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Genotyping single nucleotide polymorphisms for allele-selective therapy in Huntington disease

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Abstract

Background

The huntingtin gene (*HTT*) pathogenic cytosine-adenine-guanine (CAG) repeat expansion responsible for Huntington disease (HD) is phased with single nucleotide polymorphisms (SNPs), providing targets for allele-selective treatments.

Objective

This prospective observational study defined the frequency at which rs362307 (SNP1) or rs362331 (SNP2) was found on the same allele with pathogenic CAG expansions.

Methods

Across 7 US sites, 202 individuals with HD provided blood samples that were processed centrally to determine the number and size of CAG repeats, presence and heterozygosity of SNPs, and whether SNPs were present on the mutant *HTT* allele using long-read sequencing and phasing.

Results

Heterozygosity of SNP1 and/or SNP2 was identified in 146 (72%) individuals. The 2 polymorphisms were associated only with the m*HTT* allele in 61% (95% high density interval: 55%, 67%) of individuals.

Conclusions

These results are consistent with previous reports and demonstrate the feasibility of genotyping, phasing, and targeting of *HTT* SNPs for personalized treatment of HD.

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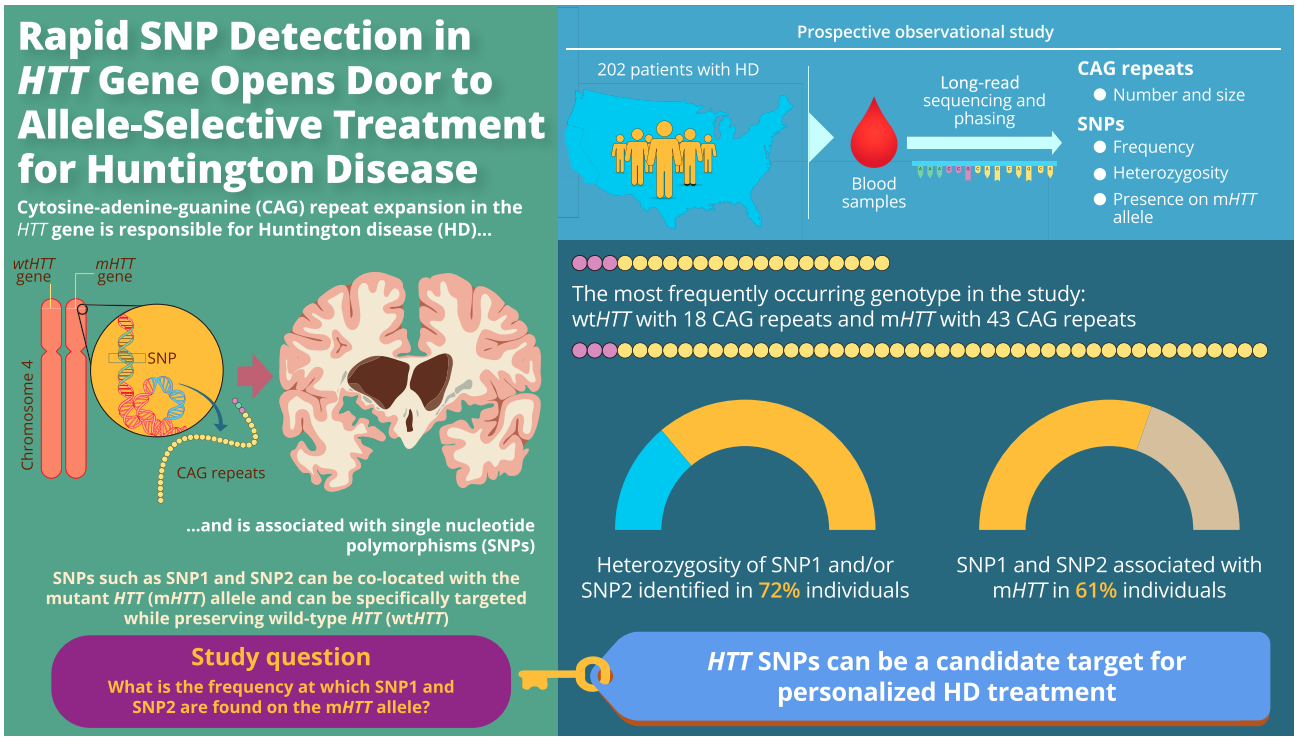
Glossary

