Exploratory Whole-Genome Analysis in Familial Hemiplegic Migraine

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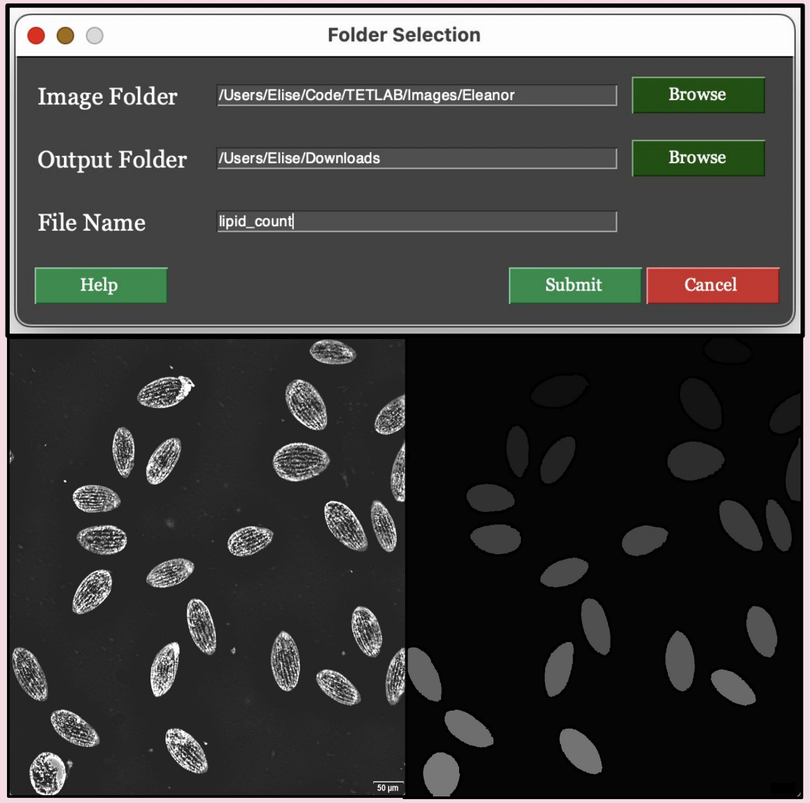
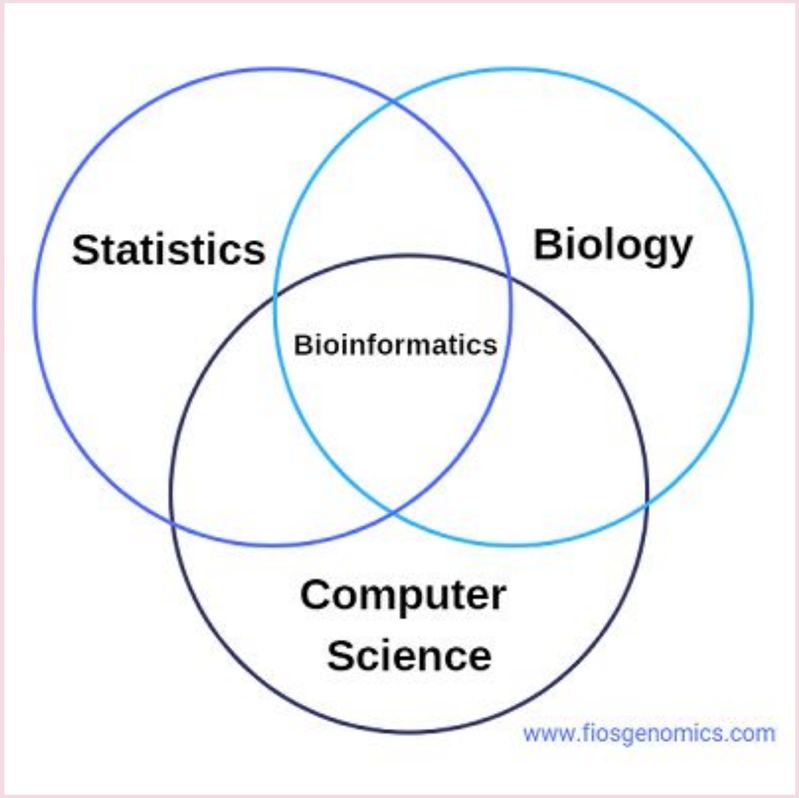
The Major

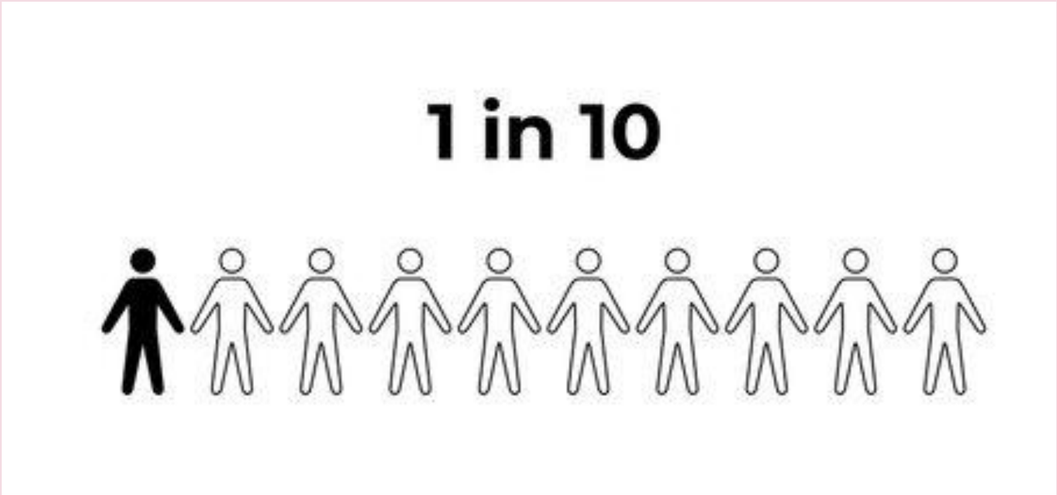
Using computational methods to analyze biological data





Rare Disease

- 10,000+ known rare diseases
- 5% have an FDA-approved treatment
- Unexpected breakthroughs



Overview

Goal: Identify rare, potentially disease causing variants in a patient with Familial Hemiplegic Migraine (FHM) using whole-genome sequencing and a variant filtering pipeline.

