Elucidating the Diagnostic Yield and Allelic Characteristics of *FGF14* Repeat Expansions in Adult Ataxia Through Whole Genome Sequencing

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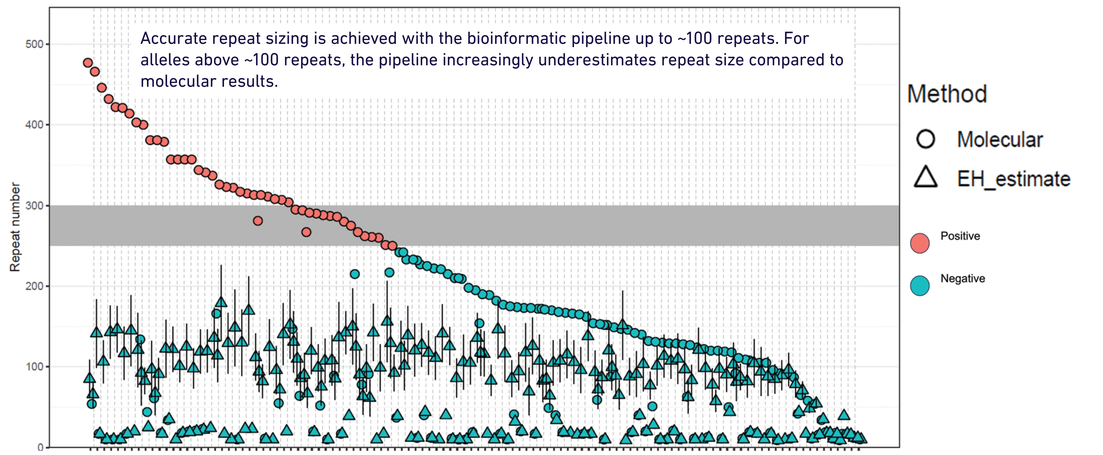
BACKGROUND

- Late-onset hereditary cerebellar ataxia (LOCA) is a group of progressive neurodegenerative disorders that remain challenging to manage without a molecular diagnosis. Even with modern next-generation sequencing, a up to 40% of adults with late-onset ataxia remain without a genetic answer.[1]
- Recent discoveries have shifted this landscape - most notably with identification of the intronic GAA expansion in *FGF14* as a cause of late-onset spinocerebellar ataxia 27B (SCA27B), which cohort studies now report as a common genetic cause and range from 10-60% among LOCA referrals.[2,3]
- Due to the size of the expansion, unbiased screening for *FGF14* GAA expansion has technical limitations. We present molecular findings from a clinical whole genome sequencing (WGS) cohort to further characterize the allelic architecture and phenotypic correlation of this genetic cause of adult ataxia.

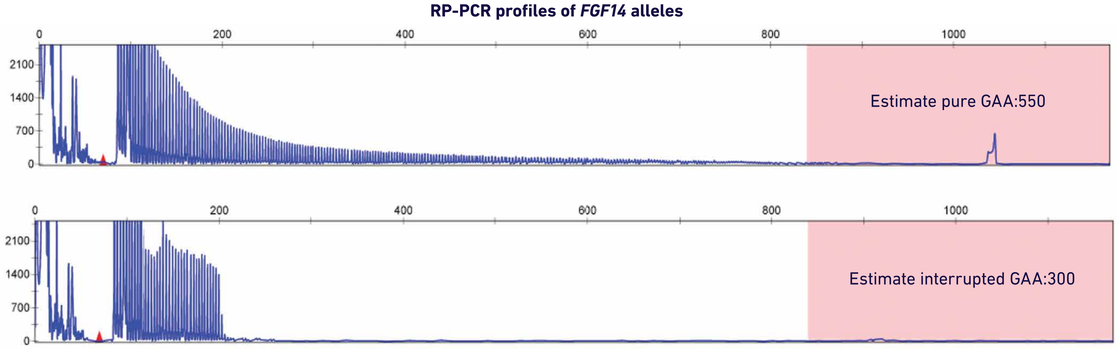
METHODS

- We screened *FGF14* GAA locus in individuals with progressive neurodegenerative condition(s) who were referred for clinical WGS with SR-WGS in 141 individuals.
- Short tandem repeat (STR) calling was performed on WGS using 150 base pair paired-end reads at 40X read coverage on average per genome.
- Repeat-primed PCR and gel sizing were performed to characterize the repeats.