CAG = cytosine-adenine-guanine; **HD** = Huntington disease; **m*HTT*** = mutant *HTT*; **SNP** = single nucleotide polymorphism; **wt*HTT*** = wild-type *HTT*; **UHDRS** = Unified Huntington Disease Rating Scale.



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Neurology® Genetics

Most individuals with Huntington disease (HD) are heterozygous for the cytosine-adenine-guanine (CAG) repeat, having one wild-type (wt*HTT*) and one abnormally expanded mutant huntingtin gene (*mHTT*) allele.[1] Allele-selective targeting of the mHTT transcript offers a personalized approach to HD treatment[1] and has the potential advantage of keeping wtHTT protein relatively intact. It has been suggested that wtHTT protein is required for normal neurologic function and may be neuroprotective in the adult brain.[2–4] One approach is to target specific single nucleotide polymorphisms (SNPs) found on the *mHTT* allele. Multiple SNPs have an increased frequency in HD but do not affect diagnosis or disease course.[5,6] According to previous reports, 65%–70% of individuals with HD of European ancestry carry SNP rs362307 (SNP1), SNP rs362331 (SNP2), or both SNPs.[1] According to the Genome Aggregation Database (gnomAD.broadinstitute.org), SNP1 and SNP2 frequencies vary by population and are higher in Latinos and Africans, respectively, whereas both are lower in Asian populations. For selective treatment to be feasible, an individual must be heterozygous for a target SNP and it must be colocated on the same allele or haplotype phased, with the expanded CAG repeat. In this observational study, individuals with HD were recruited from HD clinics in the United States (US), genotyped, and experimentally phased to evaluate the prevalence of SNP1 and SNP2 on the same allele as the expanded CAG repeat.

Methods

Ambulatory men and women aged 25–65 years with diagnostic motor features of HD (Unified Huntington Disease Rating Scale [UHDRS][7] Diagnostic Confidence Score of 4 and stage I or II HD with UHDRS Total Functional Capacity scores ≥7) were eligible. At one clinic visit, blood samples were collected in PAXgene Blood DNA and RNA tubes (PreAnalytiX, Switzerland) as per manufacturer's instructions and shipped frozen for processing.

Blood samples were processed at a central laboratory using 3 steps. First, the number of CAG repeats was confirmed by PCR and the size was determined using a Bioanalyzer (3500 Genetic Analyzer, Applied Biosystems, San Francisco, CA). Second, zygosity at the targeted SNP(s) was determined by Sanger sequencing. Finally, for samples with confirmed normal and expanded CAG repeats and SNP heterozygosity at either SNP1 or SNP2, a PacBio (Menlo Park, CA) long-read sequencing investigational assay determined the haplotype phase of the SNP with the CAG expansion.

Demographic information was descriptively summarized. The frequency of SNP1 or SNP2 T variant on *mHTT* was determined as the posterior chain product probability of

the frequency of heterozygosity at either SNP and the frequency of the U variant on the *mHTT* allele. The probability was calculated using a beta distribution model, which provides the mode and 95% highest density intervals. SNP prevalence was compared across sites and by sex and ethnicity.

Standard protocol approvals and data availability

This observational study was conducted in accordance with the Declaration of Helsinki with ethics committees' approval from all 7 participating US centers. Anonymized data will be shared on request from any qualified investigator.

Results

Participants

From February 2017 to September 2018, 203 individuals with HD were enrolled (table); 1 individual was excluded for older age.

