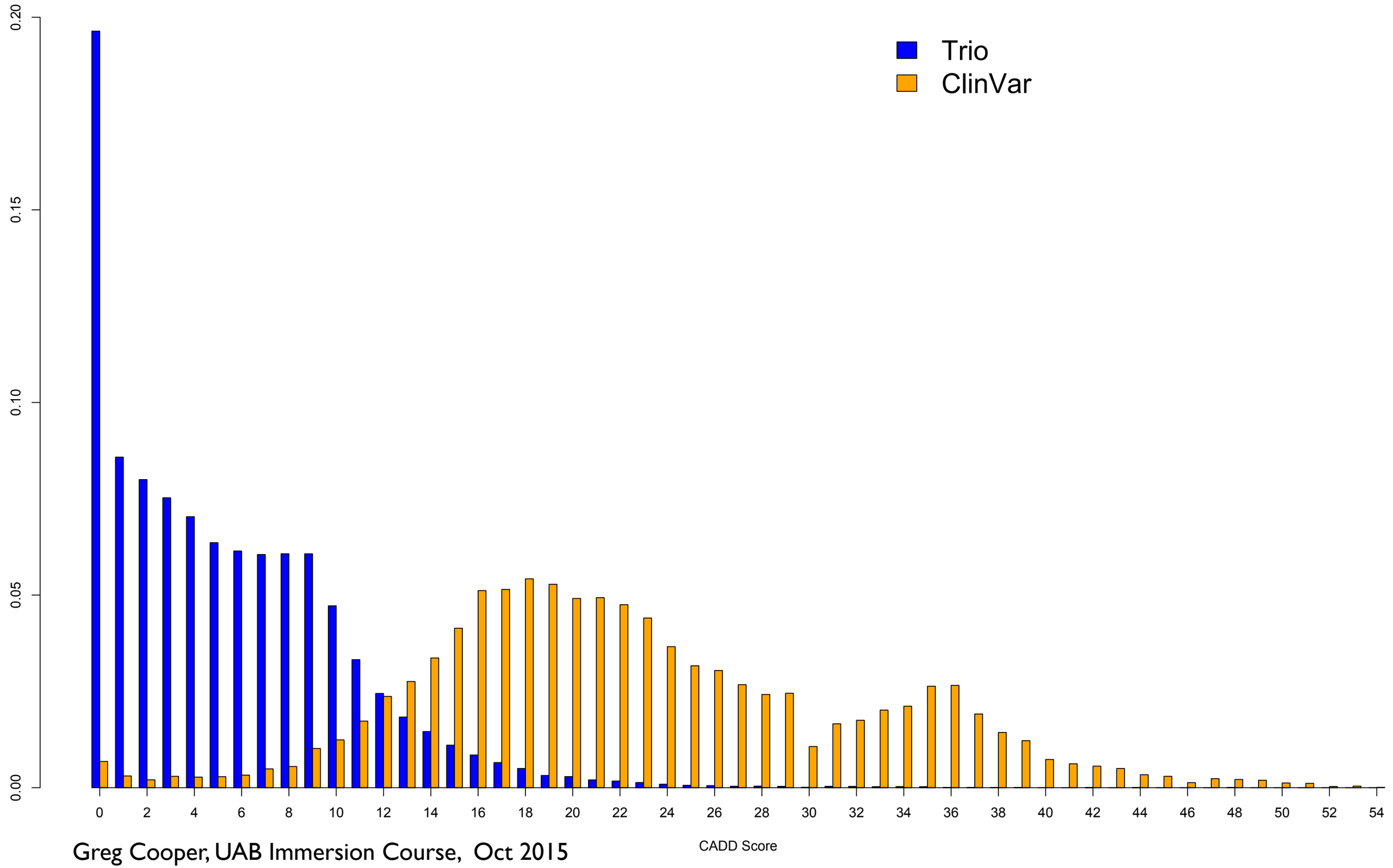
2. Build a support vector machine (SVM) that estimates, for a given variant, whether it is likely to be observed or simulated, based on its combined annotation profile

    Domain-specific information (e.g. polyPhen) via "missing" indicators

    Some interaction terms, like STOP * relCDSpos, also included

3. Score all possible ~8.6 billion possible SNVs of hg19

CADD Scores for Known Pathogenic Mutations



Greg Cooper, UAB Immersion Course, Oct 2015

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  - 8 individuals with diverse ancestry (HapMap samples)
- Then looked for genes in which:
  - 1 or more FSS-affected individuals harbored a "rare" variant, i.e. was not present in dbSNP or present in one of the 8 HapMap samples, AND…

"Brute Force" Resolution of Mendelian Disease

Number of genes in which each affected has at least one...	FSS24895	FSS24895 FSS10208	FSS24895 FSS10208 FSS10066	FSS24895 FSS10208 FSS10066 FSS22194	Any 3 of 4: FSS24895 FSS10208 FSS10066 FSS22194
Non-synonymous cSNP, splice site variant or coding indel (NS/SS/I)	4,510	3,284	2,765	2,479	3,768

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~30-50% of suspected Mendelian diseases can be traced to causal genes with just a handful of exomes!

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Variant Annotations and Disease Prediction

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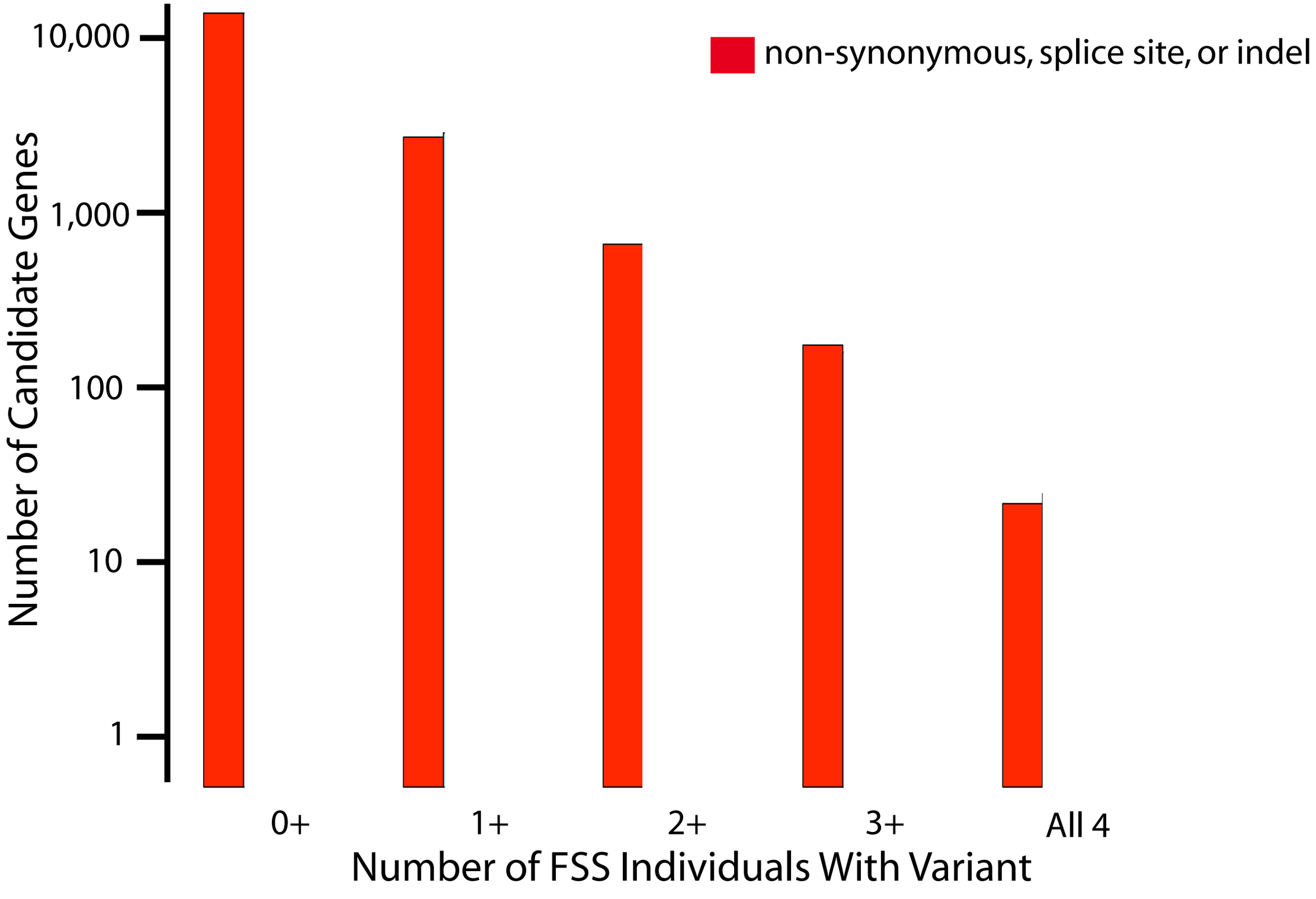

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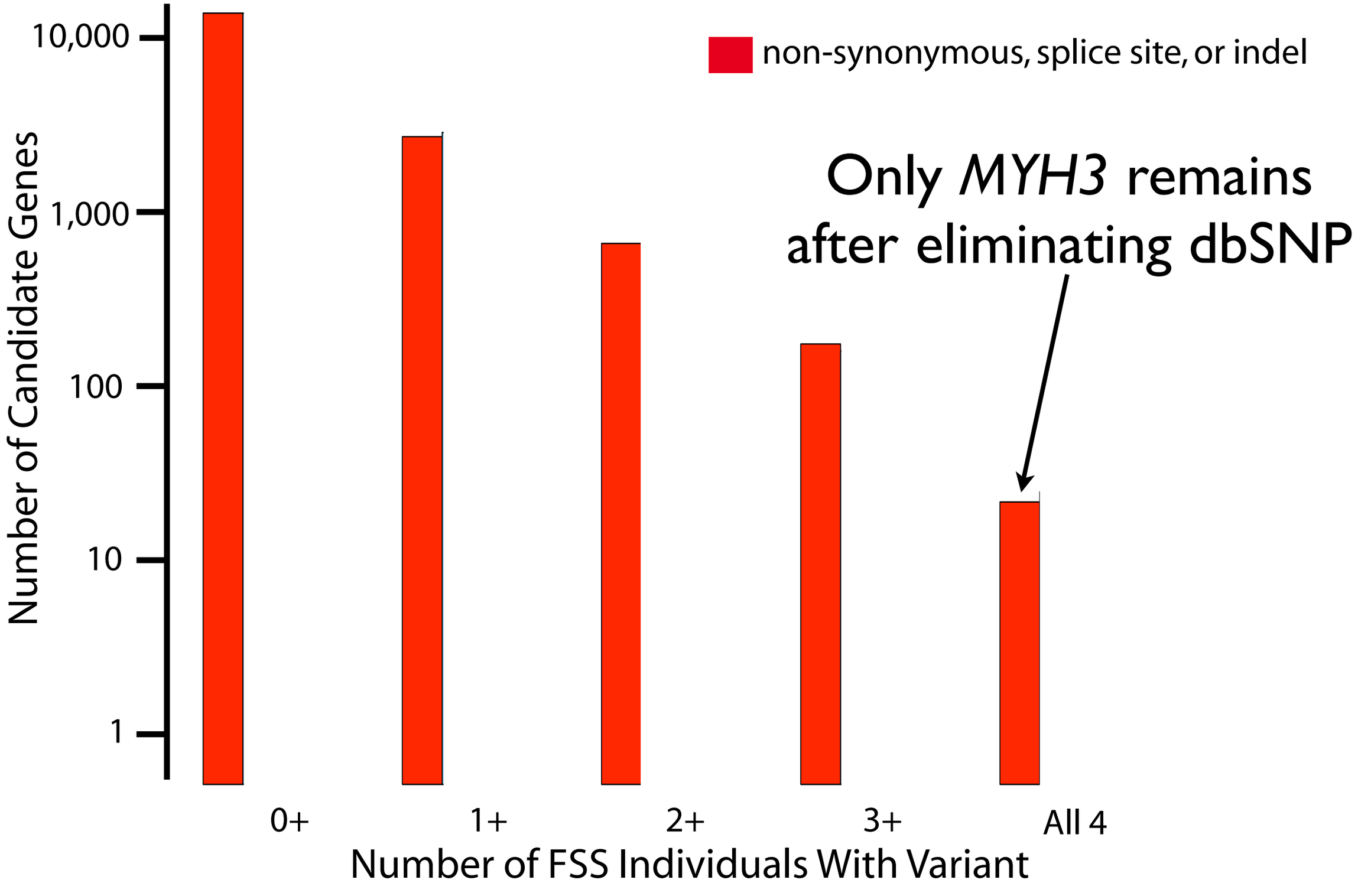