Background

- Migraines affect ~14% of the population
- FHM affects ~0.01%
- Autosomal dominant inheritance
- Three known genes:
  - CACNA1A
  - ATP1A2
  - SCN1A

Symptoms

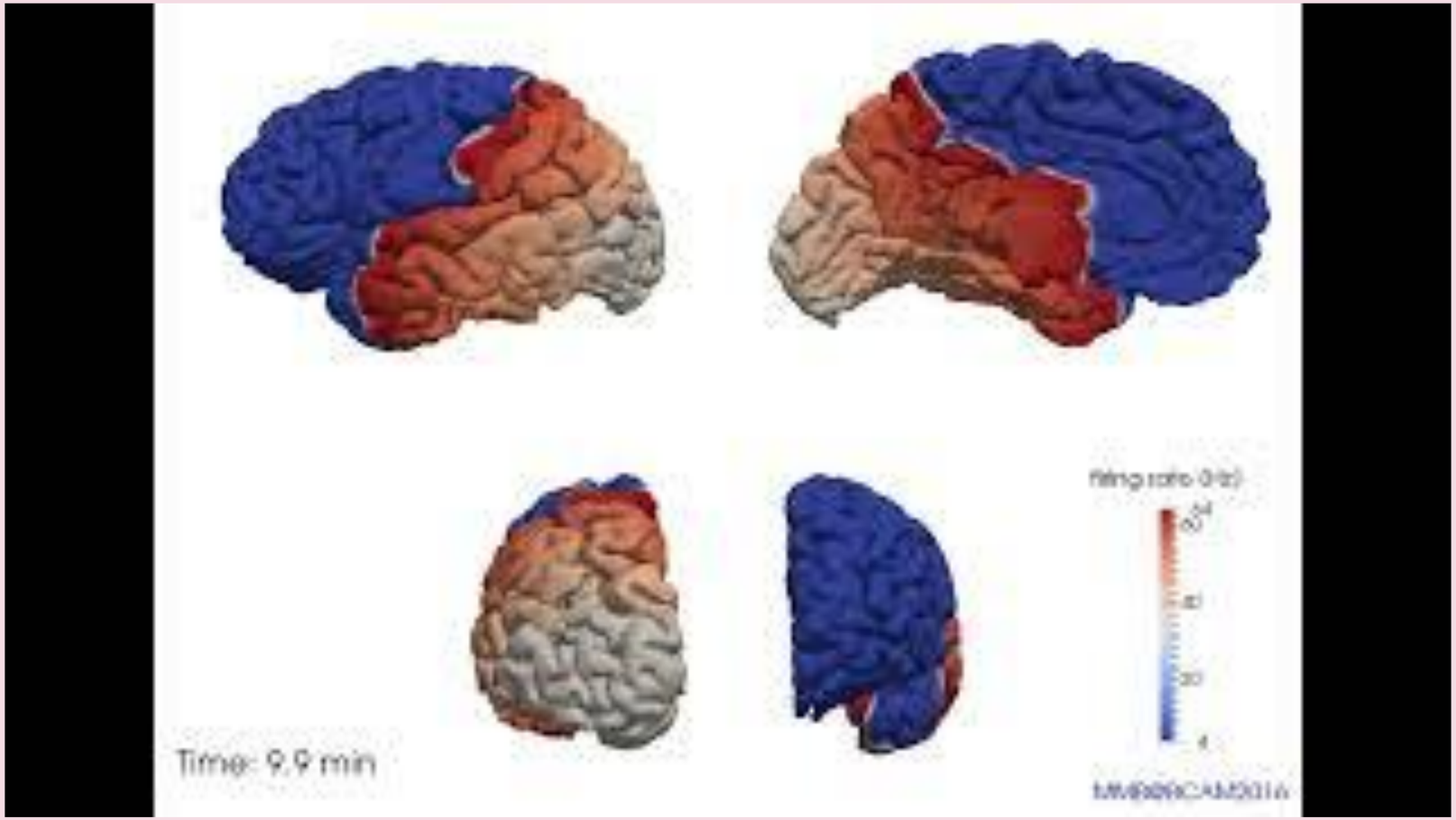
Common:

- Hemiparesis/hemiplegia
- Severe headache
- Sensory disturbances
- Difficulty speaking

More rare:

- Confusion or drowsiness
- Fever
- Seizures
- Cognitive impairment
- Coma
- Stroke

