

# Hidden Diagnoses in Mitochondrial Disease: Under-detection of *ATAD3A*–*ATAD3C* Duplications in Clinical Genome Sequencing

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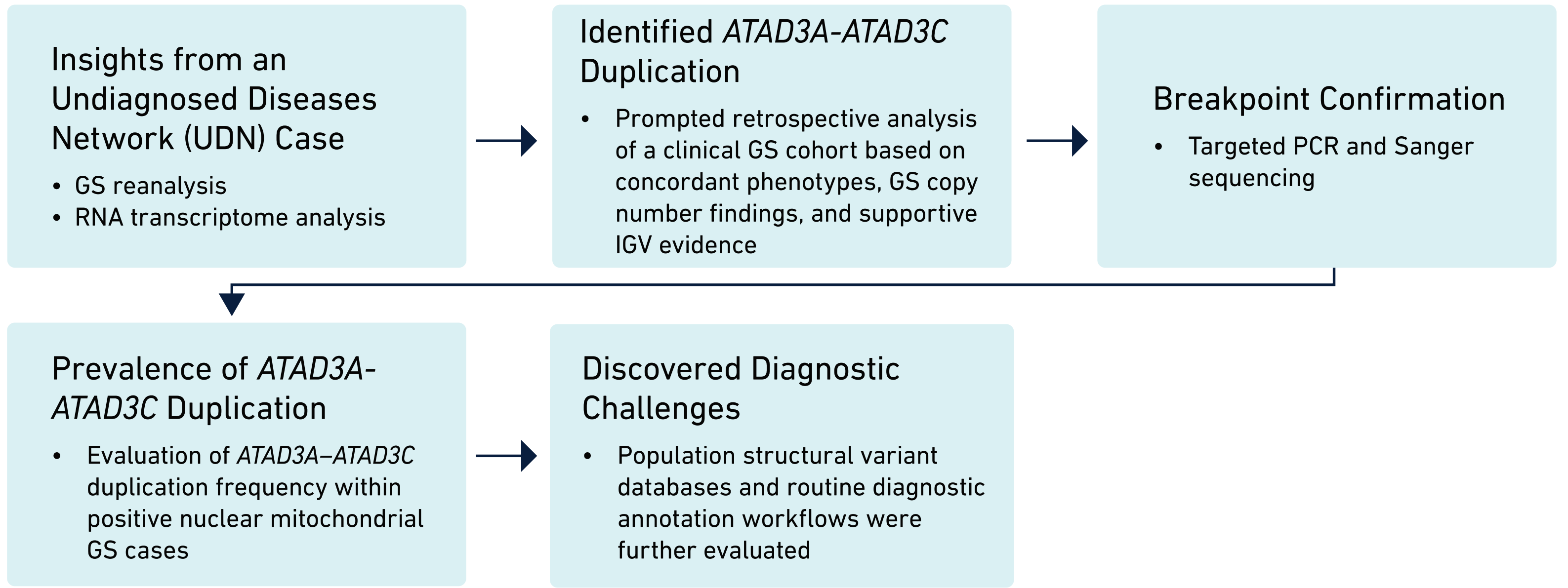
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## Background

- Recurrent *ATAD3* locus duplications are an emerging cause of severe neonatal and pediatric mitochondrial disease.<sup>1,2</sup>
- ATAD3A*–*ATAD3C* duplications are associated with a severe neonatal phenotype, including hypertrophic cardiomyopathy, persistent hyperlactatemia, corneal clouding/cataracts, encephalopathy, and early neonatal death.<sup>1,2,3</sup>
- The pathogenic mechanism is dominant-negative, as >91% of *ATAD3* hexamers incorporate at least one dysfunctional fusion monomer despite monoallelic inheritance.<sup>1</sup>
- Detection is challenging due to high homology among *ATAD3A*, *ATAD3B*, and *ATAD3C*.
- This complexity can lead to misinterpretation of copy number variants (CNVs) in routine genome sequencing (GS). As a result, affected individuals may remain undiagnosed despite clinical GS.
- Study objective:
  - Evaluate the frequency of *ATAD3A*–*ATAD3C* duplications in a clinical cohort
  - Identify factors contributing to under-recognition in diagnostic pipelines