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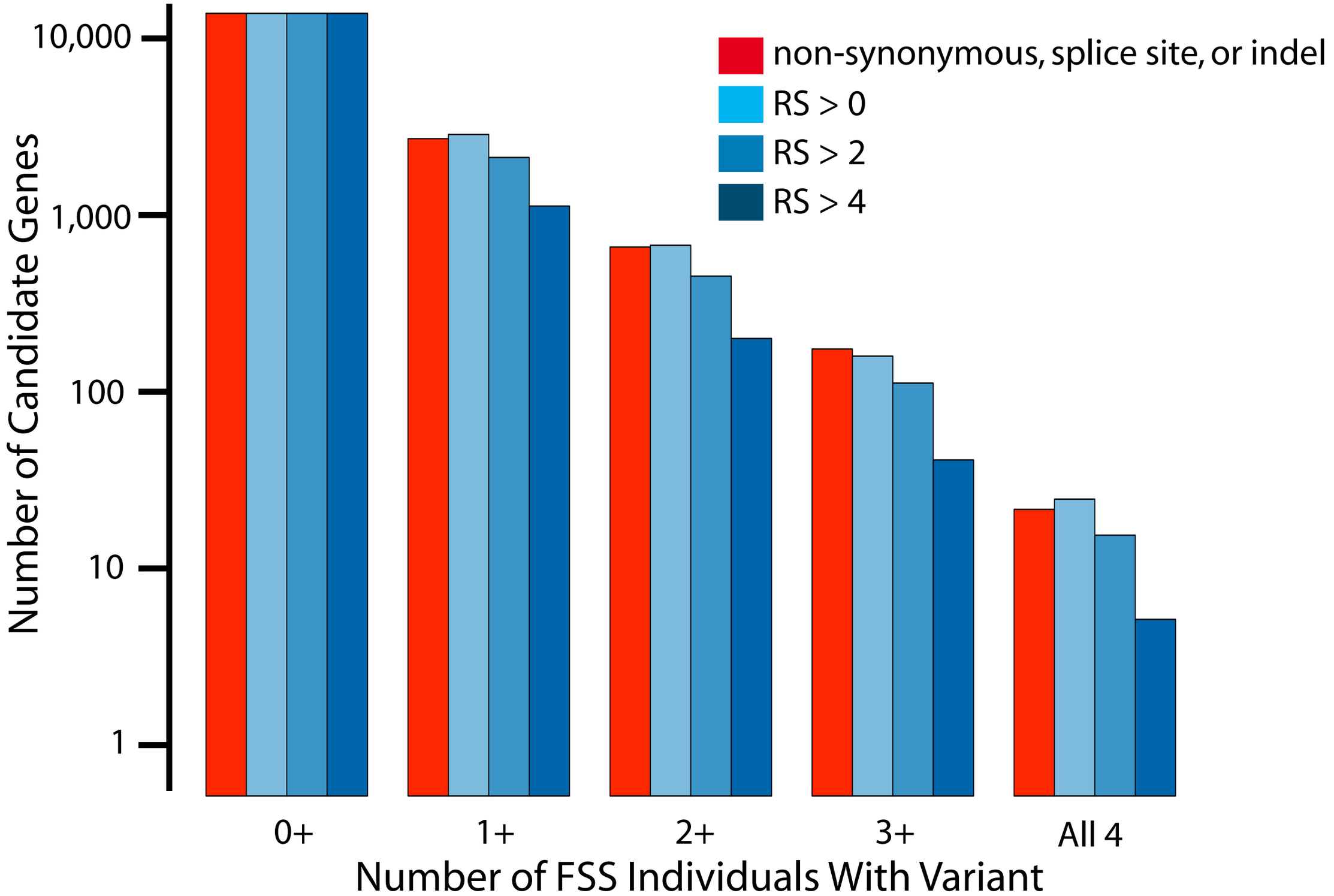

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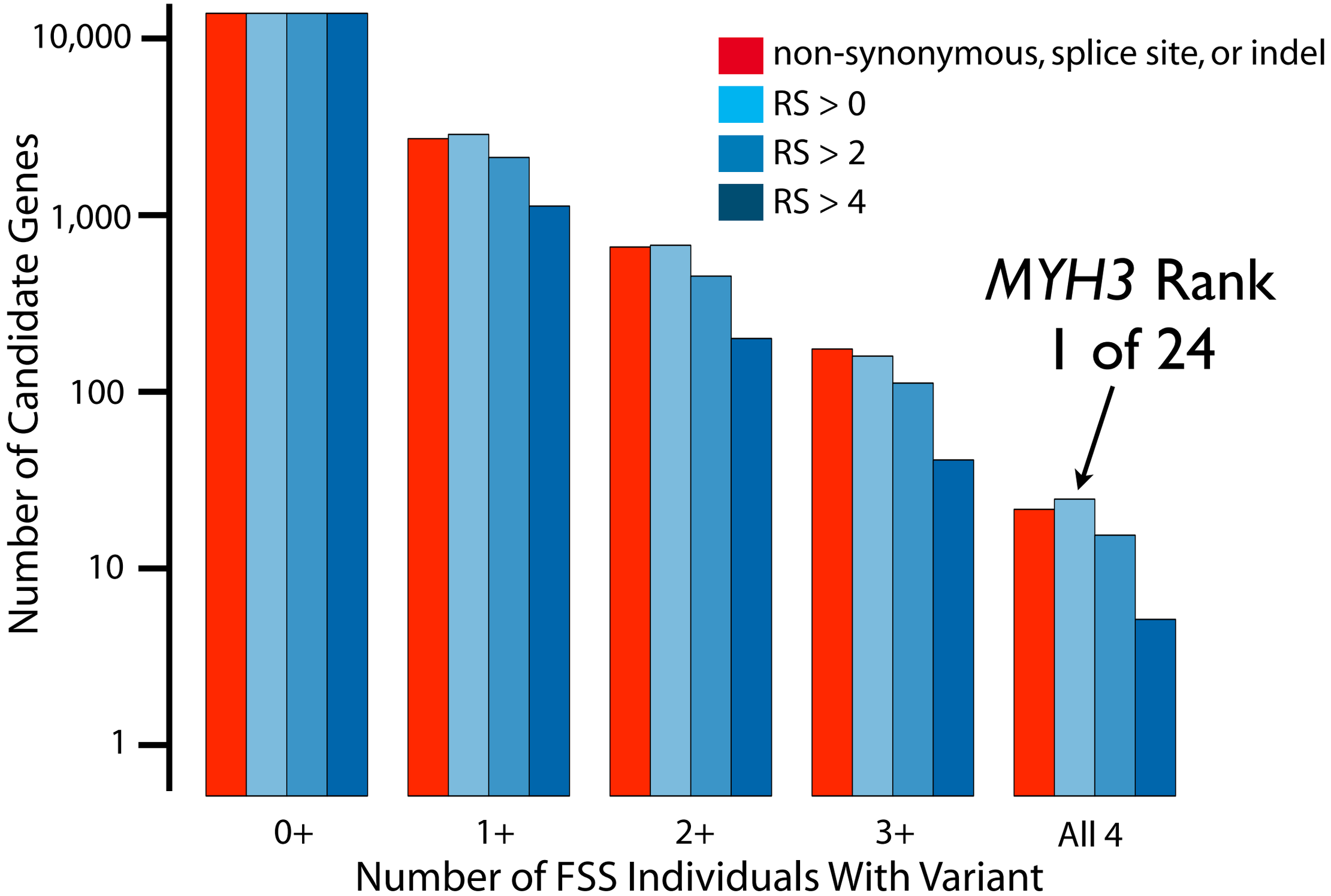

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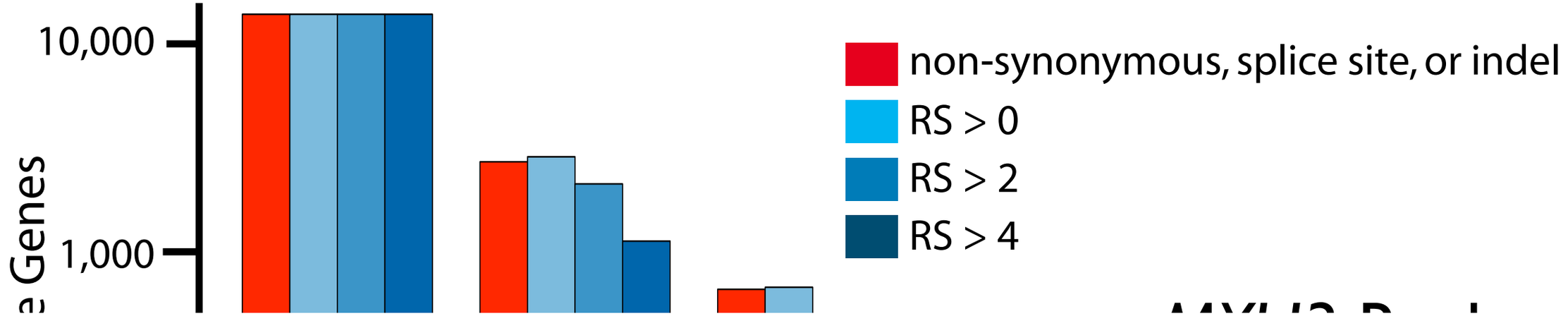
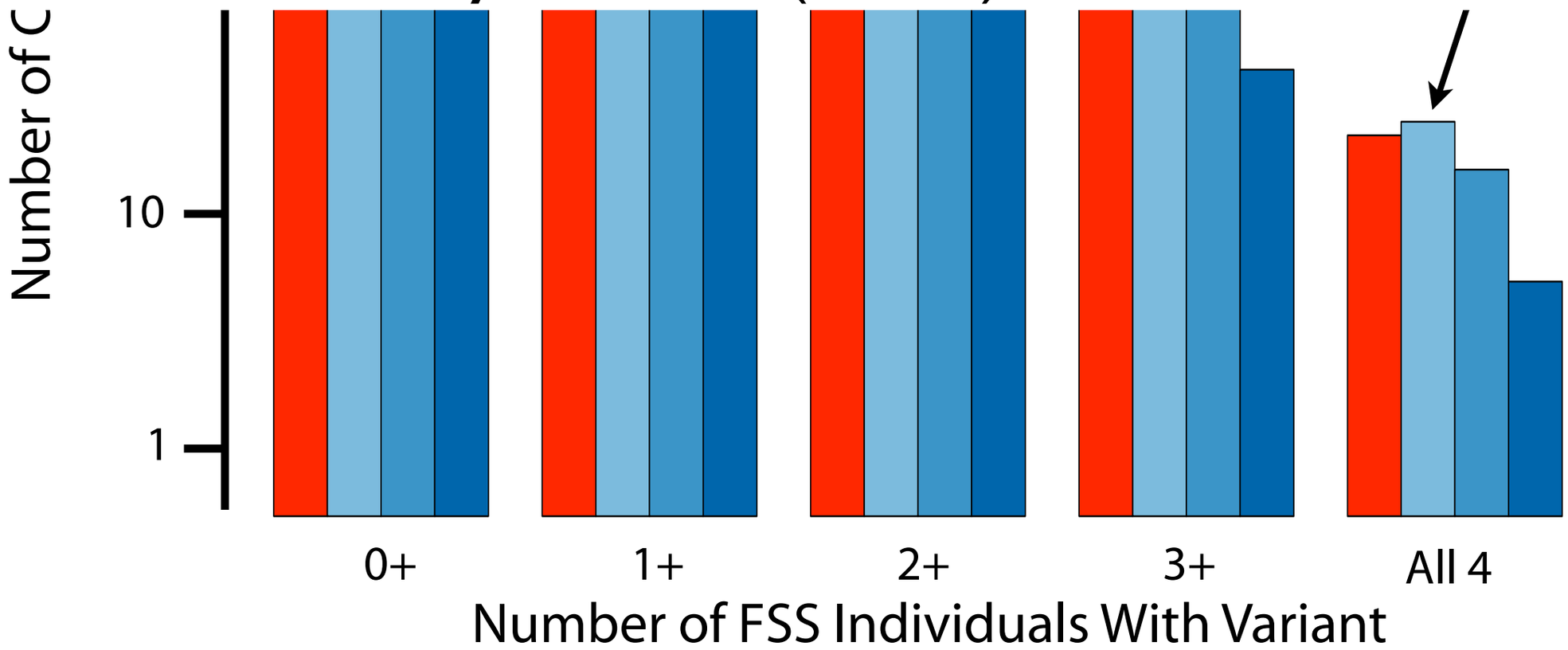


Similar results for Miller syndrome (*DHODH*), Kabuki syndrome (*MLL2*), others



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Error Rates in Genomic Data

All genomics datasets harbor both random and structured errors:

- Many sources of error, *e.g.* depth of coverage, chemistry issues, PCR mistakes, software limitations, etc.