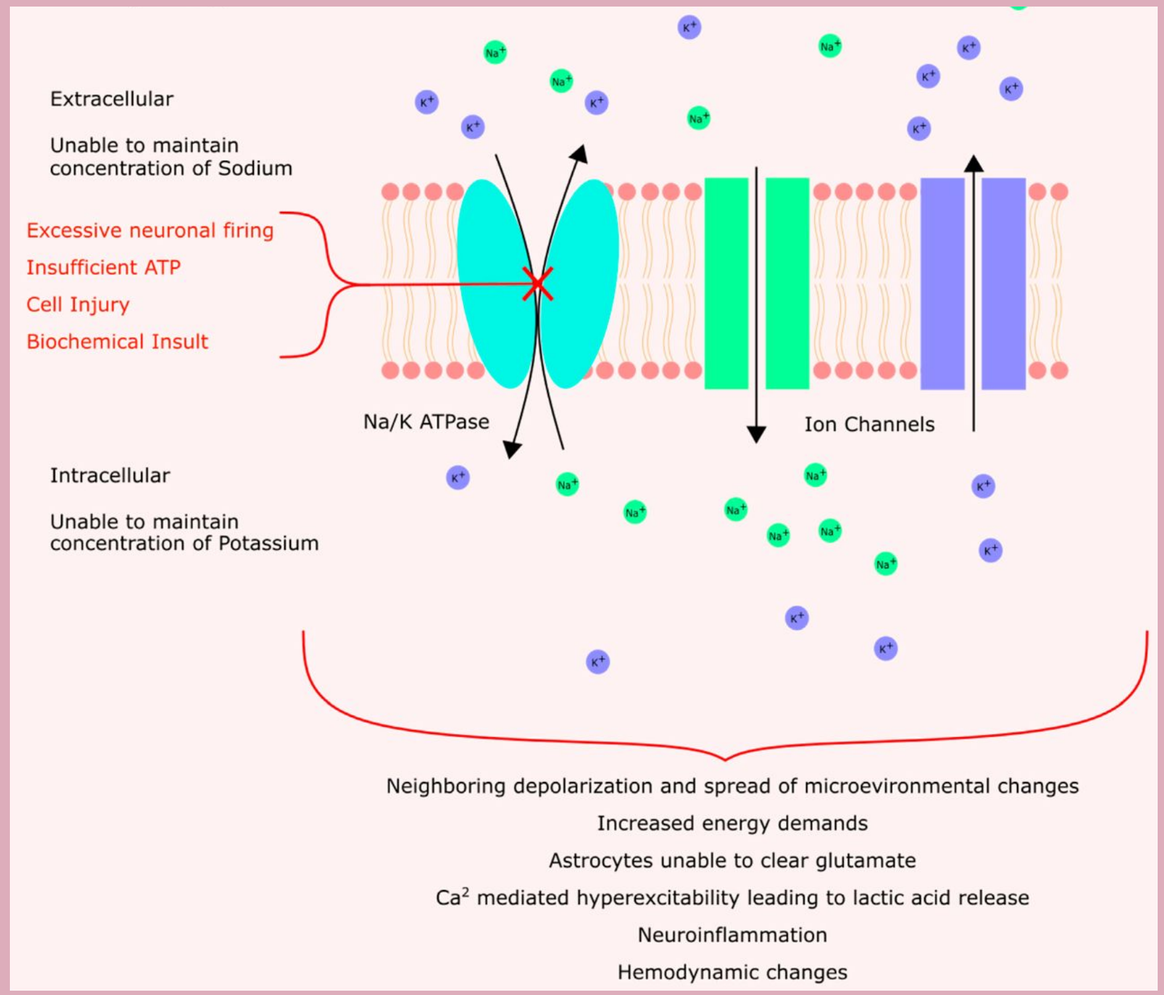
Biological Mechanism



Cortical Spreading Depression

Biological Mechanism

- Wave of neuronal depolarization
- ↑ extracellular K+
- ↑ intracellular Na+
- ↑ intracellular Ca2+
- Inflammation and vasoconstriction



Hill et. al 2024

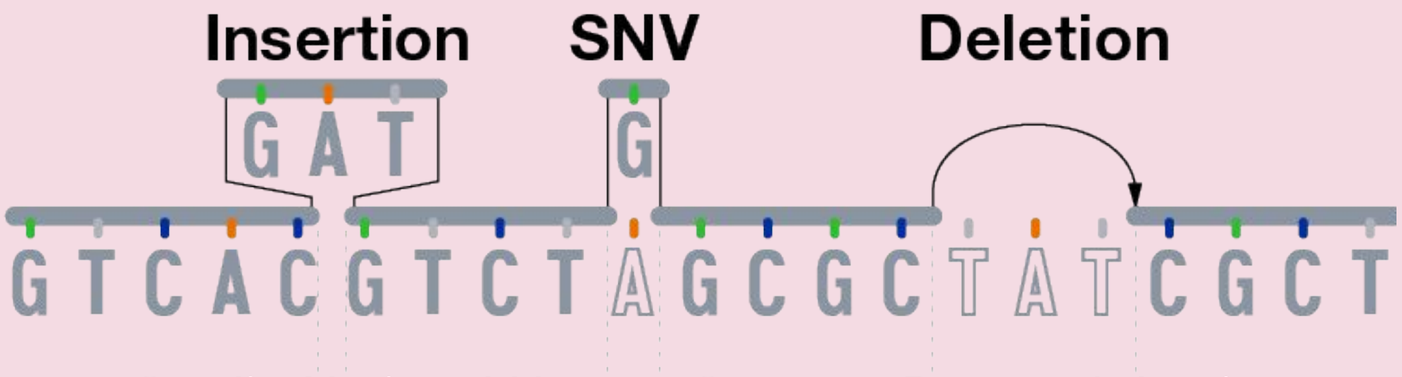
Research Question

Context:

- Subject is diagnosed with familial hemiplegic migraine (FHM)
- An affected parent tested negative for known mutations
- Family history includes migraine and epilepsy
- Known genes account for 50-70% of cases

Hypothesis: Given the overlap between hereditary epilepsy and the known FHM genes, additional variants in genes related to ion-channels or epilepsy may be responsible for cases of FHM with no known mutation.