## Methods



## Results

### Identification of Highly Identical Shared Sequence Blocks

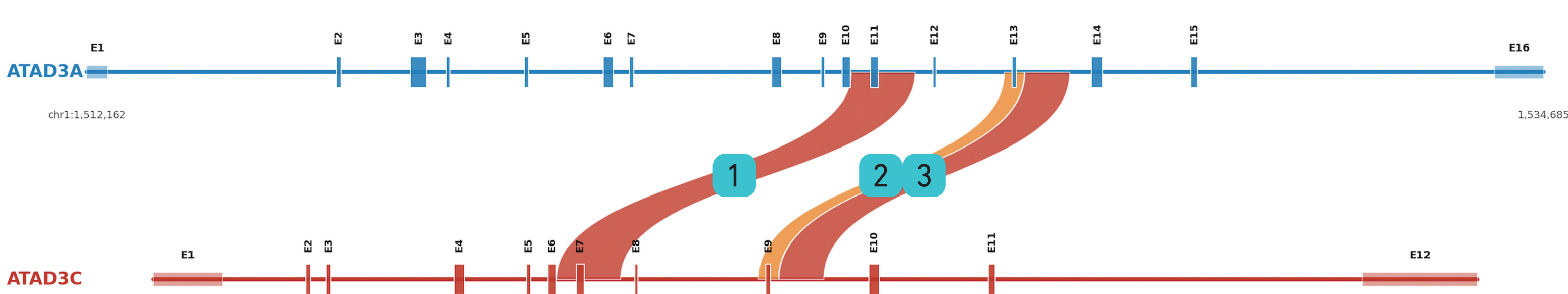


**Figure 1.** Genomic ribbon map showing three shared sequence blocks between *ATAD3C* (bottom) and *ATAD3A* (top) aligned to a common coordinate scale.

|         | <i>ATAD3C</i> (hg38)                                  | <i>ATAD3A</i> (hg38)                                   | Length (bp) | Identity |
|---------|-------------------------------------------------------|--------------------------------------------------------|-------------|----------|
| Block 1 | chr1:1,455,930–1,456,914<br><i>ATAD3C</i> intron 6–7  | chr1:1,523,978–1,524,962<br><i>ATAD3A</i> intron 10–11 | 984 bp      | 99.49%   |
| Block 2 | chr1:1,459,046–1,459,353<br><i>ATAD3C</i> intron 9    | chr1:1,526,346–1,526,653<br><i>ATAD3A</i> intron 13    | 307 bp      | 99.67%   |
| Block 3 | chr1:1,459,364–1,460,057<br><i>ATAD3C</i> intron 9–10 | chr1:1,526,664–1,527,357<br><i>ATAD3A</i> intron 13–14 | 693 bp      | 98.41%   |

**Table 1.** Genomic coordinates, length, and percentage nucleotide identity of shared sequence blocks identified between *ATAD3C* and *ATAD3A* (hg38).

### *ATAD3A*–*ATAD3C* Duplication in the GS Cohort and Breakpoint Analysis

A retrospective review of our laboratory GS cohort identified one previously reported *ATAD3A*–*ATAD3C* duplication case and three additional suspected cases with concordant phenotypes and supportive IGV findings. Breakpoint characterization and confirmation were subsequently performed using Sanger sequencing.