- All institutions, machines, technicians, protocols, samples, etc introduce varying degrees and types of subtle yet systematic and highly "significant" sources of error

- Even when errors are rare at large, they can be proportionally enriched in subsets of variants

Functional Variants are Proportionally Error-Enriched

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Irony of variant annotation is that the more "interesting" a variant is, the more likely it is to be the result of an error

false positive rate ≠ false discovery rate

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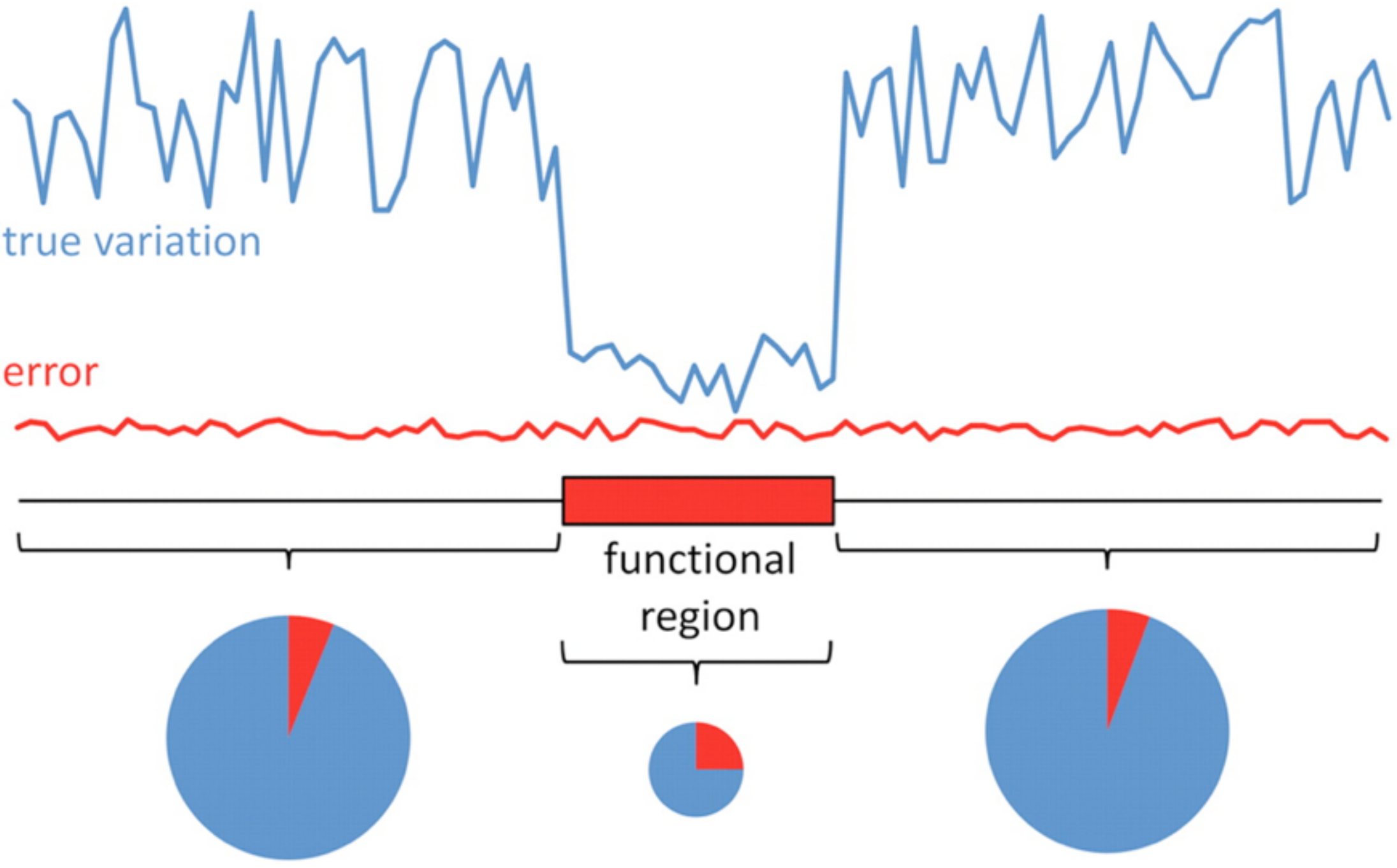

Greg Cooper, UAB Immersion Course, Oct 2015

MacArthur and Tyler-Smith 2010 *Hum Mol Gen*

De Novo Mutations and Errors

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- Excludes false negative variants in parents

De Novo Mutations and Errors

Table 1: Overview of all variants detected per proband and impact of the prioritization steps for selecting candidate non-synonymous *de novo* mutations

Trio	1	2	3	4	5	6	7	8	9	10	Average
High-confidence variant calls	20,810	21,658	21,338	22,647	17,694	22,333	21,369	22,658	24,085	22,962	21,755
After exclusion of nongenic, intronic and synonymous variants	5,556	5,665	5,691	5,991	4,607	5,567	5,716	5,628	5,985	5,994	5,640
After exclusion of known variants	165	159	157	155	120	136	120	149	96	171	143
After exclusion of inherited variants	4	7	3	7	7	2	2	6	6	7	5

Vissers *et al* 2010 *Nature Genetics*

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After exclusion of known variants	165	159	157	155	120	136	120	149	96	171	143
After exclusion of inherited variants	4	7	3	7	7	2	2	6	6	7	5

- After aggressive QC/false positive control, 51 candidate de novo variants in 10 probands
- 9 of which validated
- FDR = 82%
- In genomes, typically observe many hundreds of plausible candidates, of which <100 are real

Vissers *et al* 2010 *Nature Genetics*

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  - Biologically rare annotations are ALWAYS enriched for errors, sometimes dramatically so
  - Rigorous statistical analysis is important, but manual review of both genetic and non-genetic information is a critical step in evaluating mutations in both clinical and research projects

Greg Cooper, UAB Immersion Course, Oct 2015

Other General Comments on Finding Causal Variants

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  - Less than 50% of rare, likely Mendelian diseases to which exome sequencing has been applied has yielded good hits
  - Even when a gene is known for a disease, protein-coding alterations often explain only a subset of cases

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Developmental Delay and Intellectual Disability

1 - 2% of kids are born with one or more of:

- intellectual disability
- developmental delay
- heart defects
- craniofacial and skeletal abnormalities
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Most of these problems have genetic causes

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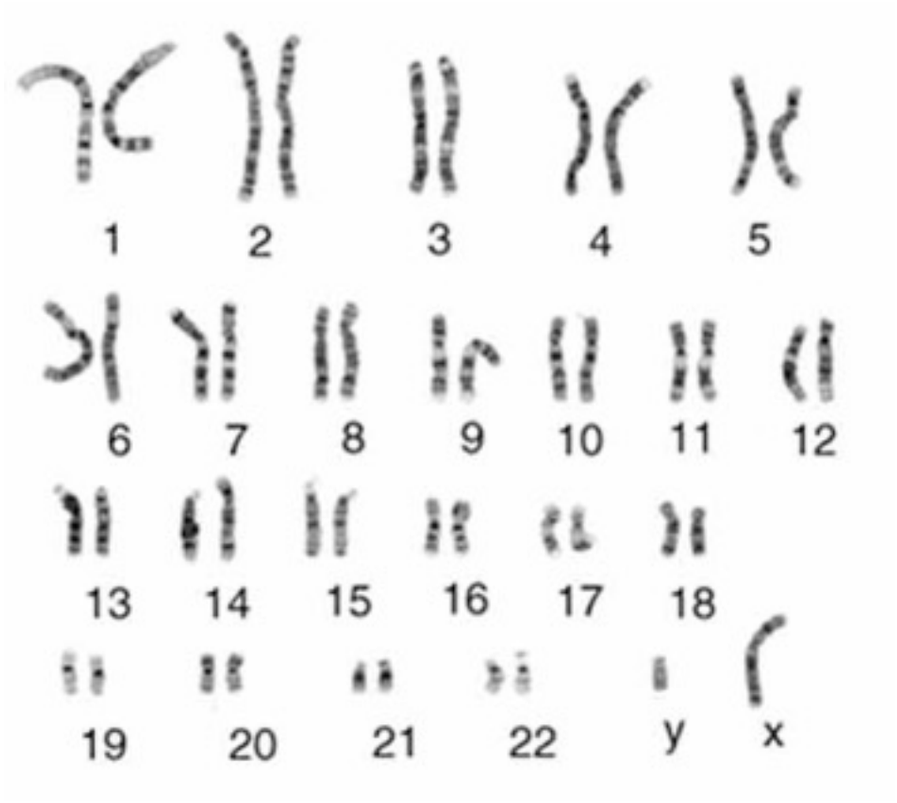
Genomic Diagnosis of DD/ID



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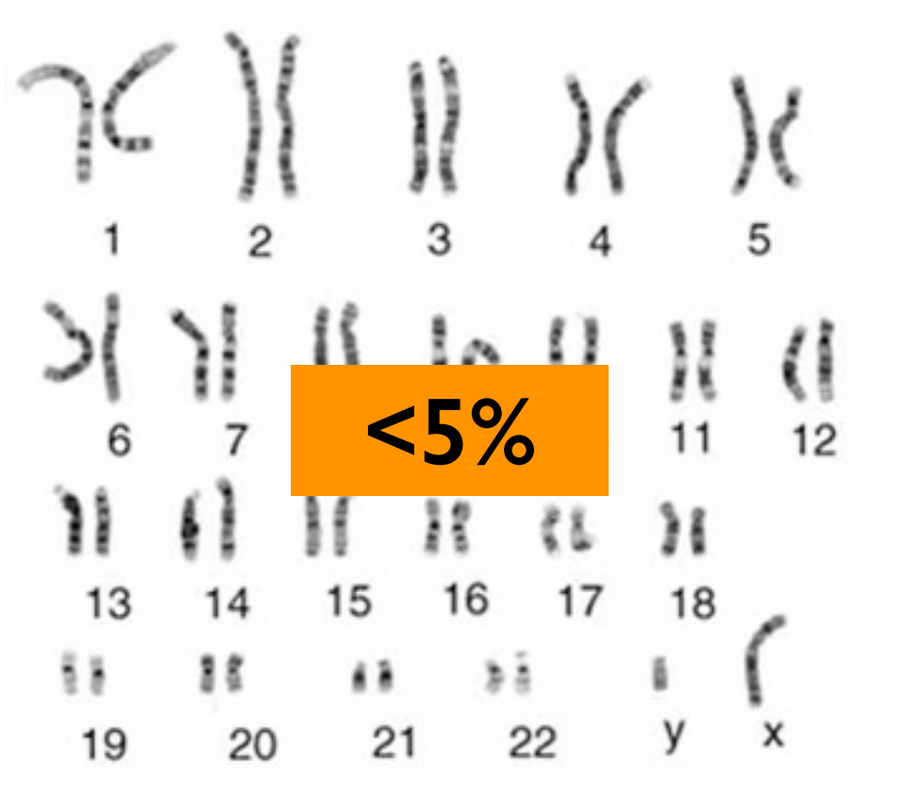

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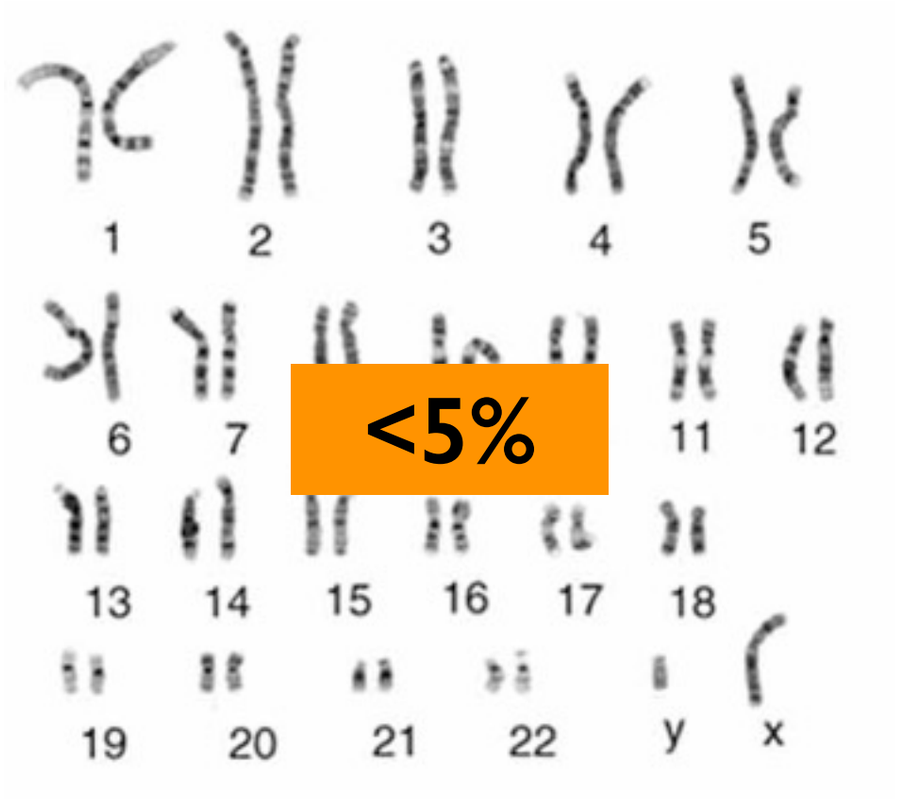