SNP heterozygosity and phasing

Nearly three-quarters of individuals (146/202; 72%) were heterozygous for SNP1 only (n = 52), SNP2 only (n = 46), or both SNPs (n = 48) (figure 1). Thus, approximately one-quarter of individuals (56/202; 28%) were found to have the same SNP on both alleles or did not have a SNP on either allele. Among the heterozygotes, the sequencing results to determine SNP haplotype phasing with CAG expansion were available for 128 individuals, of which 108 (84%) had SNP1 only (n = 41), SNP2 only (n = 28), or both SNPs (n = 39) present on the *mHTT* allele (figure 1). For the other 20 heterozygous individuals, target SNPs were found on the wt*HTT* allele. Two individuals had expanded CAG repeats on both alleles. Sixteen individuals had inconclusive phasing results because of failed quality control on multiple sample processing attempts (up to 4) and interpretable data could not be provided.

The most frequently occurring genotype for *HTT* was a normal allele with 18 CAG repeats and mutant allele with 43 CAG repeats (figure 2). The prevalence of SNP1 and/or SNP2 was consistent across study sites and independent of the sex of individuals. Because of the small numbers of individuals with different ethnicities, no conclusions regarding ethnicity and SNP prevalence could be made.

Overall, the frequency at which SNP1 and/or SNP2 were associated only with the m*HTT* allele in this observational cohort was 0.61 (95% high-density interval: 0.55, 0.67), based on the probability of both SNP heterozygosity and phasing on the m*HTT* allele ([146/202] × [108/128]).

Table Characteristics of study participants

Characteristics	Individuals with HD (N = 203)
Age, y, mean (SD)	49.7 (10.0)
Sex, n (%)	
Male	104 (51)
Female	99 (49)
Race, n (%)	
White	193 (95)
Black or African American	6 (3)
Native American or Alaska Native and White[a]	2 (1)
Asian	1 (0.5)
Native Hawaiian or other Pacific Islander	1 (0.5)
Ethnicity, n (%)	
Hispanic or Latino	12 (6)
Not Hispanic or Latino	191 (94)
CAG repeats, median (range)	43 (38–62)

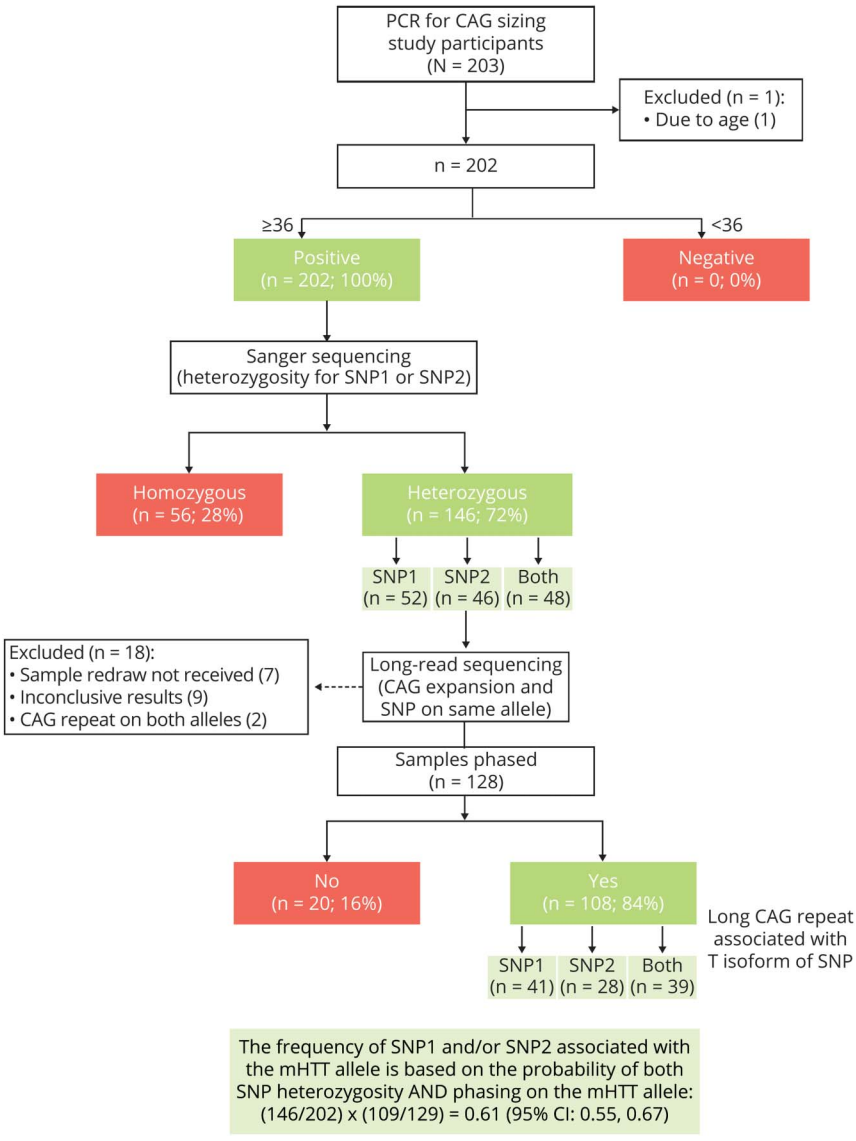
Abbreviations: CAG = cytosine-adenine-guanine; HD = Huntington disease.
[a] Two participants indicated American Indian and White.