|              | Duplication                                                                 | Confirmed by Sanger | Inheritance Pattern |
|--------------|-----------------------------------------------------------------------------|---------------------|---------------------|
| Case 1 (UDN) | chr1:1456616_1524660dup ( <i>ATAD3A</i> intron 11:: <i>ATAD3C</i> intron 7) | Yes                 | De novo             |
| Case 2       | chr1:1456616_1524660dup ( <i>ATAD3A</i> intron 11:: <i>ATAD3C</i> intron 7) | Yes                 | De novo             |
| Case 3       | chr1:1456616_1524660dup ( <i>ATAD3A</i> intron 11:: <i>ATAD3C</i> intron 7) | Yes                 | De novo             |
| Case 4       | chr1:1456616_1524660dup ( <i>ATAD3A</i> intron 11:: <i>ATAD3C</i> intron 7) | Yes                 | De novo             |
| Case 5       | chr1:1460744_1526509dup ( <i>ATAD3A</i> exon 13:: <i>ATAD3C</i> intron 9)   | Yes                 | Unknown             |

**Table 2.** Characterization of recurrent and novel *ATAD3A*–*ATAD3C* duplications. Breakpoint coordinates are based on hg38.

### Phenotype-guided Interrogation of the *ATAD3* Locus in a UDN Family

- An infant presented with:**
- Severe, unexplained multisystem phenotype
- Key clinical features:**
- Cardiac: Hypertrophic cardiomyopathy with decreased biventricular function
  - Neurologic: Hypotonia; diffuse cerebral dysfunction
  - Sensory: Congenital cataracts; bilateral hearing loss
  - Respiratory/Feeding: Respiratory issues; swallowing difficulties
- GS:**
- Negative
- Enrolled in UDN:**
- Reanalysis of GS and analysis of RNA transcriptome sequencing. Phenotype-driven prioritization and targeted inspection of the *ATAD3* locus.