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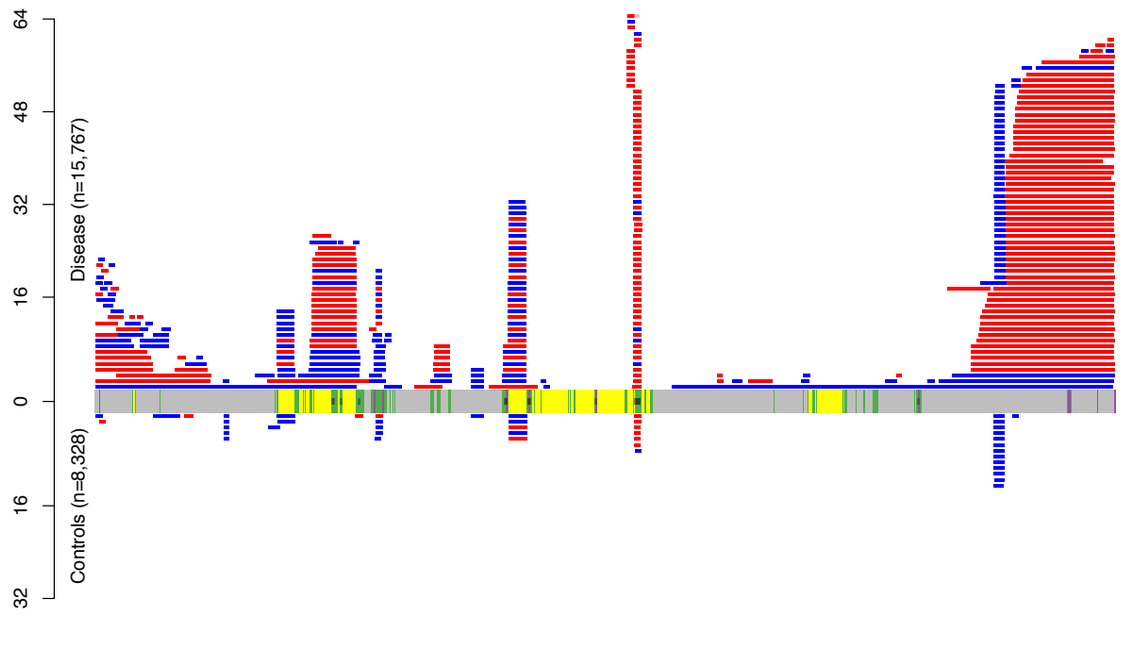

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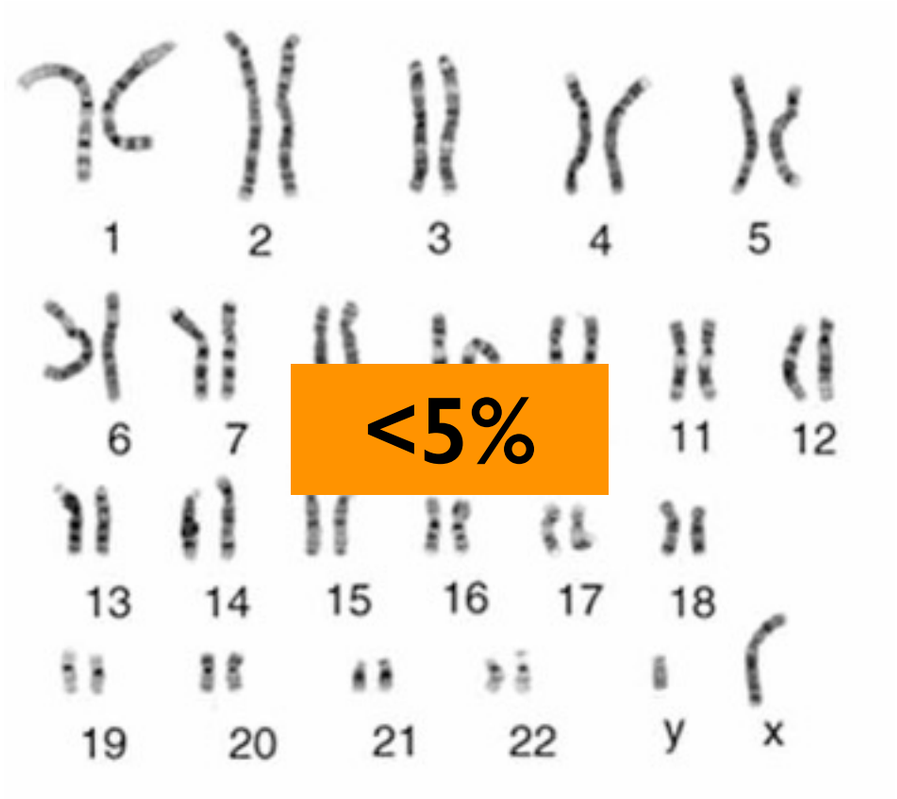

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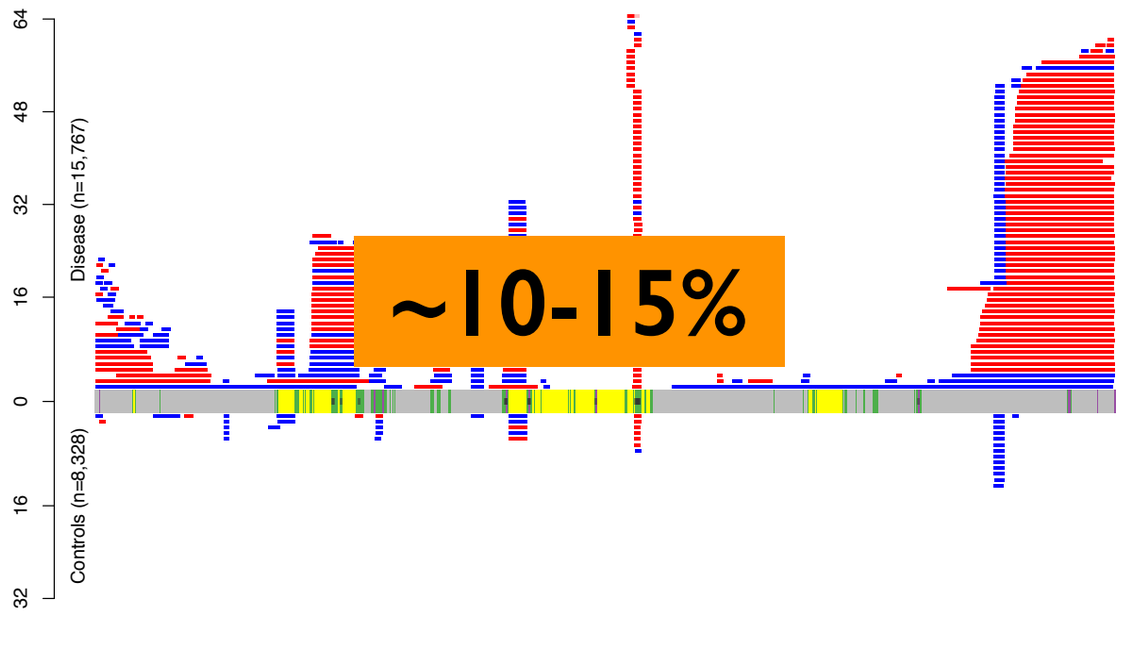

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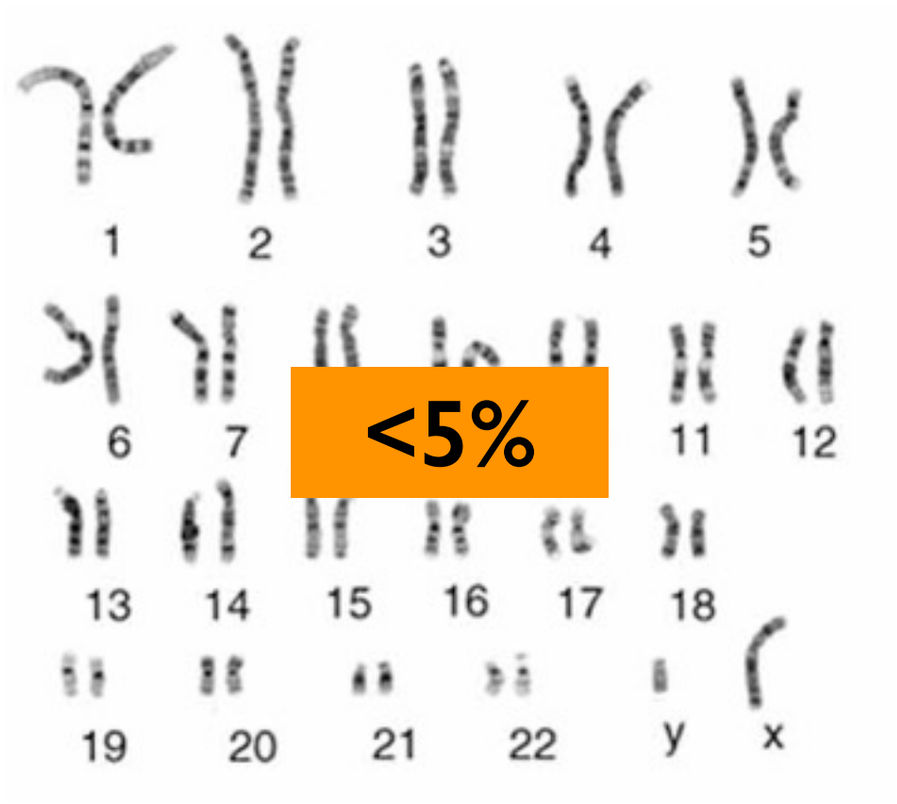

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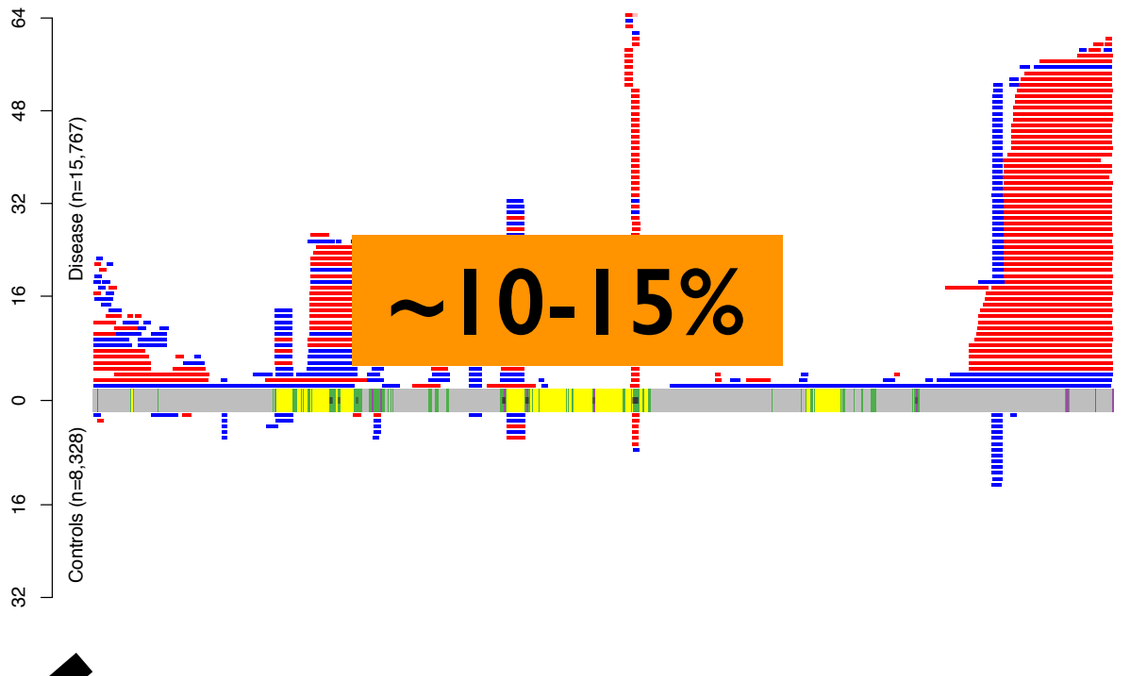

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CGTATACCGGGTCATGCACGTGTAGAGCGAG
TTAGCTCGCTGGCTAAAGAGGGTCGACATCC
GCGAGTTTATGAGGAAGAATCGGCAGCTTGA
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Now