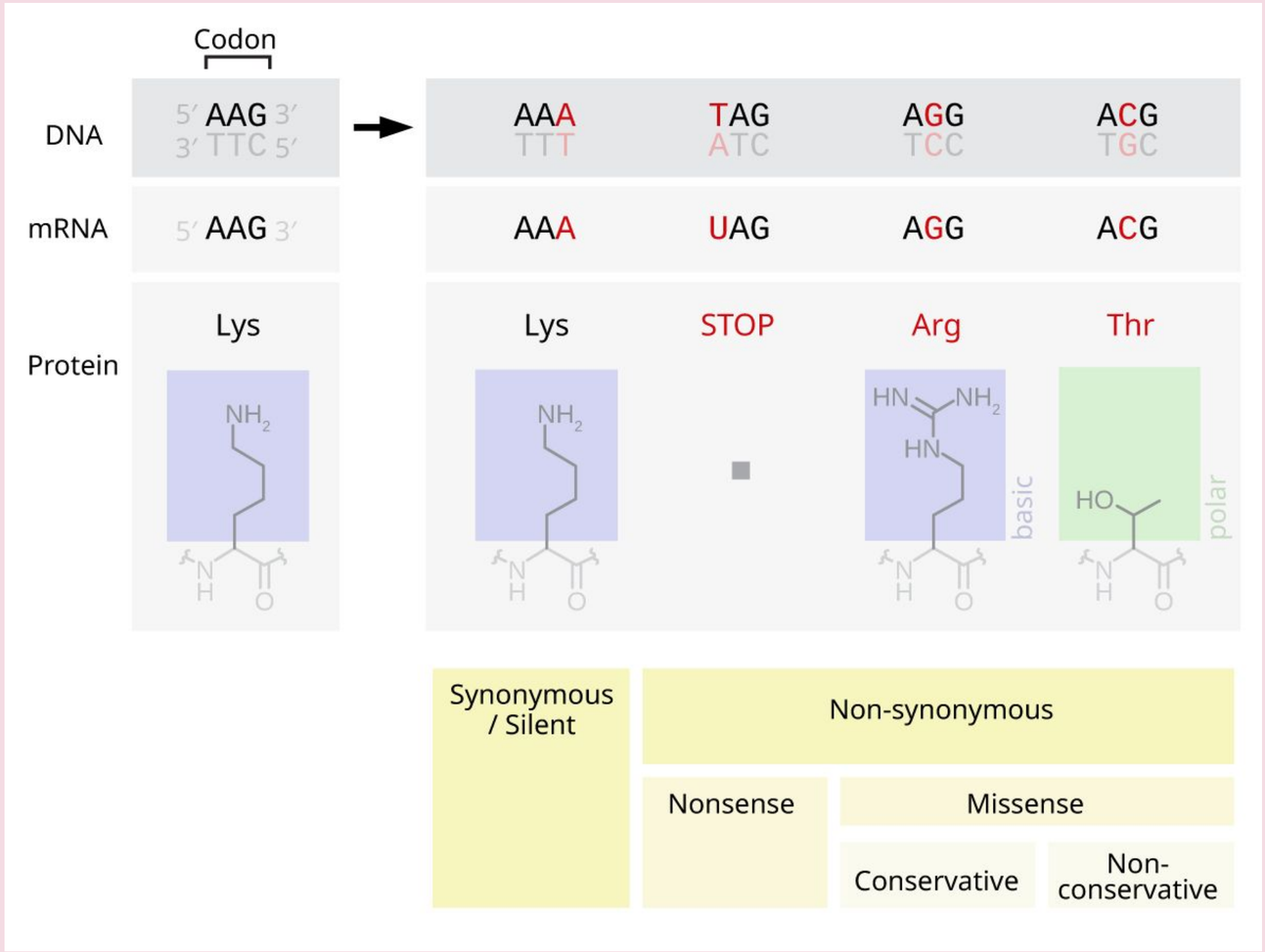
Genomic Variation



Credit: National Human Genome Research Institute

Base Pairs → Codon → Amino Acid → Protein

Genomic Variation



The big rug

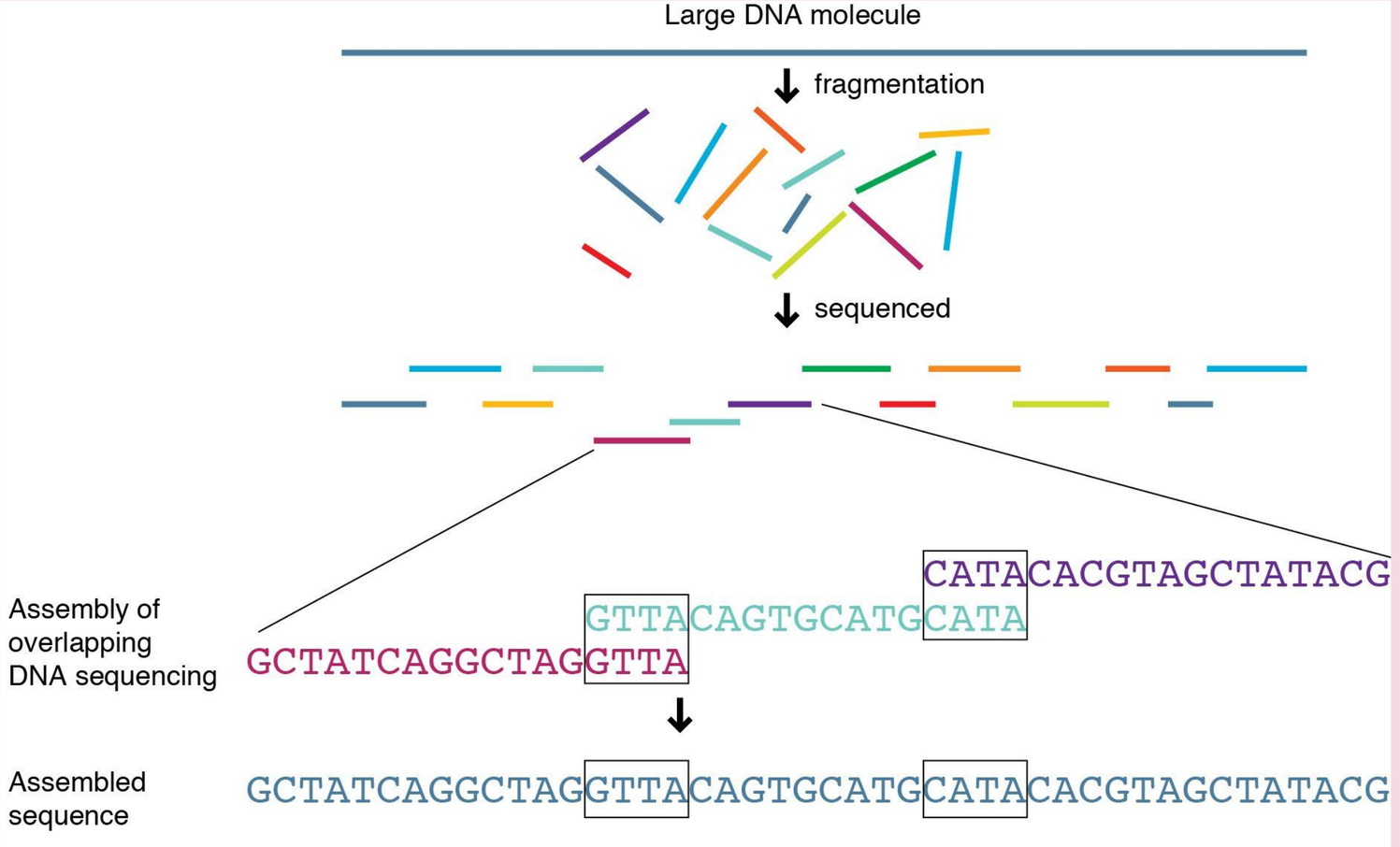
The big zru g

Credit: Jonsta247 on Wikipedia

Whole Genome Sequencing

Process:

- Fragmentation
- Amplification
- Sequencing
- Alignment



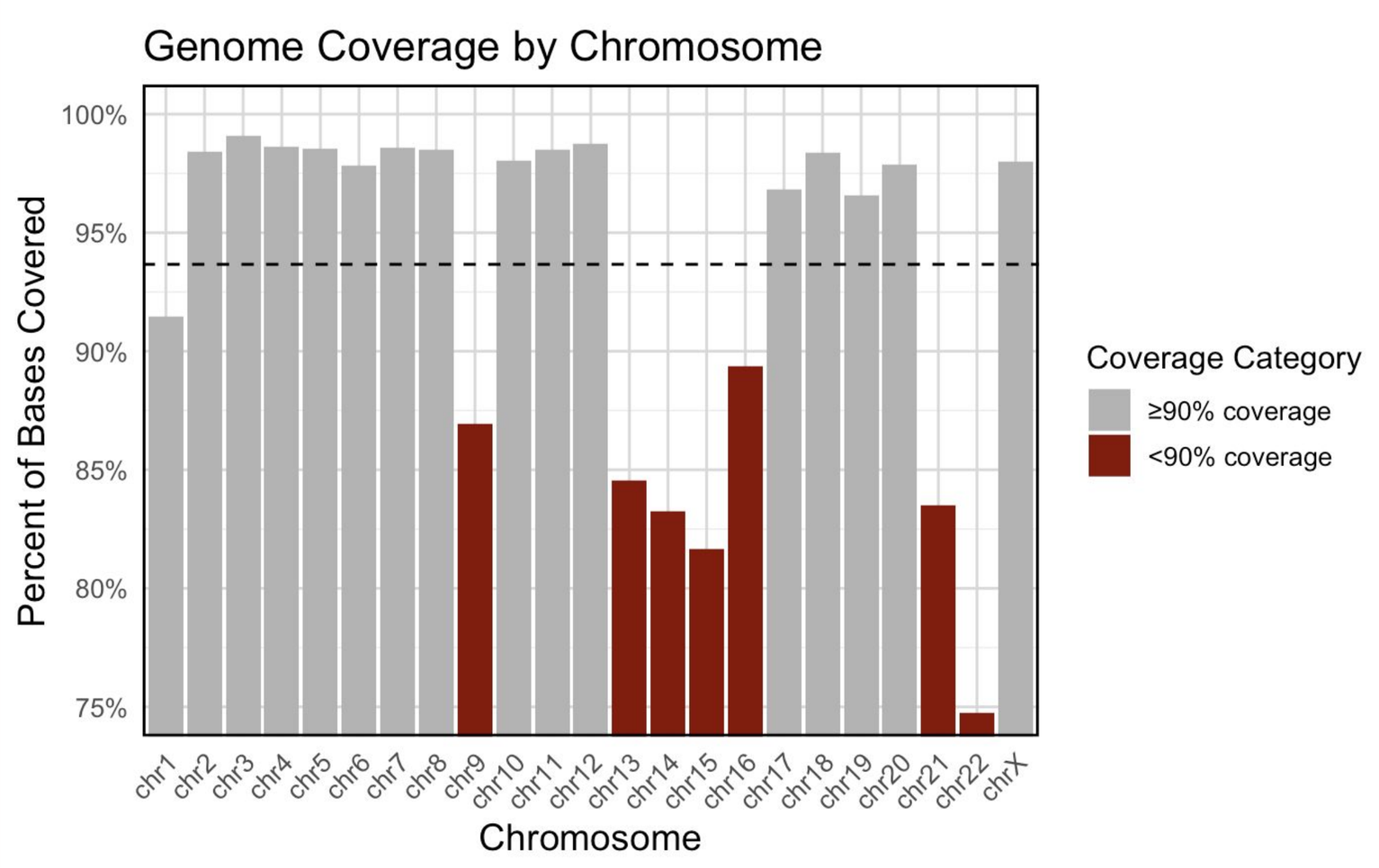
Credit: National Human Genome Research Institute

Data & Methods

Analysis pipeline:

- Raw file from lab
- Quality control
- Base quality score recalibration
- Variant Calling
- Hard filtering
- Variant annotation