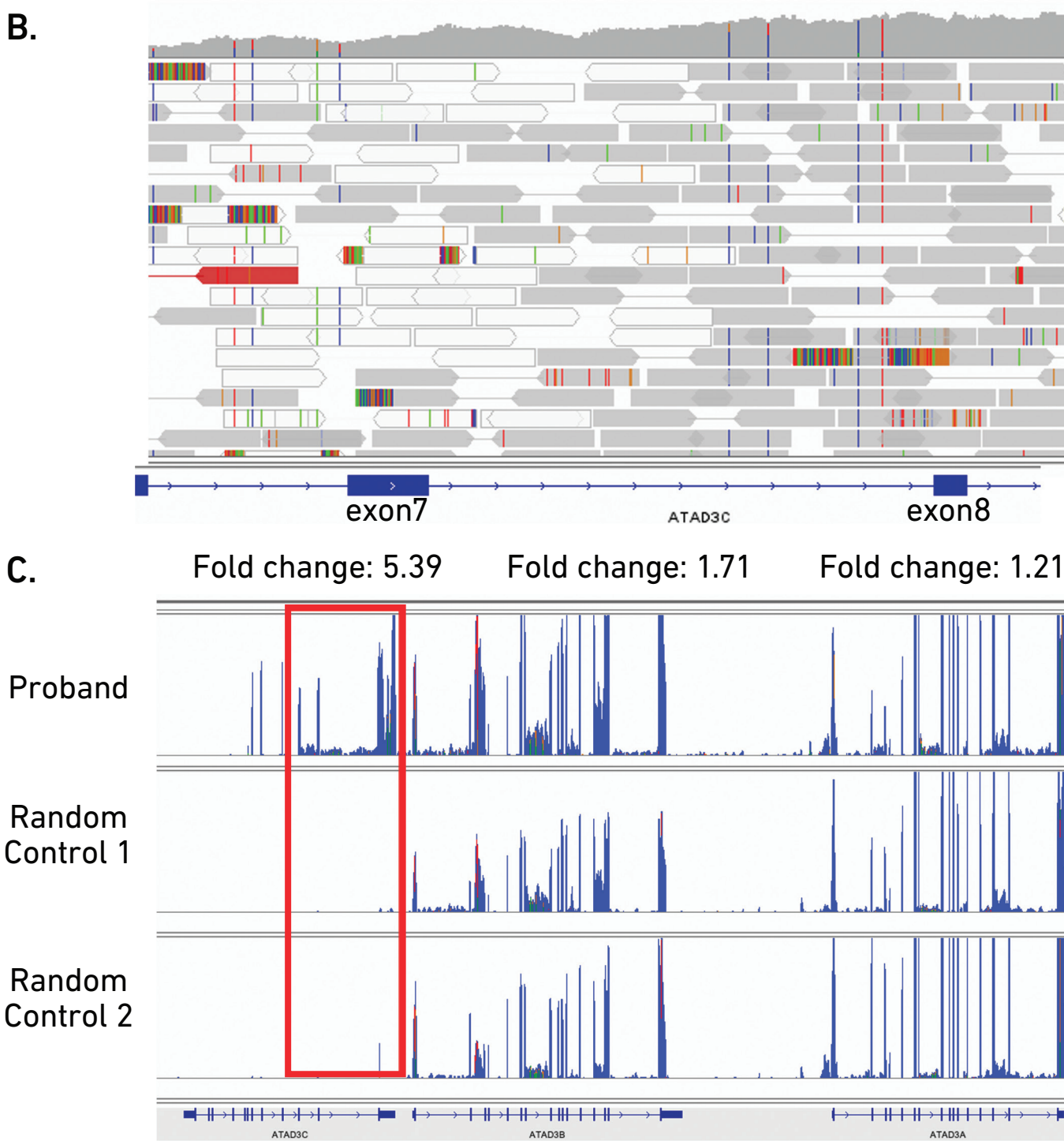
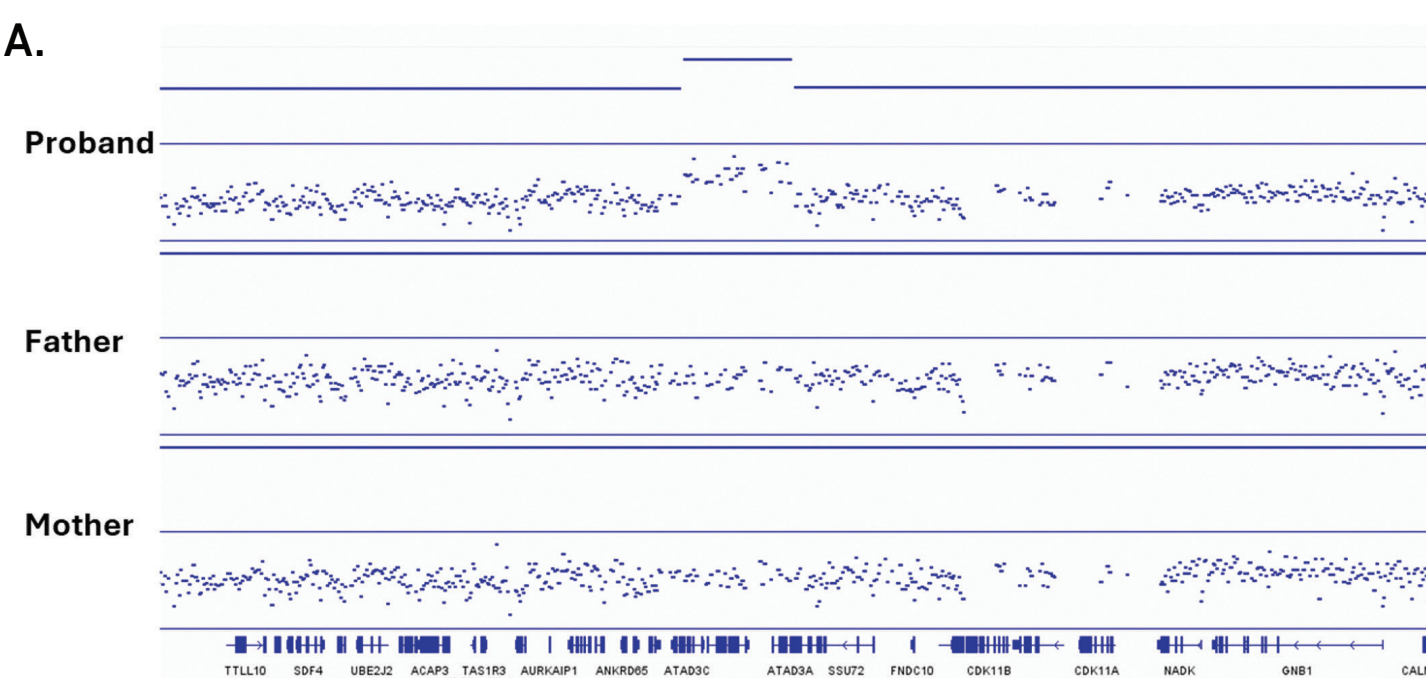