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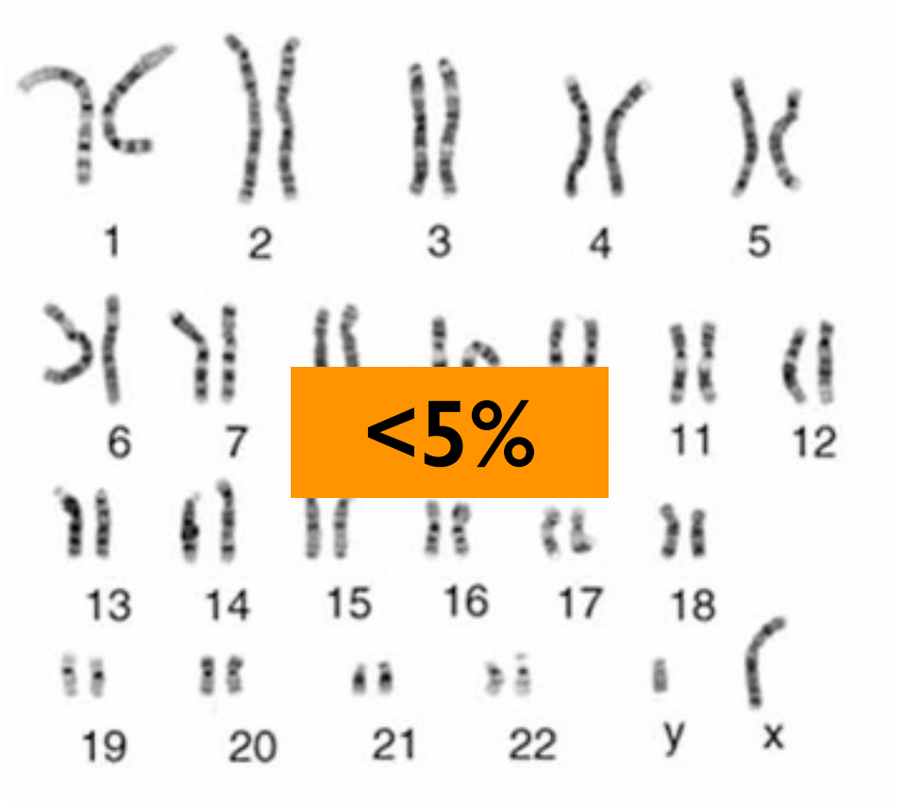

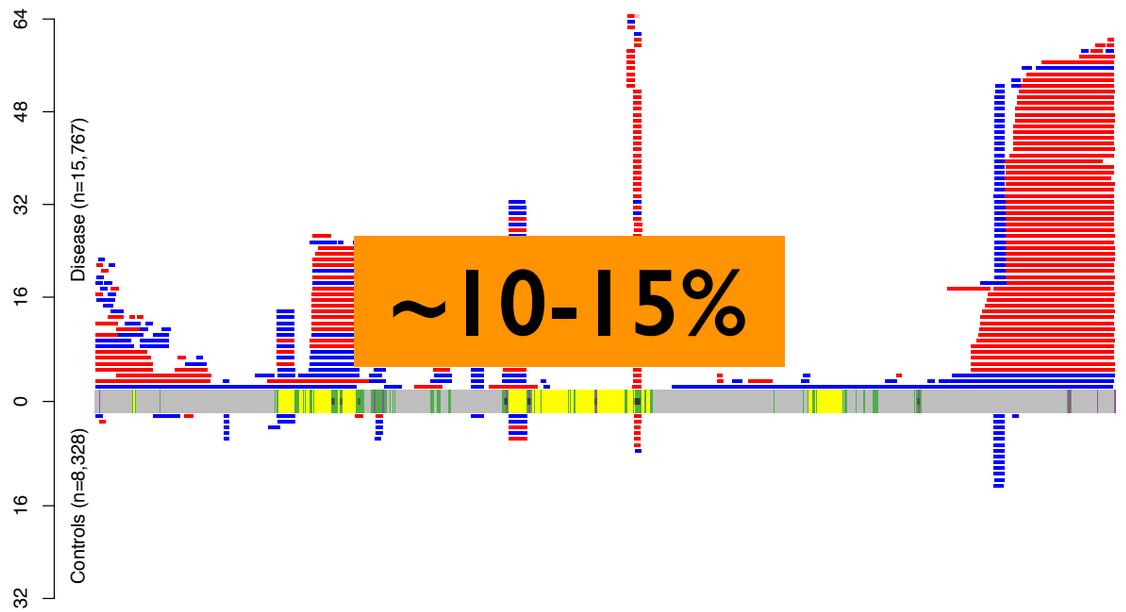

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Now

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HudsonAlpha Pediatric Genomic CSER Project

HUDSONALPHA
INSTITUTE FOR BIOTECHNOLOGY

pediatric genomics

1. Recruit 450 parent-offspring trios over 4 years with a DD/ID affected proband without a specific diagnosis

2. Conduct exome (now whole genome) sequencing to identify variants that are DD/ID causal and therefore diagnostic

3. Return pathogenic variants to families

4. Evaluate overall impact of genetic information return on families' health and well-being

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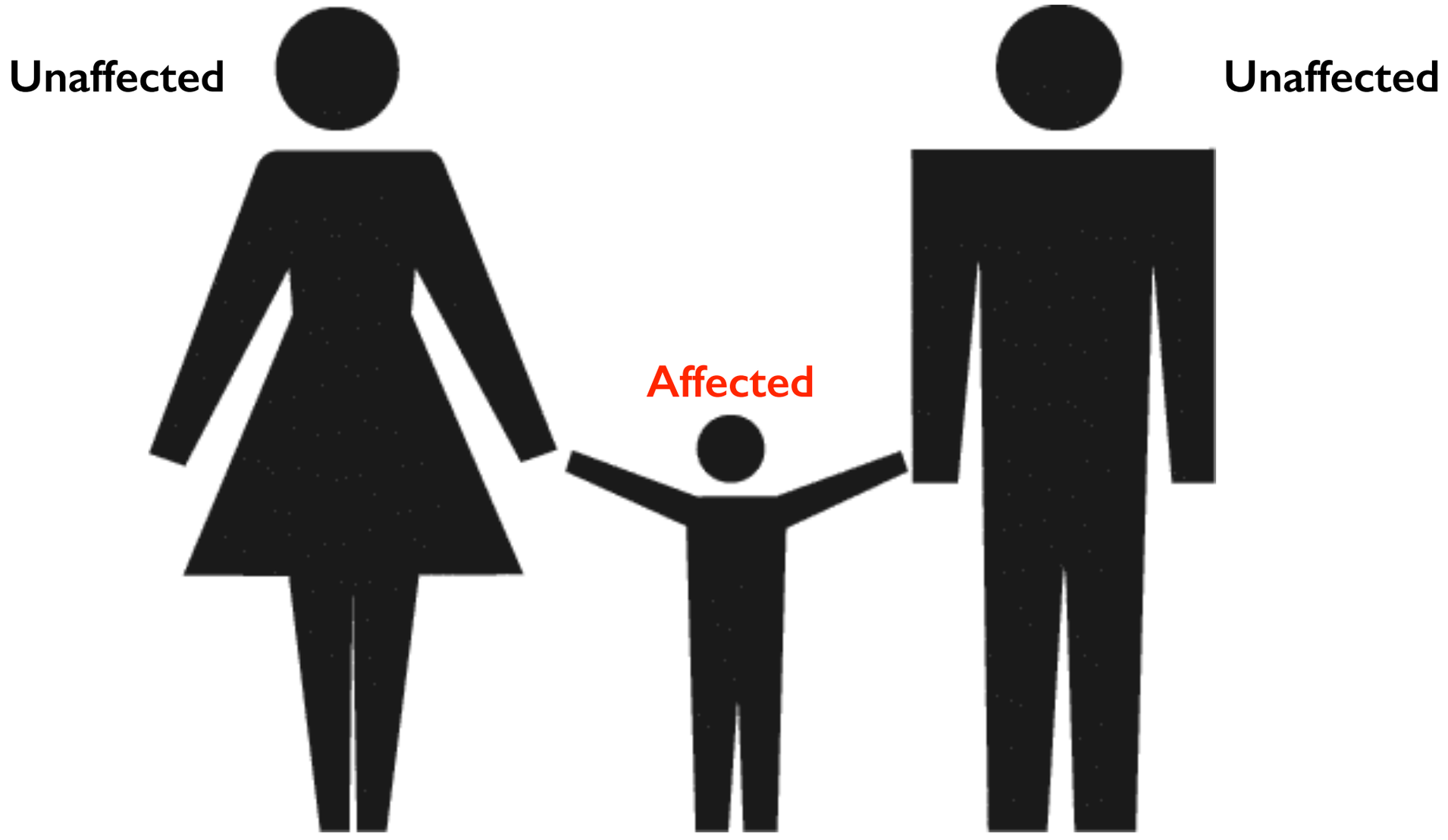
Familial Variant Analysis Paradigms



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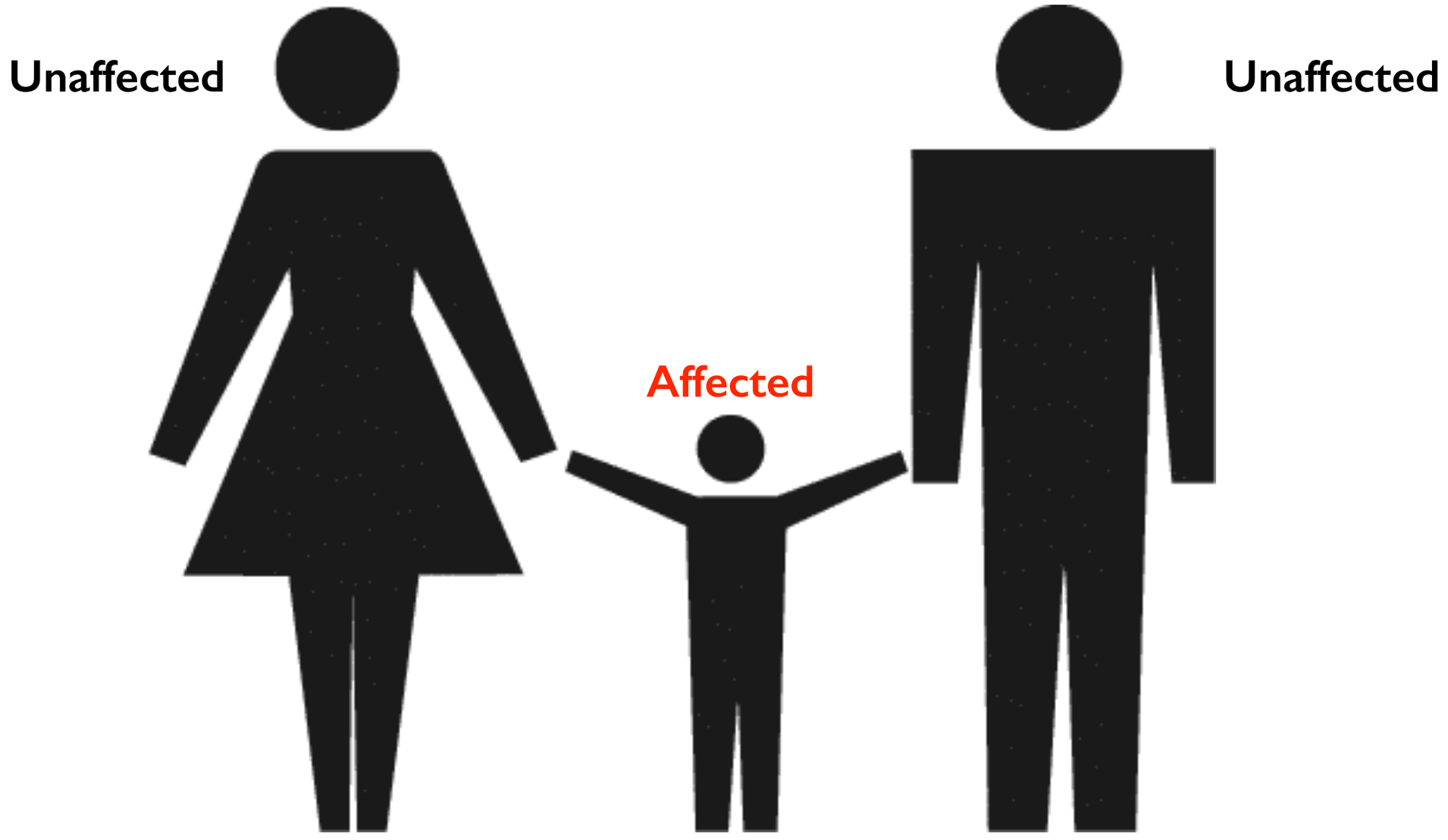