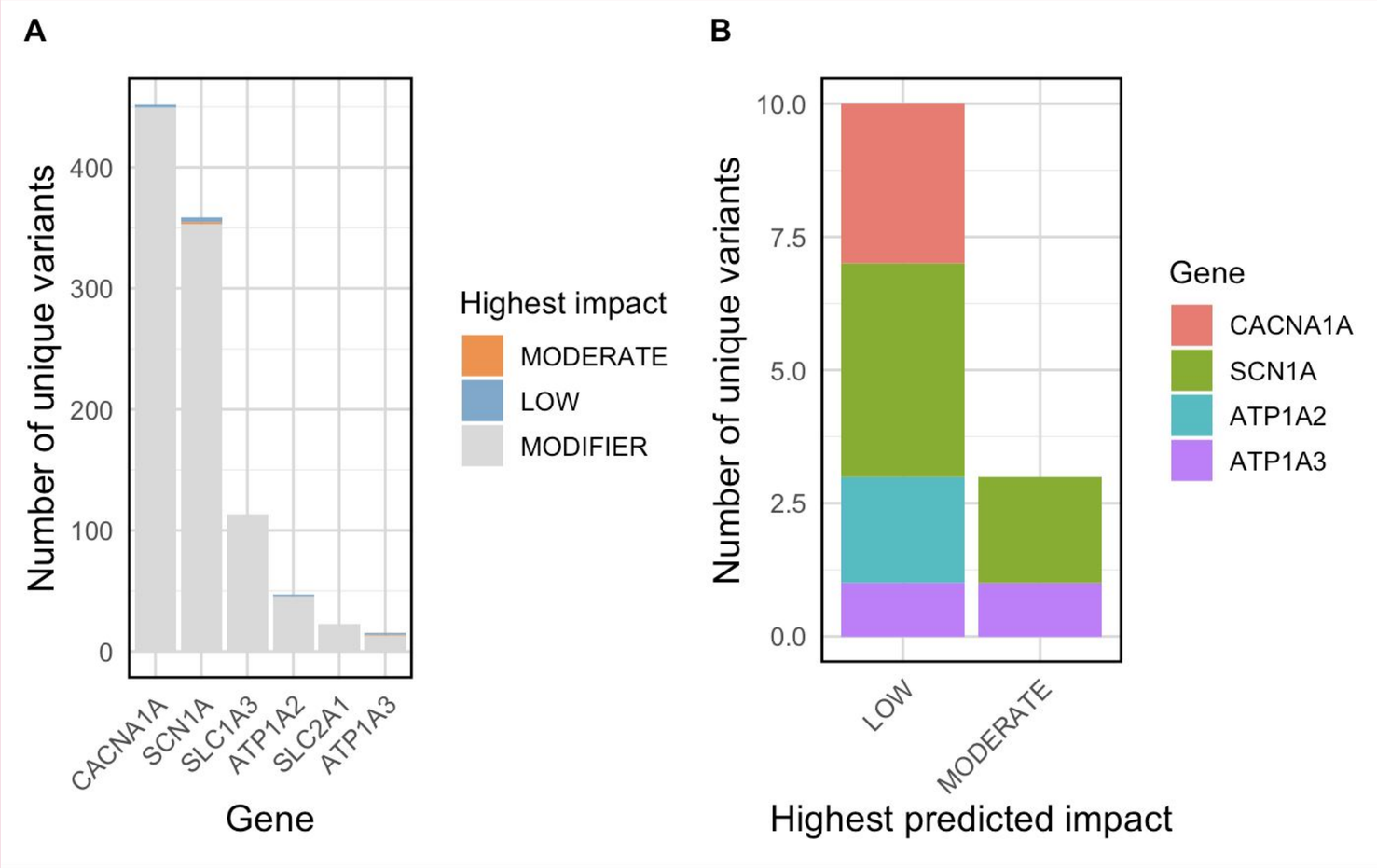
Mean Coverage: 93.67%
Mean Read Depth: 11.01
Mean Base Quality: 34.77



Initial Results

- **Known genes**
  - CACNA1A
  - ATP1A2
  - SCN1A
- **Strong evidence**
  - SLC1A3
  - SLC2A1
  - ATP1A3

Most of the mutations in known genes were modifier impact.



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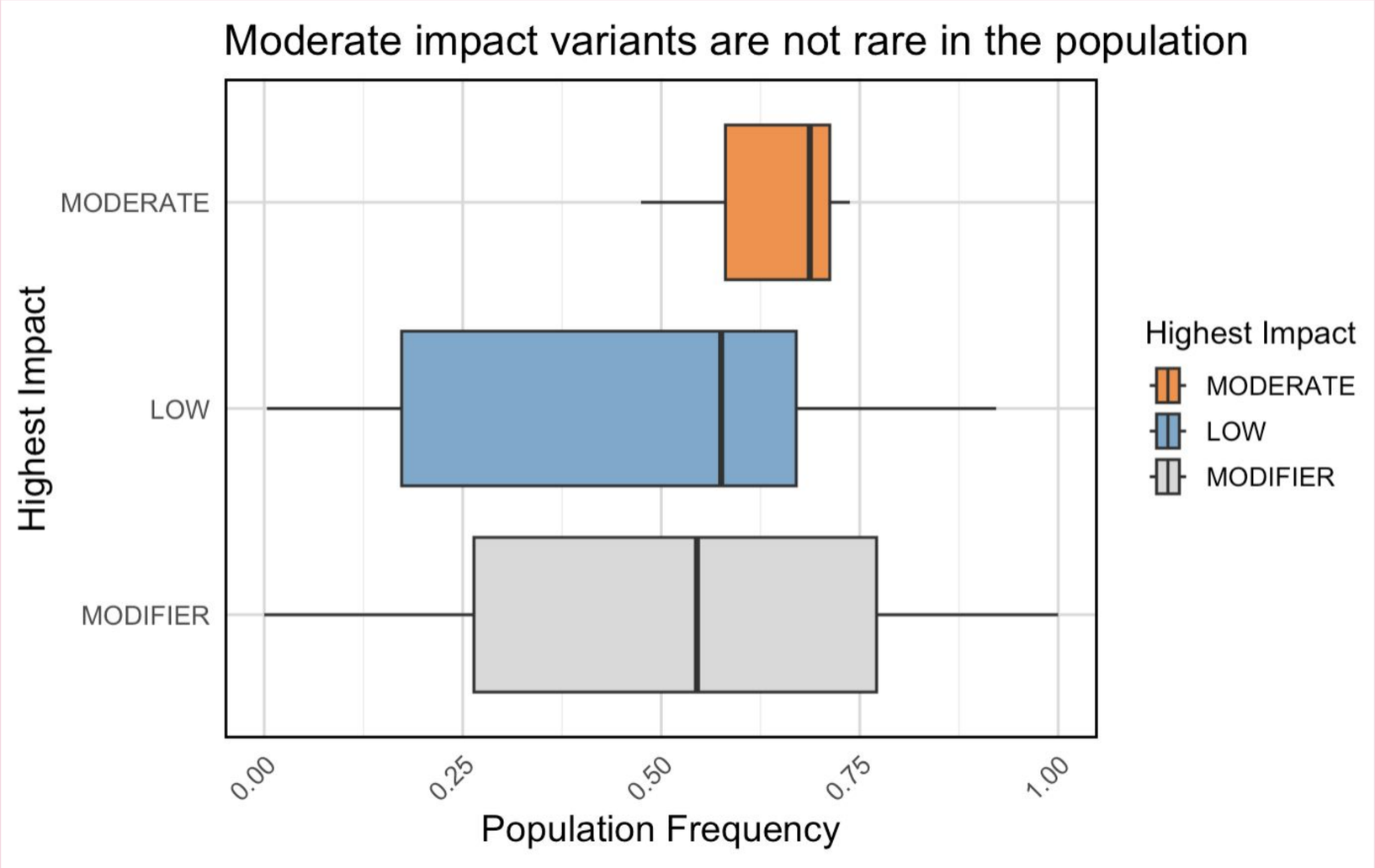