**Figure 2.** (A) GS-based normalized log<sub>2</sub> coverage plot of the *ATAD3* locus in the UDN family demonstrating a de novo *ATAD3* duplication. (B) Representative higher-resolution IGV view of one boundary of the duplicated region. Clear soft-clipped reads supporting a definitive breakpoint were not identified. (C) Distinct RNA expression pattern demonstrating increased expression of *ATAD3C* (fold change = 5.39), and approximately 1.21–1.7-fold increased expression of *ATAD3B* and *ATAD3A* compared with controls.

### High Prevalence of *ATAD3* Duplications Among Positive Nuclear Mitochondrial GS Cases and the Associated Diagnostic Challenges

Among positive nuclear-encoded mitochondrial GS cases, *ATAD3A*–*ATAD3C* duplications contributed approximately 2.3% (4/175) of diagnoses in our cohort, suggesting that this locus may represent a relatively frequent cause of mitochondrial disease.

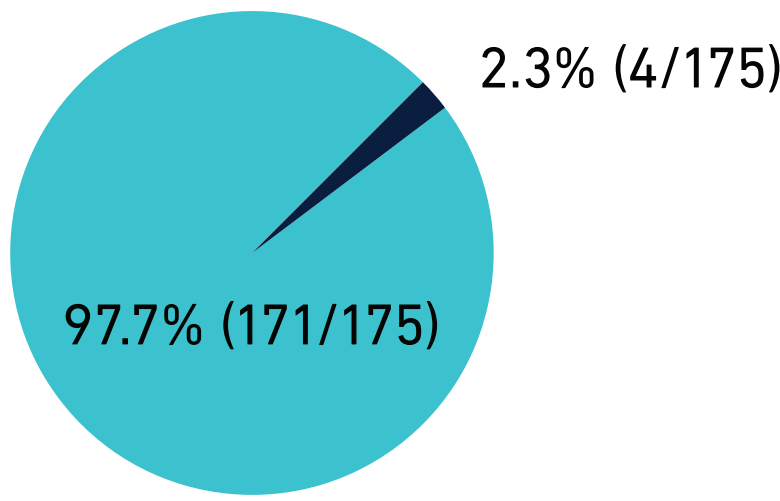


**Figure 3.** The prevalence of *ATAD3A*–*ATAD3C* duplication among nuclear mitochondrial positive cases.

Multiple copy number changes have been reported within the *ATAD3* locus, with deletions and duplications involving *ATAD3B* observed at relatively high allele frequencies in the general population. The presence of recurrent benign copy number variation within this locus may overlap with potentially pathogenic rearrangements, making it challenging to determine whether an observed duplication represents a true disease-causing event.

