A Rare Single Nucleotide Variant Causing a False-Negative *HTT* CAG Repeat Expansion Result in the Evaluation of a Patient for Huntington Disease

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Introduction

- Huntington Disease (HD) is an autosomal dominant, neurodegenerative disease resulting from the expansion of a CAG trinucleotide repeat tract in exon 1 of *HTT*.
- The length of the normal CAG repeat is ≤ 26, while symptomatic individuals usually have repeat lengths of ≥36 (Table 1).
- Repeat-primed PCR is the most commonly used method to test for CAG expansions in HD.
- We present the case of a 58 year-old man with chorea, gait instability, dysarthria, and bilateral caudate atrophy on MRI. Testing for *HTT* CAG repeat expansion at an outside laboratory showed homozygous, non-pathogenic allele size of 15 repeats (15/15).
- The patient's father had similar clinical symptoms and *HTT* test results (17/17), and autopsy findings were consistent with HD.
- Given high clinical suspicion of HD and the patient's family history, *HTT* testing was repeated at our institution with a different assay. Brain tissue from the patient's deceased father was also tested at our institution.

Table 1. Mutation category and phenotype based on CAG repeat length in the *HTT* gene

CAG length	Result category	Phenotype
≤26	Normal	Normal
27-35	Intermediate	Normal, but unstable transmission to offspring
36-39	Reduced penetrance (RP)	Variable phenotype: normal to HD
≥40	Expanded	Fully penetrant HD

Materials and methods

- The number of CAG repeats in exon 1 of *HTT* was evaluated by the AmplideX® PCR/CE *HTT* kit (Asuragen, Inc.) using genomic DNA isolated from blood and postmortem brain tissue.[1]
- A two-primer, anchor-primed PCR was performed (Fig. 1A), and the products were analyzed by capillary electrophoresis (ABI 3500xL) (Fig. 1B).
- Peaks were compared to the ROX 1000 size standard and converted from size in base pairs to number of CAG repeats using the AmplideX® PCR/CE *HTT* Macro. True alleles were distinguished as the highest fragment peaks.
- In addition, sizing PCR was performed and the PCR products were separated by agarose gel electrophoresis. The larger amplicons were excised, purified, and analyzed by Sanger sequencing (Fig. 1C, red arrow).
- Identified sequencing variants were aligned to the Human reference genome GRCh38 and interrogated using publicly available databases (gnomAD and ClinVar).
- A literature review was conducted to identify other cases of false negative *HTT* testing.

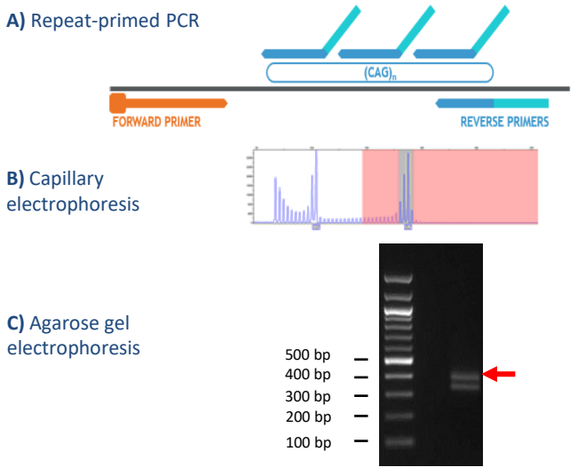

Figure 1. *HTT* two-primer PCR assay configuration (A), capillary electrophoresis (B), and PCR amplification for Sanger sequencing (C)

Results

- AmplideX® PCR/CE *HTT* revealed a heterozygous *HTT* genotype in the reduced penetrance range for both the patient (15/38) and the father (17/37) (Figure 2).
- Sanger sequencing of the larger amplicons (corresponding to the 37 and 38 repeat alleles for the patient and father, respectively) identified a C to G single nucleotide variant (SNV) in the expanded allele immediately upstream of the first CAG repeat in both the patient and father (*HTT* c.51C>G, p.Phe17Leu) (Figure 3).
- No other variants were identified.
- This variant is not reported in publicly available databases (gnomAD or ClinVar) and has been reported only once in the English literature in the context of a false-negative result with apparent homozygous allele sizing.[2]

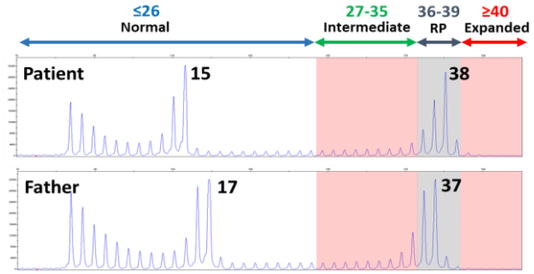

Figure 2. Electropherograms showing a heterozygous *HTT* genotype for the patient (15/38) and father (17/37)

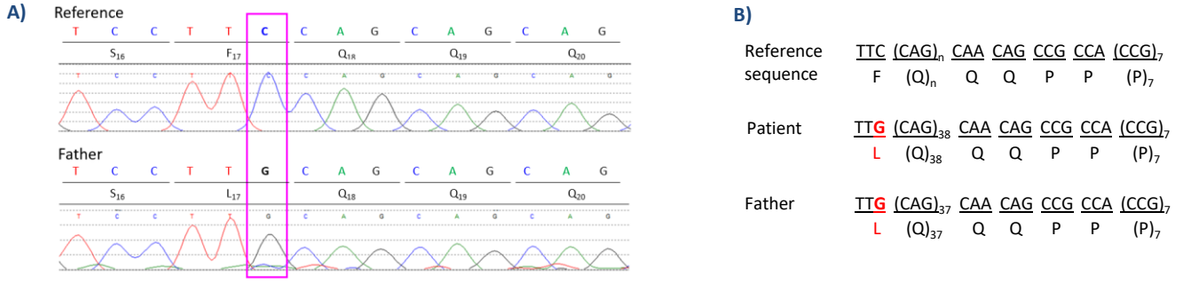

Figure 3. Sanger sequencing demonstrates a SNV (*HTT* c.51C>G, p.Phe17Leu) on the allele of 37 repeats (father) as shown in the pink box (A). The same SNV was also confirmed by Sanger sequencing on the patient's allele of 38 repeats (B).

Conclusions

- We present a case of a patient with a high clinical suspicion of HD and initially normal *HTT* CAG testing. Repeat testing identified a repeat allele which explained the patient's symptoms.
- The failure of the first assay to amplify the expanded repeat allele is likely explained by the SNV interfering with primer annealing resulting in amplification of only the normal allele.
- The same SNV was also found in the patient's father's autopsy brain tissue.
- The design of the AmplideX® PCR/CE *HTT* kit (Asuragen, Inc.) avoids common and rare polymorphisms that are known to interfere with *HTT* testing.
- While potentially rare, this case highlights the importance of orthogonal confirmation of *HTT* test results in the setting of high clinical suspicion and apparent homozygous sizing of the *HTT* CAG tract.

Acknowledgements:
We thank the Harvard Brain Tissue Resource Center for providing brain tissue from the father's autopsy for genetic analysis.

References:
1. AmplideX® PCR/CE *HTT* Kit Protocol Guide. Asuragen, Inc.
2. Margolis RL, Stine OC, Callahan C, et al. Two novel single-base-pair substitutions adjacent to the CAG repeat in the Huntington disease gene (IT15): Implications for diagnostic testing. *Am J Hum Genet.* 1999;64(1):323-6.