Correlation between SR-WGS estimated GAA repeat number and molecular sizing (N=112)



RP-PCR profiles of *FGF14* alleles

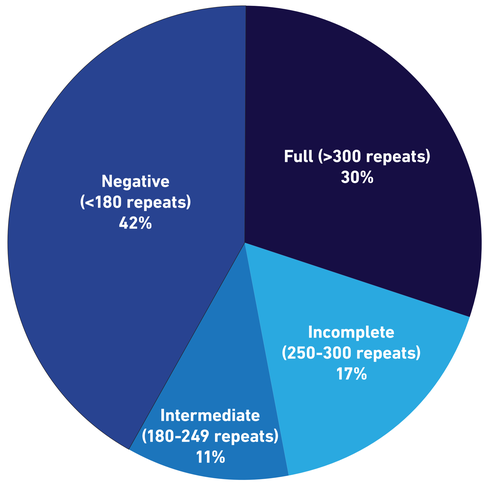


The upper panel shows a pure GAA expansion (~550 repeats), characterized by a regular stutter pattern. The lower panel shows a suspected interrupted allele (~300 repeats), indicated by loss of the regular stutter signal. The red line denotes the cutoff of 250 repeats.*

*The PCR will include unique sequences that extend beyond the repeat track; for 250 repeats, the length will be over 750 + ~100bp.

RESULTS

46.8% (66/141) cases had a *FGF14* GAA repeat expansion

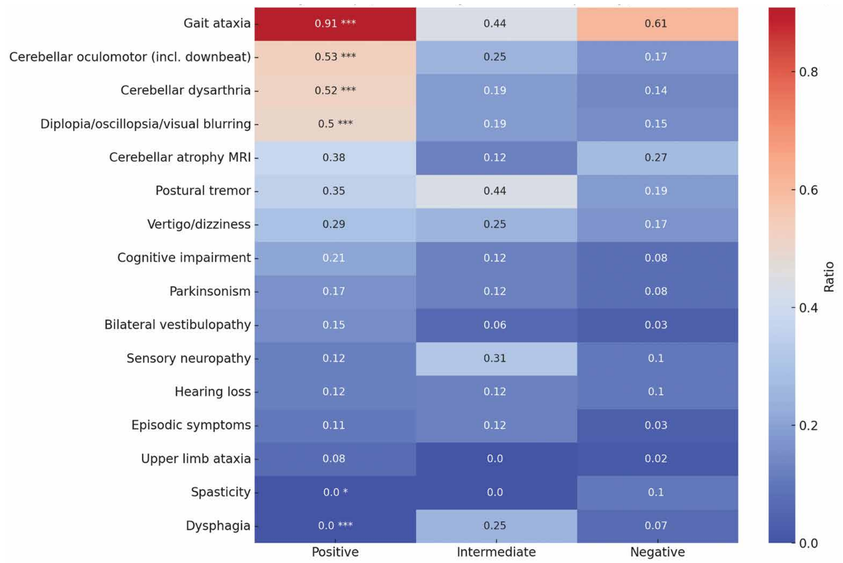


Cases with a positive molecular diagnosis (>250 repeats)	
Age of onset (range[n] in yrs)	23-81 (53)^
Age at time of testing (range[n] in yrs)	4-90 (66)*
Gender	Male (28):Female (38)
Repeat size	250-550 (66)

^ Number of cases with the information available

* Cases with a positive molecular diagnosis of >250 repeats but with first age of test <30 years(4-25) were not reported positive due to lack of literature support but were included in the table.

Feature Ratios by Group (Ordered by Positive Frequency, Oculomotor Combined)



Clinical features such as gait ataxia, cerebellar oculomotor dysfunction, dysarthria, diplopia, and oscillopsia/visual blurring were significantly more prevalent among individuals with a positive *FGF14* GAA repeat expansion compared to those without the expansion.

* p<0.05, ** p<0.01, *** p<0.001

CONCLUSION

We applied a strategy to detect *FGF14* repeat expansions in patients referred for WGS. Our analysis demonstrated that *FGF14* expansions account for a substantial proportion (~46.8%) of late-onset cerebellar ataxia (LOCA). Incorporating *FGF14* repeat expansion analysis into WGS pipelines can markedly improve the diagnostic yield. Further methodological development to resolve motif structure and interruptions will enable more accurate characterization and reporting of these variants.

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References:
1) Spinocerebellar ataxia 27B: A novel, frequent and potentially treatable ataxia. PMID 38279833.
2) Deep intronic FGF14 GAA repeat expansion in late-onset cerebellar ataxia. PMID 36516086.
3) An update on the adult-onset hereditary cerebellar ataxias: Novel genetic causes and new diagnostic approaches. PMID38760634.