Expanded Gene Panel

Type	Name	Description	Rationale
Gene Family	CACN	Calcium voltage-gated channel subunits	Mutations in CACNA1A known to cause FHM
Gene Family	ATP	ATPases	Mutations in ATP1A2 known to cause FHM, mutations in ATP1A3 strongly believed to cause FHM
Gene Family	SCN	Sodium voltage-gated channels	Mutations in SCN1A known to cause FHM
Gene Family	SLC	Solute carrier families	Mutations in SLC1A3 and SLC2A1 strongly believed to cause FHM
Gene Family	PRRT	Dispansins	Mutations in PRRT2 strongly believed to cause FHM
Gene Family	KCN	Potassium channels	Mutations in KCNK18 associated with autosomal dominant migraine with aura, mutations in KCNC1, KCNT1, KCNQ2, KCNQ3 associated with epilepsy
Gene	PNKD	Myofibrillogenesis Regulator 1	Two families with FHM had mutations in this gene
Gene	CSNK1D	Casein kinase 1 delta	Associated with FASP, a sleep disorder linked to migraines
Gene	ALPK1	Alpha kinase 1	Associated with ROASH syndrome, which causes migraine headaches
Gene	TREX1	Three prime repair exonuclease 1	Associated with RVCL, a small vessel disease which impacts the brain
Gene	COL4A1	Collagen type IV alpha 1 chain	Mutations can cause small vessel disease in the brain
Gene	COL4A2	Collagen type IV alpha 2 chain	Mutations can cause small vessel disease in the brain
Gene	NOTCH3	Notch receptor 3	Associated with CADASIL, a hereditary disorder with migraine symptoms. Included due to strong phenotypic overlap

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Gene Family	PRRT	Dispansins	Mutations in PRRT2 strongly believed to cause FHM
Gene Family	KCN	Potassium channels	Mutations in KCNK18 associated with autosomal dominant migraine with aura, mutations in KCNC1, KCNT1, KCNQ2, KCNQ3 associated with epilepsy
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Filtering Steps

3,567,427 variants

1. Expanded gene panel
   → 424 variants
2. Read depth ≤ 10
   → 204 variants
3. Rare (population frequency ≤ 1%) and High impact
   → 10 variants
4. Manual validation
   → 9 variants
5. Biological Relevance

Final Candidate Variants

Chromosome	Position	Gene	Reference Allele	Alternate Allele	Importance
3	193289900	ATP13A5	TCA	T	High. ATP13A5 is highly expressed in CNS pericytes, which maintain the blood-brain barrier
4	42465016	ATP8A1	T	TCTATCTTTAGA	Unknown. Possible alignment artifact
4	42465019	ATP8A1	G	GTTTT	
4	42465024	ATP8A1	T	TTTTTACCTGGG	
4	42465026	ATP8A1	A	ACTAATAATTTGTCCAACTAATTTTC	
5	1240633	SLC6A18	TA	A	Low. Primarily expressed in the kidneys
13	45397266	SLC25A30	TC	T	Low. Primarily expressed in the kidneys
17	7283358	SLC2A4	GA	G	Low. Involved in glucose homeostasis
17	21703469	KCNJ18	TGC	T	Medium. Mutations known to cause periodic paralysis, but expression in the skeletal muscle doesn't match CSD biology

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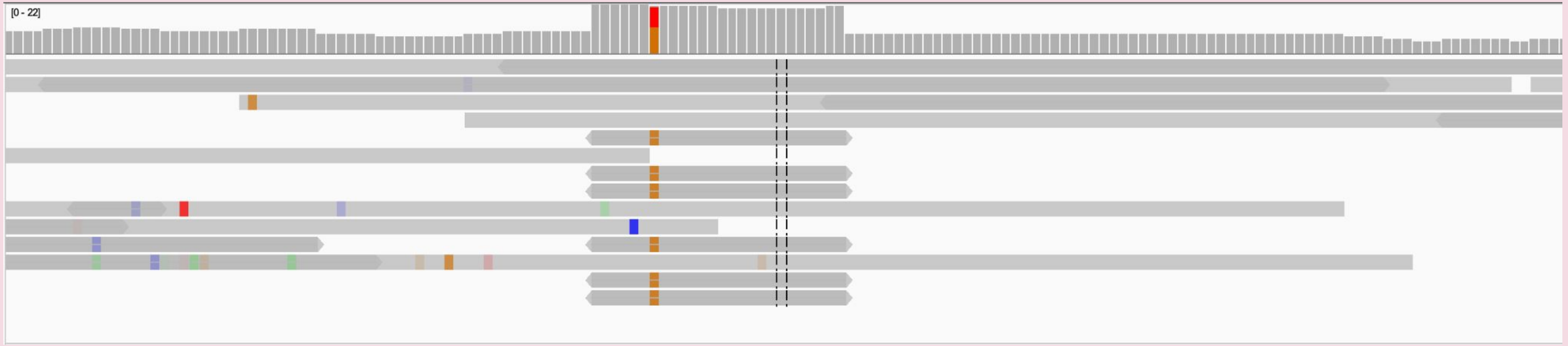
ATP8A1

- Multiple variants around 42,465,016-42,465,026
- Small insertions
- Soft clipped bases