Discussion

This is the first study to demonstrate the feasibility of rapid assessment of SNP prevalence and haplotype phasing in a relatively large number of individuals with HD (>200) using next-generation sequencing of HD transcript. Heterozygosity for SNP1, SNP2, or both SNPs was established in most individuals with HD (61%). These results suggest that clinical trials in this population are feasible and future SNP1/2 selective treatments could potentially address a significant portion of the HD population.

This study directly phased patient samples, and the results are consistent with SNP frequencies reported in previous studies using computational methods.[5,8,9] Pfister et al.[5] sequenced 24 SNPs using genomic DNA from 109 German and US individuals with HD and found an increased frequency of SNP1 (>48%) vs other SNPs. The addition of 2 SNP sites was calculated to incorporate approximately 75% of the individuals with HD tested.[5] In another study, unrelated Italian (European Caucasian) individuals with HD heterozygous for the CAG repeat (N = 327) were genotyped at 26 SNP sites, including SNP1 and SNP2.[8] Of these, 86% of individuals were heterozygous at one or more SNP loci and may be amenable to allele-selective therapy. SNP2 heterozygosity was most prevalent in this HD population (46.2%), increasing the estimated probability of heterozygosity at either SNP1 or SNP2 to 65%.[8] Using the University of British Columbia and Tissue Bank for HD Research database, direct sequencing was performed for

Figure 1 Phasing results



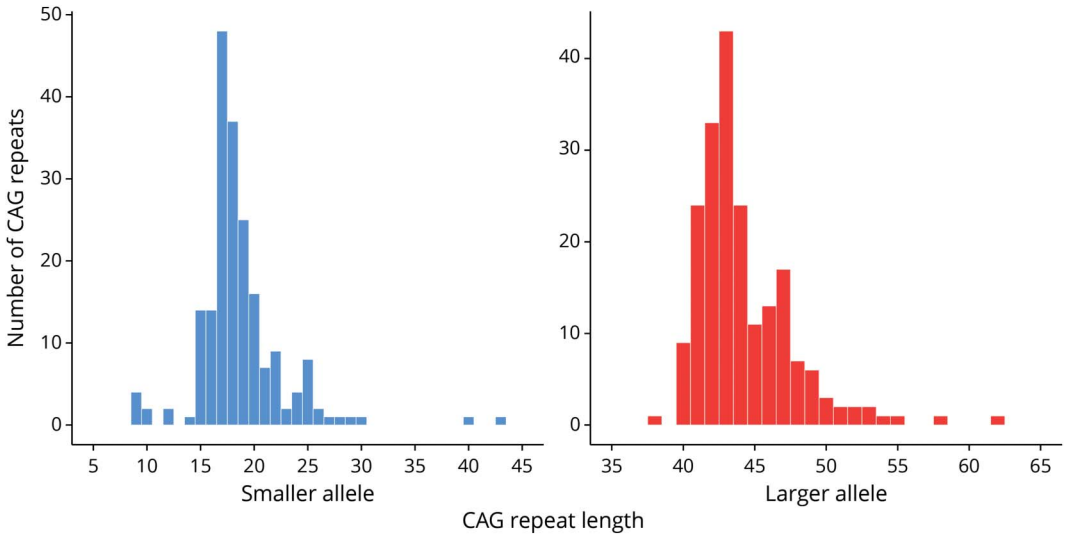
HDI = high-density interval.