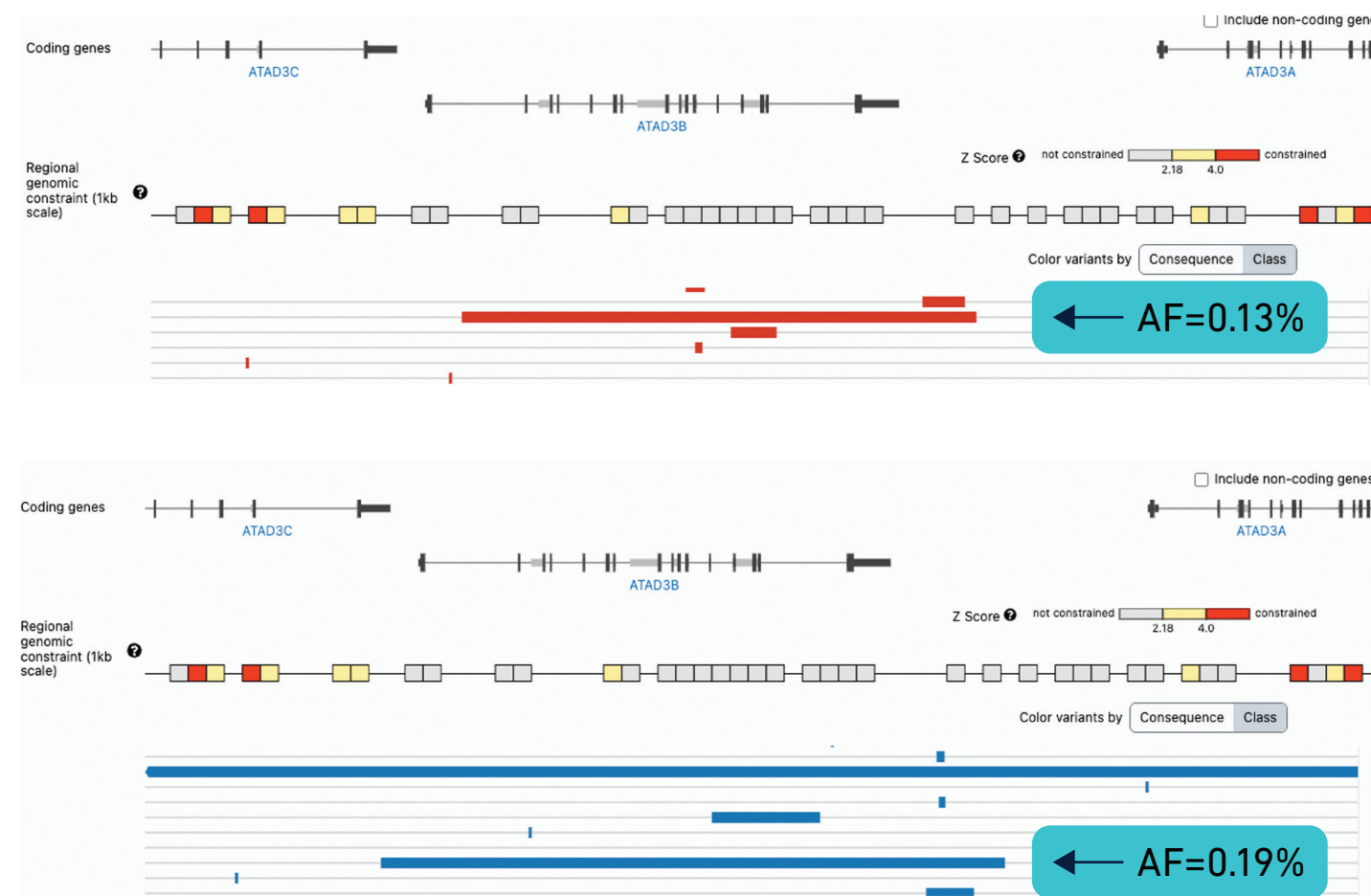


**Figure 4.** Allele frequencies of DEL\_CHR1\_AC7C0F12, a 28,390 bp deletion (red), and DUP\_CHR1\_A0368906, a 34,557 bp duplication (blue), identified in the general population from gnomAD SVs v4.1.0.

## Conclusion

- ATAD3A*–*ATAD3C* duplications are an under-recognized but clinically significant cause of pediatric mitochondrial disease.
- Phenotypic-driven manual inspection of the *ATAD3* locus enhances detection.
- Identification of these duplications and breakpoint characterization improve diagnostic accuracy and may facilitate future genotype-phenotype correlation studies.