De Novo

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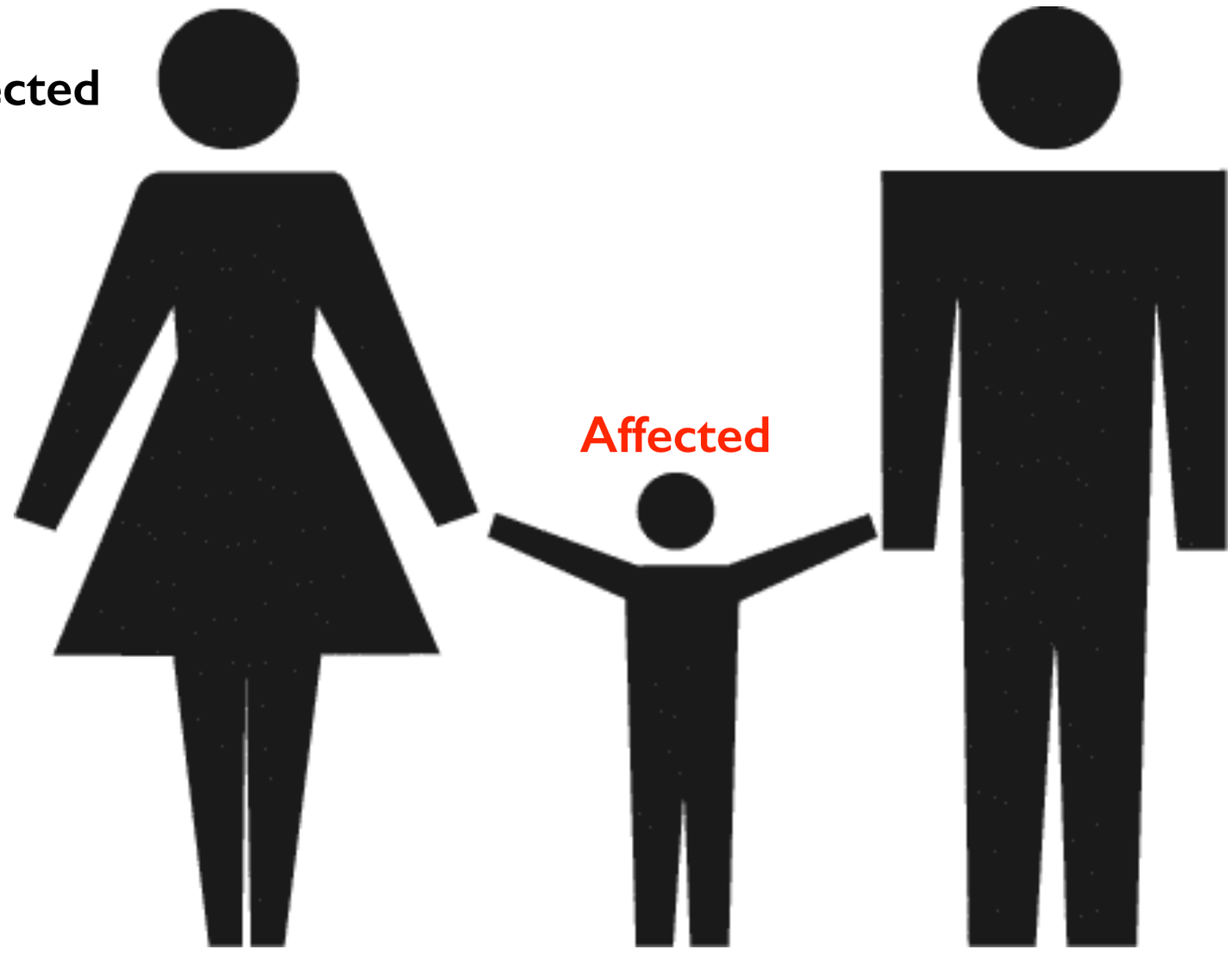

De Novo	+/+	+/-	+/+

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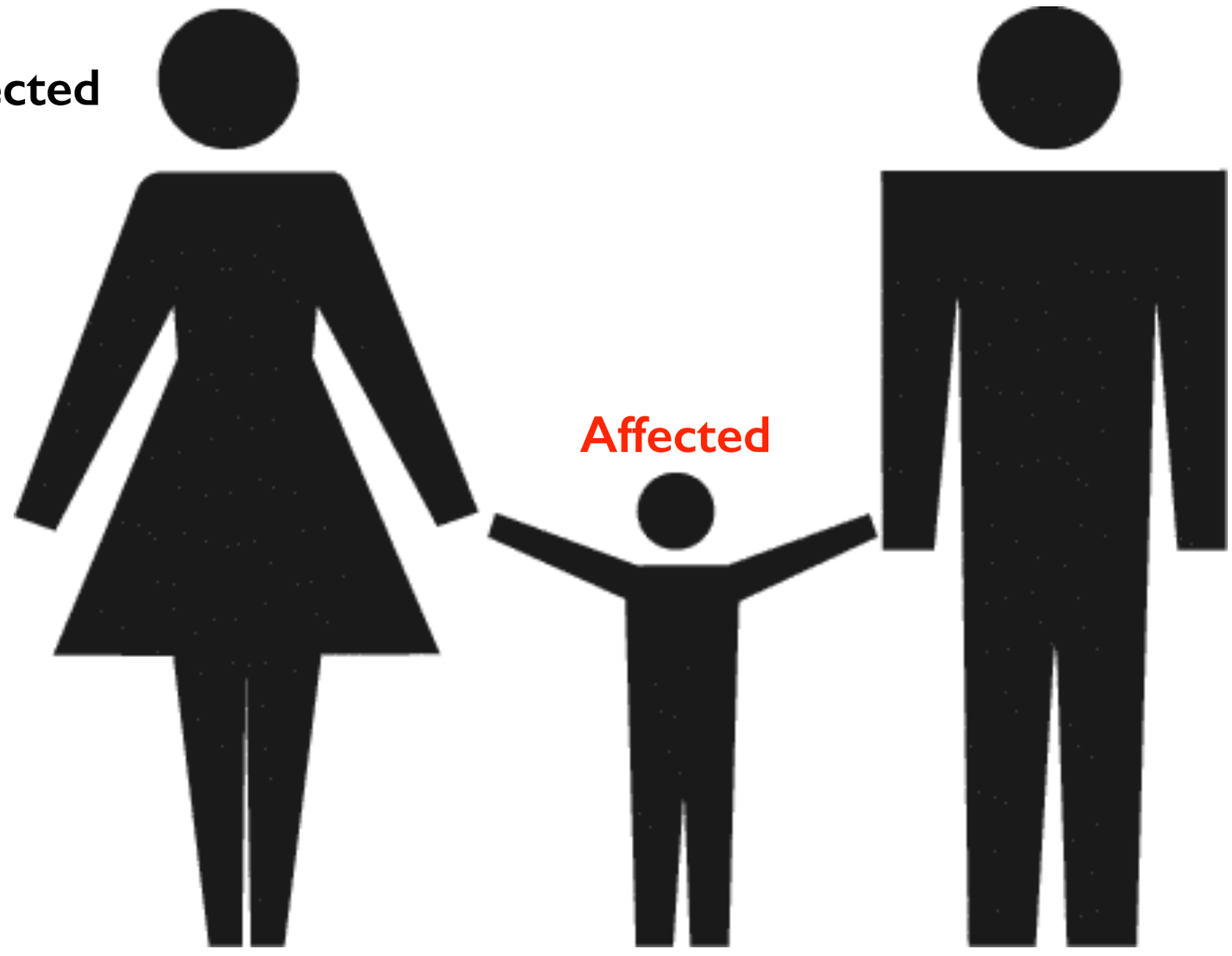

De Novo +/+ +/- +/+

Comp-Het or Recessive

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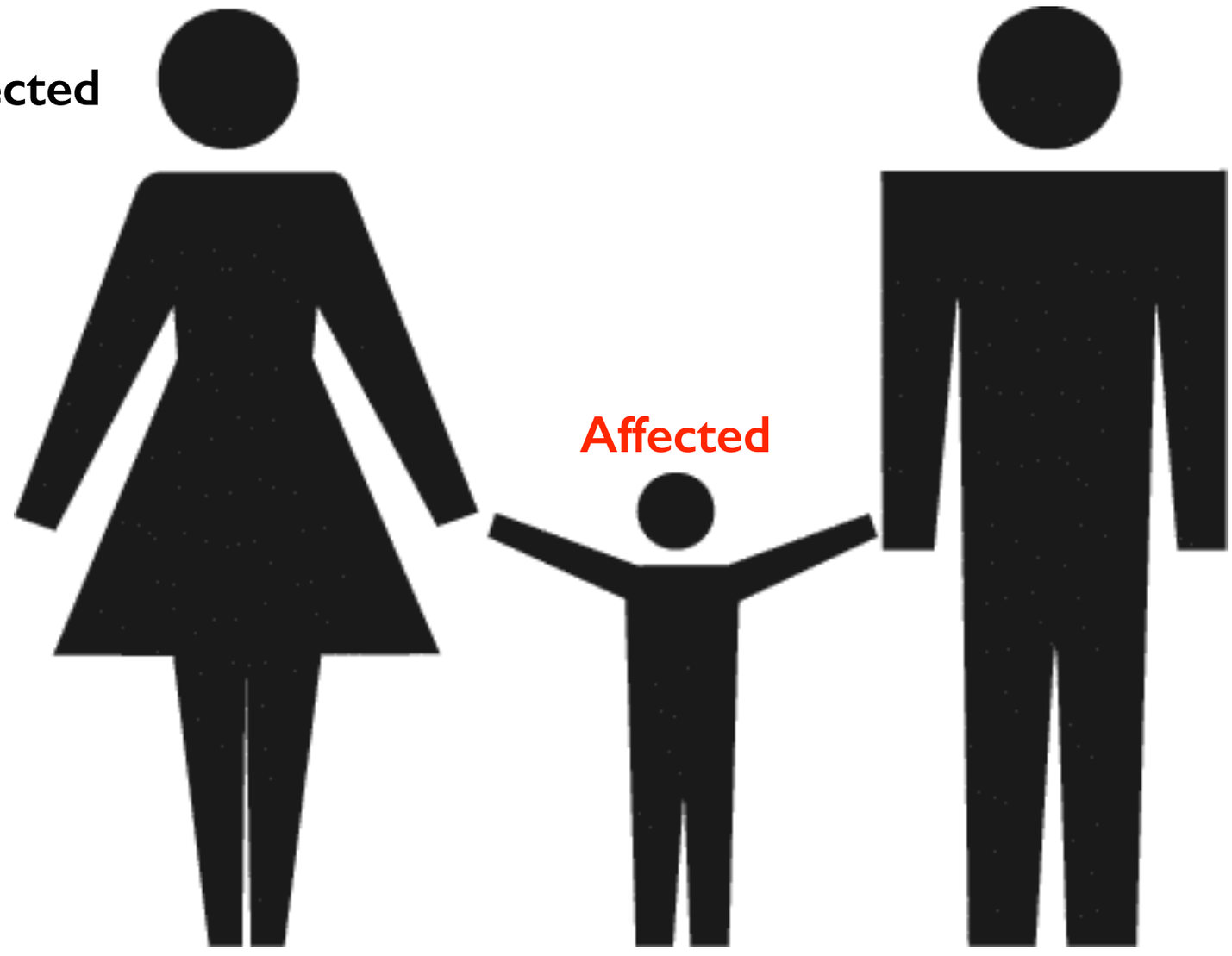

De Novo	+/+	+/-	+/+
Comp-Het or Recessive	+/-	-/-	+/-

Variant Review

- After filtering based on population frequencies, genomic annotations, and inheritance patterns, variants are manually reviewed by at least two genomic analysts
- Lists typically span a few to dozens of variants
- Goal is to evaluate the totality of evidence, integrating resources like OMIM, ClinVar, published literature, genomic annotations, etc., to make a determination as to the medical relevance of any given mutation(s)
  - genetics and correlations with previous cases is paramount