Canadians of European origin (n = 65) and confirmed in a replication group (n = 203).[9] Of 190 SNPs identified, 23% were common (minor allele frequency >0.20). The maximum coverage of a single SNP was 52%, whereas targeting between 1 and 4 SNPs was theorized to cover 89% of the HD population.

The ability to prospectively define an individual's specific *HTT* SNP haplotype permits consideration of personalized allele-selective gene-silencing methods. In the PRECISION-HD trials (NCT03225833, NCT03225846), the presence of SNP1 and/or SNP2 with the CAG expansion is determined using a similar process. Based on these results, participants receive targeted therapy with WVE-120101 or WVE-120102 for SNP1 and SNP2, respectively, aiming to selectively lower *mHTT* without affecting *wtHTT*. These investigational compounds are stereopure antisense oligonucleotides (ASOs) synthesized by precisely controlling the chirality of the phosphorothioate linkages to enable selective targeting of the SNPs of interest. In general, stereopure ASOs have increased lipophilicity and stability and enhanced RNase H1 activity than comparable stereorandom ASOs.[10]

This study confirms the feasibility of rapidly detecting SNP1 and/or SNP2 in the HD population in the United States and opens the possibility of selectively targeting mHTT transcript in eligible patients. It is important that the proof of concept of this approach may lead to the identification and targeting of other SNPs in the HD population, allowing others to potentially benefit from allele-selective treatment.

Figure 2 Length of CAG repeats among population with HD



The normal allele with 18 CAG repeats and the mutant allele with 43 CAG repeats were shown to be the frequently occurring genotype for *HTT* in the patients tested. The data did not pass the normal distribution using the Shapiro-Wilk normality test.

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Disclosure

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Jody Corey-Bloom, MD	University of California San Diego	Author	Subject recruitment/ acquisition of data; analyzed and interpreted the data; revised the manuscript for intellectual content
E. Ray Dorsey, MD	University of Rochester, NY	Author	Study design; analyzed and interpreted the data; revised the manuscript for intellectual content
Mary Edmondson, MD	HD Reach, Raleigh, NC	Author	Study design; analyzed and interpreted the data; revised the manuscript for intellectual content
Sandra K. Kostyk, MD, PhD	Ohio State University, Columbus	Author	Subject recruitment/ acquisition of data; analyzed and interpreted the data; revised the manuscript for intellectual content
Mark S. LeDoux, MD, PhD	University of Memphis and Veracity Neuroscience, LLC, Memphis, TN	Author	Study design; subject recruitment/ acquisition of data; analyzed and interpreted the data; revised the manuscript for intellectual content
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Francis Walker, MD	Wake Forest University School of Medicine, Winston Salem, NC	Author	Analyzed and interpreted the data and revised the manuscript for intellectual content
Vicki Wheelock, MD	University of California Davis Health, Sacramento	Author	Subject recruitment/ acquisition of data; analyzed and interpreted the data; revised the manuscript for intellectual content
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