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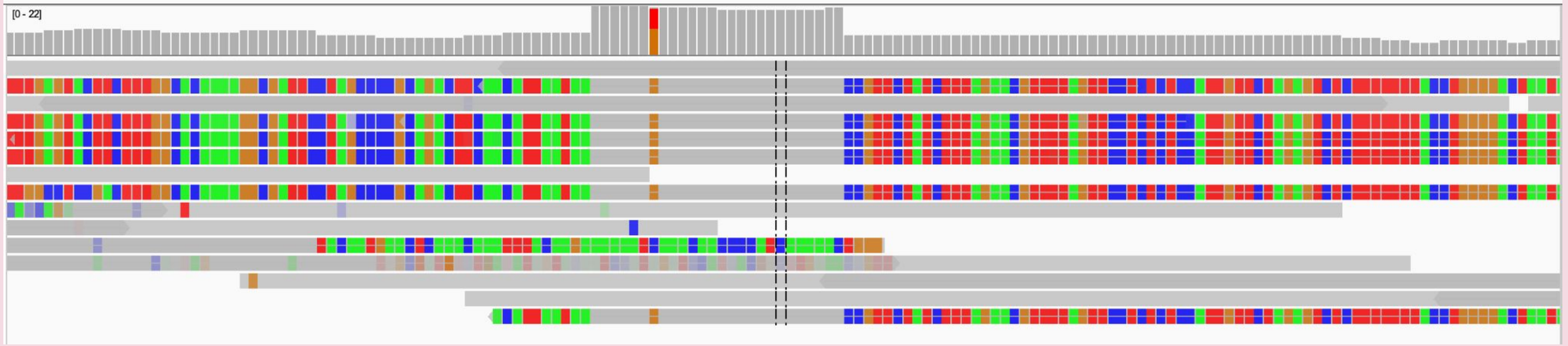

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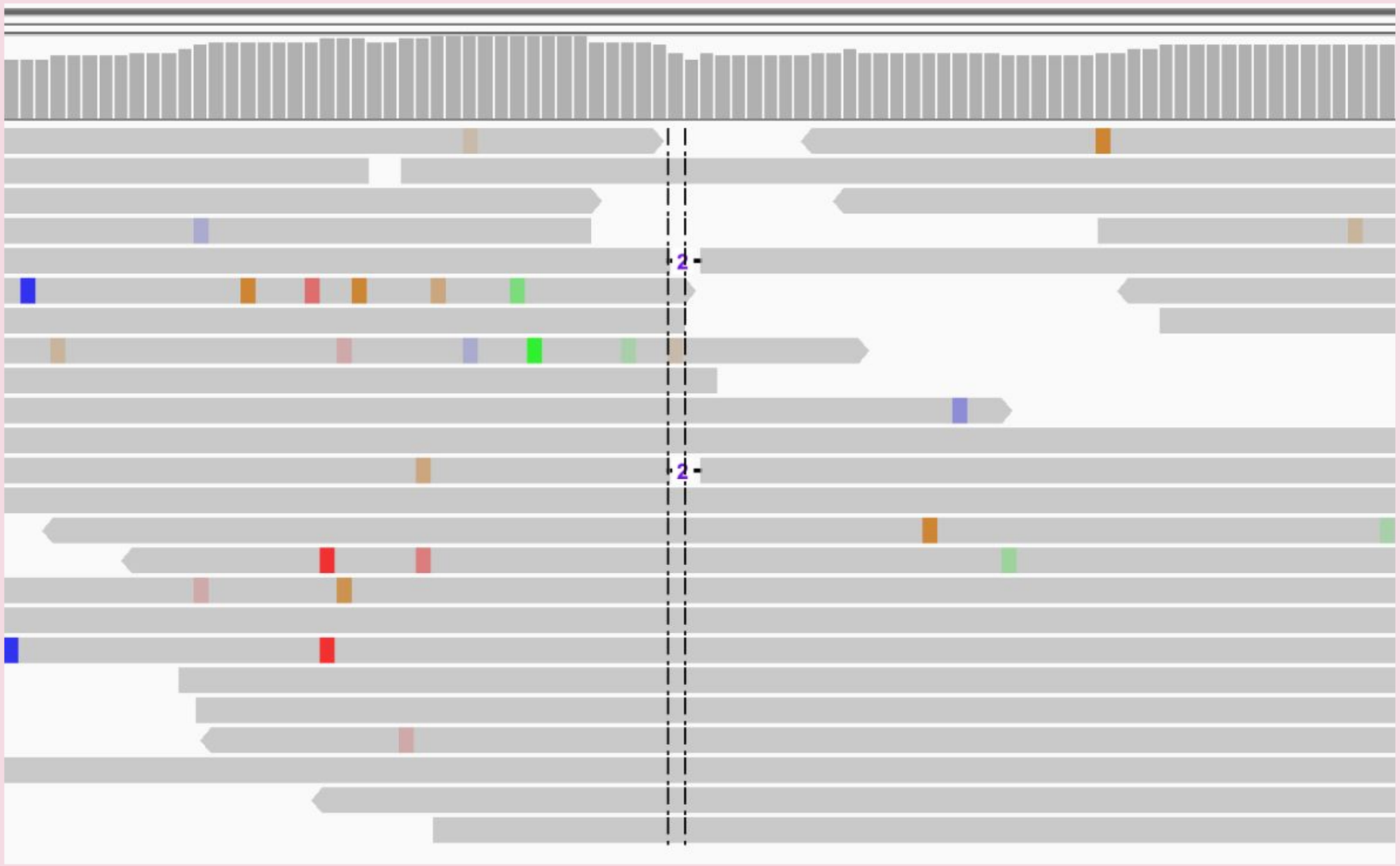
ATP13A5

- Deletion of 2 bases
- Low read support



Limitations and Future Directions

- Data quality
- Single subject
- Limited scope
- Functional effects

Conclusion

- No significant variants in known genes
- 3.5 million → 9
- ATP8A1 + ATP13A5 in CNS
- Sequencing evidence weak

Acknowledgements

- Advisor for my major
- Individual major program
- Professors and mentors
- Family and friends
- Open source tools

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Questions?