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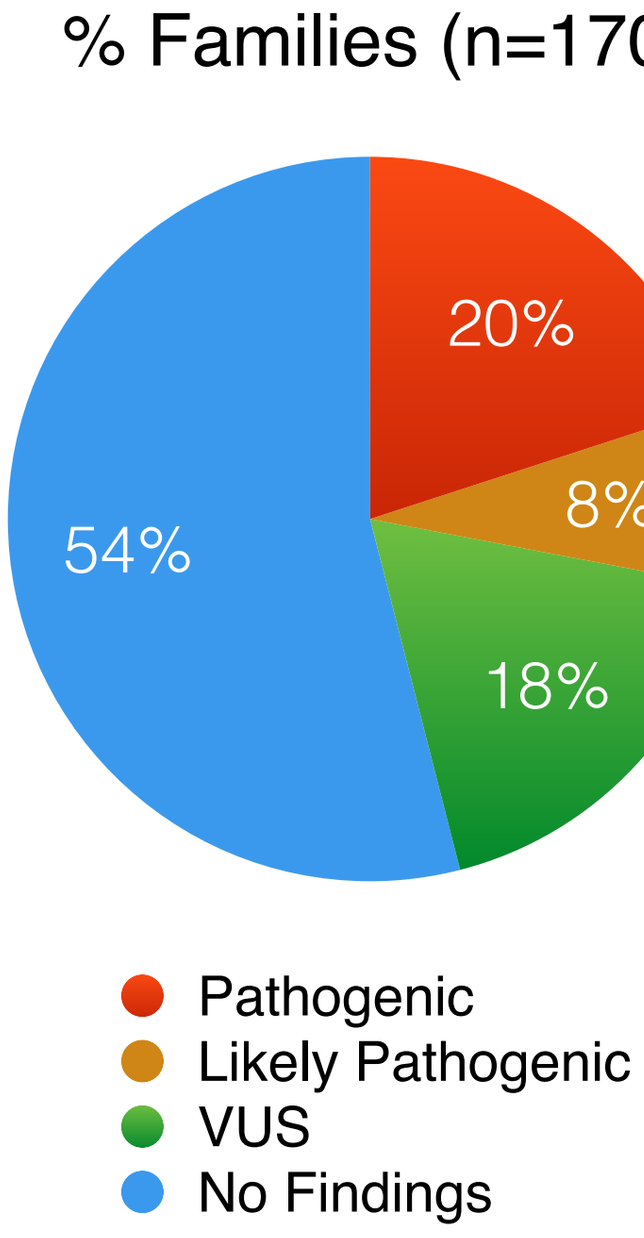
Diagnostic Finding Overview



- Genetic diagnosis for 28% of families
  - Pitt-Hopkins syndrome
  - Dravet syndrome
  - Rett syndrome
  - Rubinstein-Taybi syndrome
  - Noonan-like syndrome

- Variants of uncertain significance identified in 18% of families; may be diagnostic in the future

- Re-analysis of negative exomes using updated information identified three diagnostic variants (e.g. MTOR, DDX3X and CLPB)

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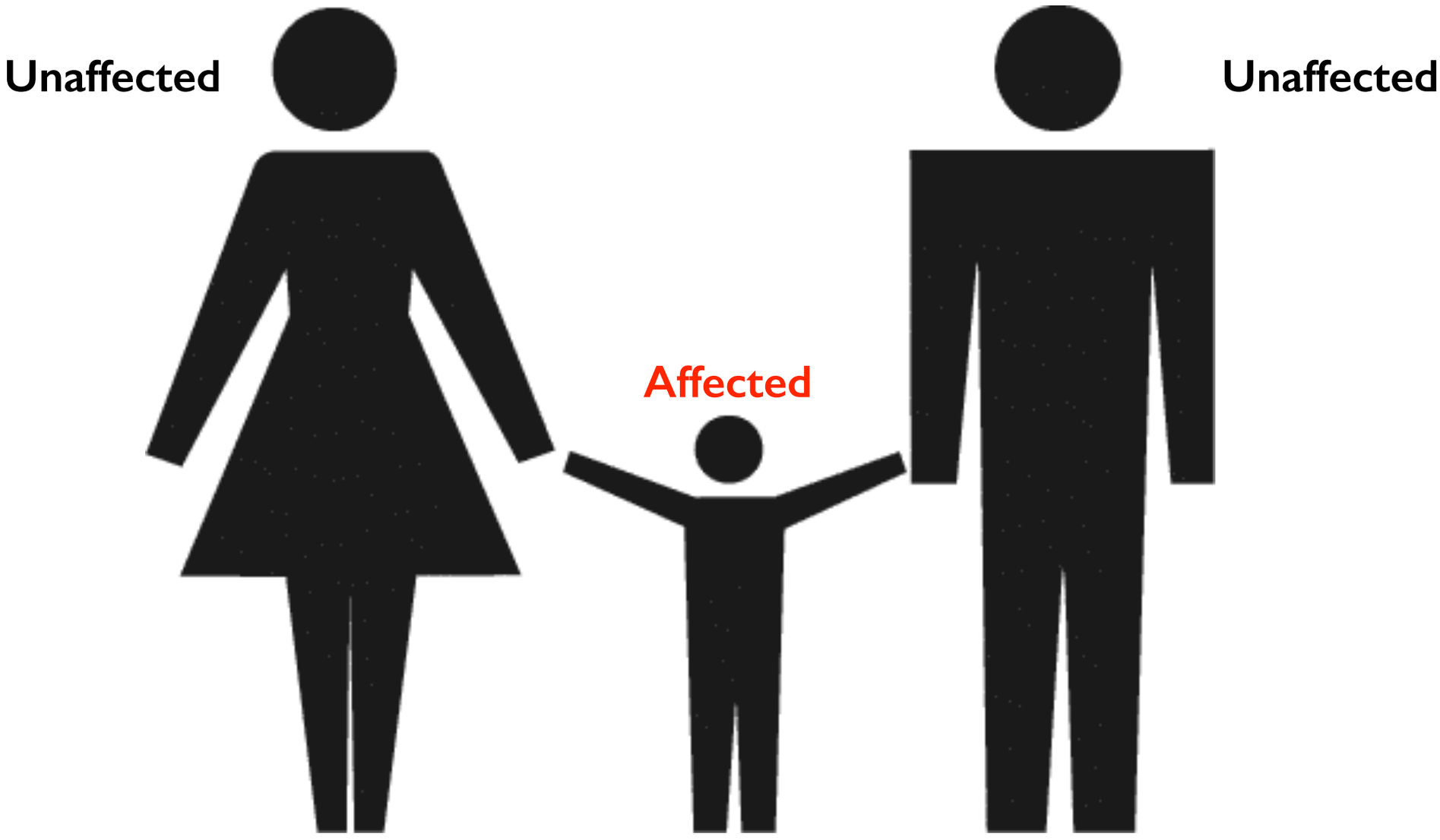
“Secondary” or “Incidental” Findings



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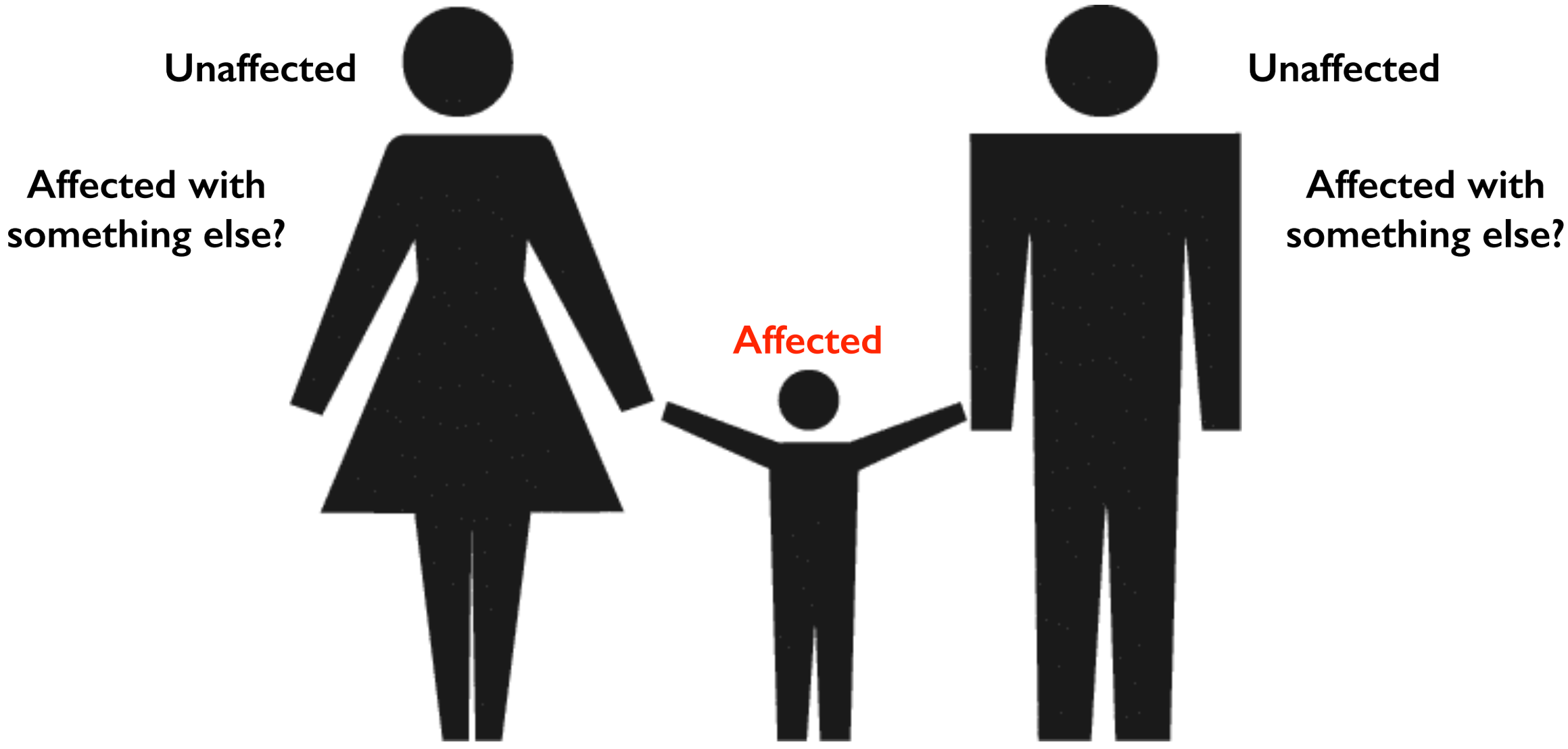

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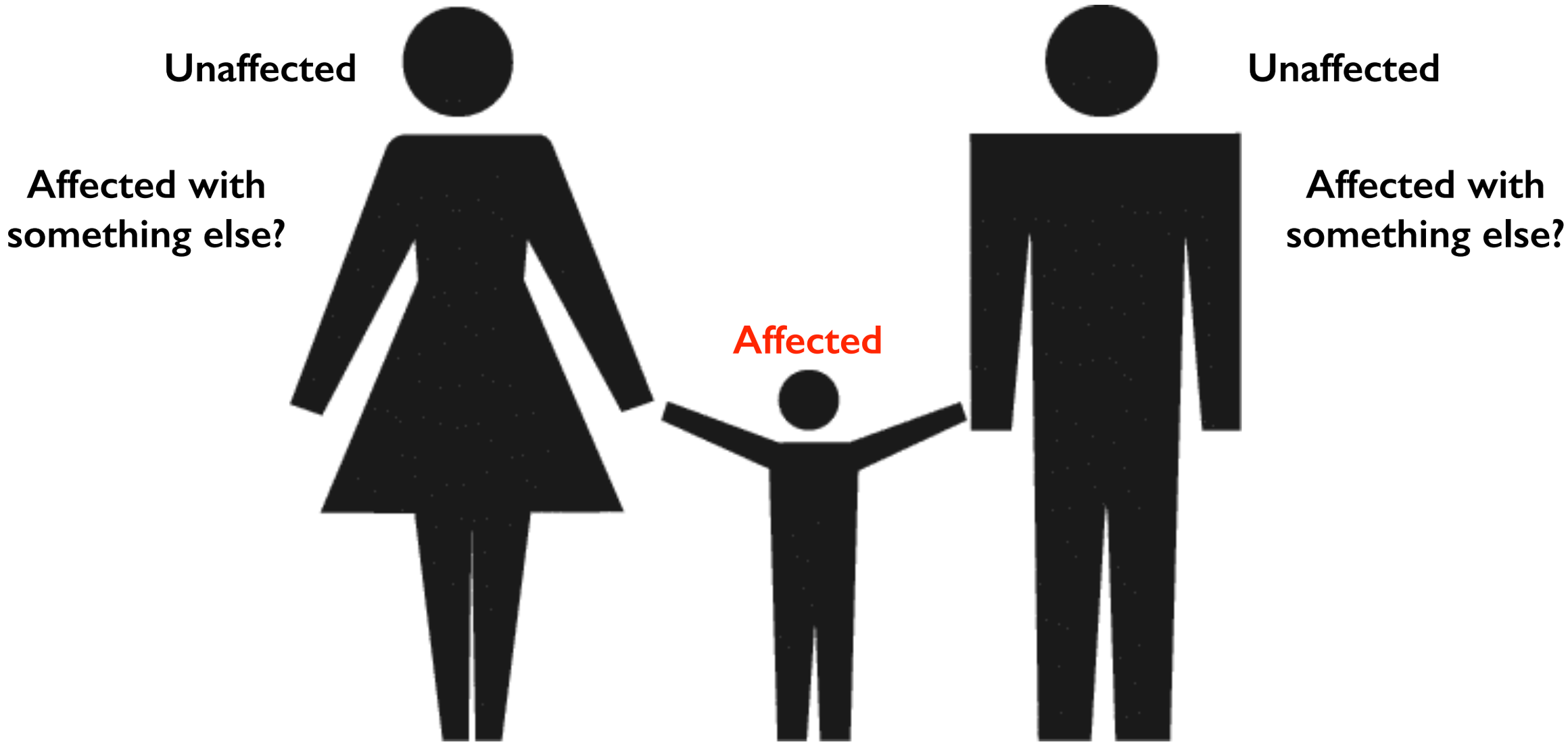

