Long-Read Genome Sequencing for Cerebellar Ataxia

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Background

Hereditary cerebellar ataxias are genetically heterogeneous disorders characterized by progressive cerebellar degeneration.[1] Despite advances in genetic testing, including repeat-expansion testing, targeted gene panels, exome sequencing, and short-read genome sequencing, many patients remain without a molecular diagnosis.[2]

Clinical genome sequencing, currently based on short-read approaches, has a limited ability to call structural variants, repeat expansions, and complex genomic rearrangements.[3] Long-read genome sequencing (LR-GS) enables improved detection of these variant classes and may consolidate multiple genetic tests into a single assay.[4]

Objectives

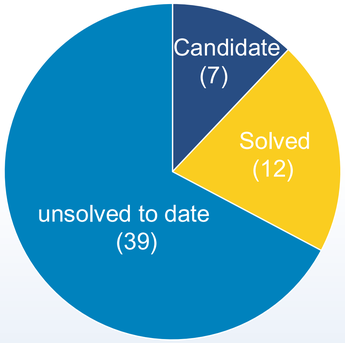
To evaluate the diagnostic yield of LR-GS in patients with cerebellar ataxia who had prior non-diagnostic clinical genetic testing, and to assess the feasibility of reanalyzing previously extracted DNA samples.

Methods

- Previously extracted DNA samples of 100 ataxia patients (probands only) with prior non-diagnostic genetic testing were obtained from a research biorepository
- 59 samples passed QC for long-read sequencing (PacBio HiFi) used for variant calling and annotation. One sample failed sequencing, leaving 58 samples for analysis.
- All 58 patients had negative ataxia repeat-expansion analysis, and a subset (n=29) had prior exome testing.

Results

Among the 58 analyzed samples, 21% yielded a diagnostic or likely diagnostic finding. Seven candidate variants were also identified. In one case, LR-GS assisted in the interpretation of two previously identified variants in *SYNE1* by establishing their phase as in trans.



Variant Information	Genes
Repeat Expansion	*FGF14, CACNA1A*
Missense	*FRMD5, KCND3, IFIH1, STUB1, AFG3L2, CHCHD10*
Frameshift	*CACNA1A*
Intronic/splice	*SACS*
Mitochondrial	*MT-TK*
Compound heterozygous (phasing)	*SYNE1*
Candidate variants	*UBR4, SAMD9L, PLD3, CACNA1G, KIF5A, ELN, GRIA3*

Why Prior Genetic Testing Did Not Detect These Variants

- Gene panels evolve over time; earlier panels may not have included genes now known to cause ataxia
- Intronic variants are generally not captured by exome sequencing, though variants near exon–intron boundaries may occasionally be detected
- Mitochondrial DNA is not routinely analyzed in many ataxia testing workflows
- Short-read sequencing cannot determine phasing of compound heterozygous variants

Conclusion

LR-GS was performed in 58% of **previously extracted DNA samples,** demonstrating the feasibility of applying long-read sequencing to archived specimens, not originally prepared as high-molecular-weight samples. Pathogenic or likely pathogenic variants were identified in 12 of 58 cases (21%) with prior negative genetic testing.

Although many variants could theoretically be detected by short-read genome sequencing, LR-GS enables comprehensive detection of multiple variant classes in a single assay and simplifies the diagnostic workflow.[2,3]

Discussion

- **Fifty-eight percent of previously extracted DNA samples were successfully sequenced and analyzed**, demonstrating the feasibility of reanalyzing archived specimens.
- LR-GS identified pathogenic variants in 12 of 58 cases (21%) with prior negative genetic testing.
- While most variants could theoretically be detected by short-read genome sequencing, LR-GS provided additional diagnostic value by enabling **phasing of *SYNE1* variants** and detecting a **mitochondrial DNA variant** not identified on prior testing.
- LR-GS can **streamline genetic diagnosis** and **improve detection of complex genomic variants**, including mitochondrial variants, in hereditary ataxia.[4]

Acknowledgements

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References

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