ACMG gene list (57 genes), mostly dominant mutations that result in medically “actionable” conditions

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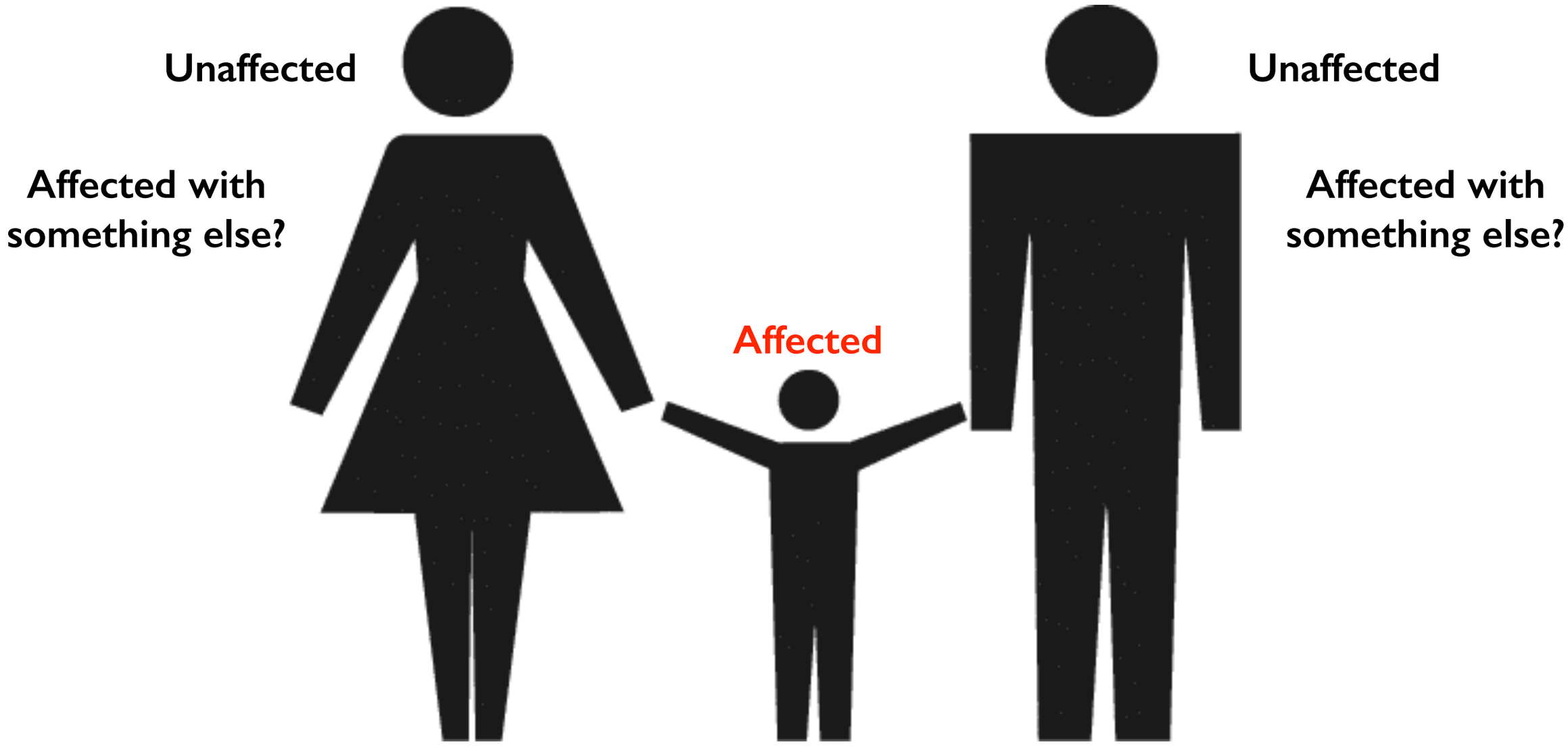

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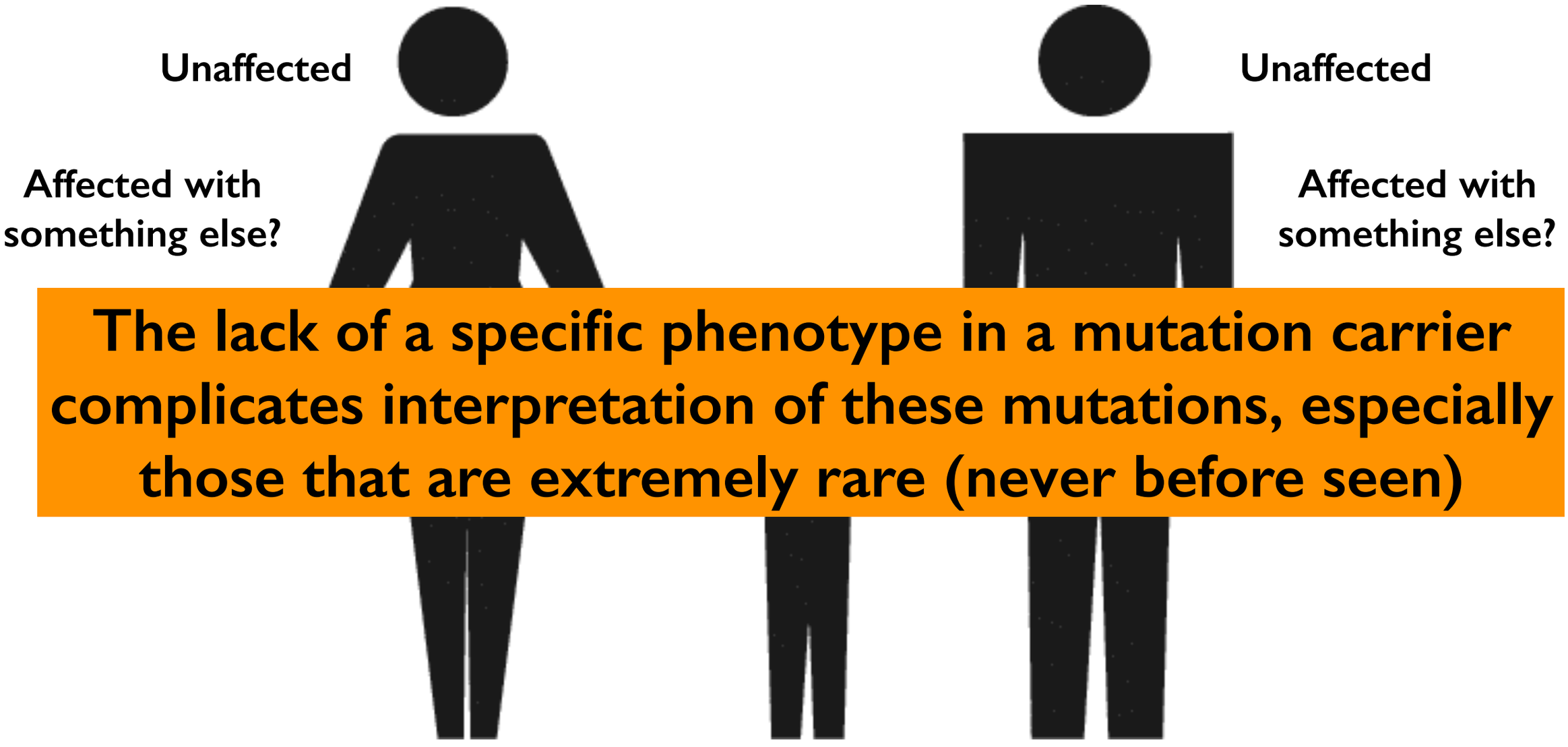

The lack of a specific phenotype in a mutation carrier complicates interpretation of these mutations, especially those that are extremely rare (never before seen)

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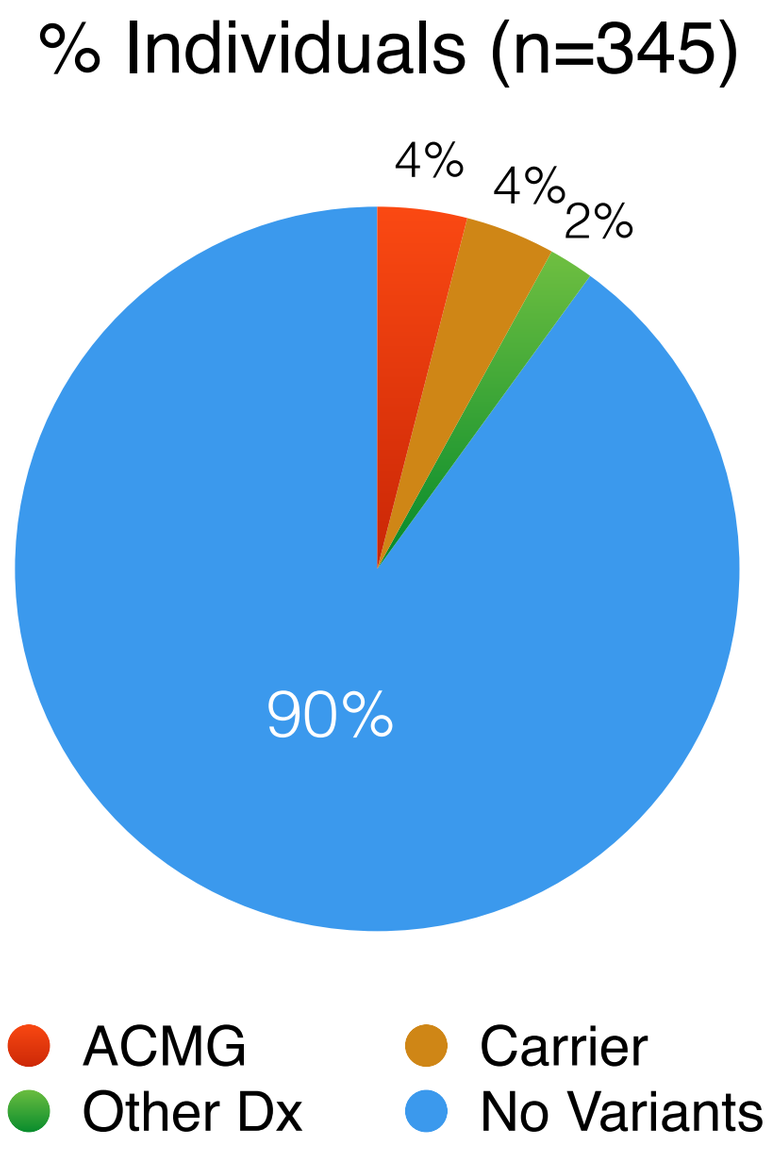
Secondary Findings Overview



• Identified secondary variants are linked to many different diseases/conditions

- Cystic Fibrosis (carrier)
- Tay-Sachs disease (carrier)
- Sickle cell disease (carrier)
- Breast cancer
- Colorectal cancer
- Cardiomyopathy
- Malignant hyperthermia susceptibility
- Carnitine deficiency
- Albinism

- Return or not driven by preference
- Uncertain genetics and risk/benefit to interventions lead to difficult decisions

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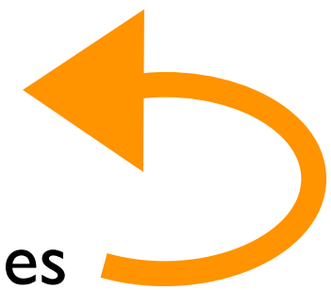

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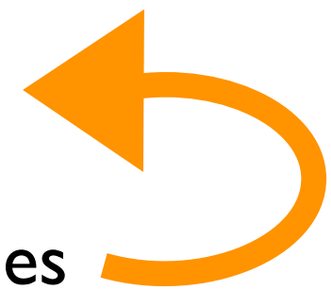

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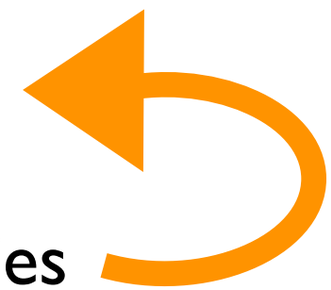

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- Data sharing is another area that needs improvement
  - best evidence is more genotype-phenotype correlation, which can only emerge from vast data networks for rare variants and rare diseases
- "Proving" variant causality is very hard, and often impossible, but for clinical decisions certainty may or may not be necessary (i.e., all possible error types and consequences must be evaluated)

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