Genetic testing for Huntington's disease: past, present and future. How could genetic data be used to improve clinical practice?

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Abstract

The identification of the repeat expansion which causes Huntington's disease in 1993 soon led to a clinical genetic test for the condition, enabling people at risk to have a test to determine whether they will get the disease. The primary determinant of age at onset in Huntington's disease is CAG repeat length, but in recent years there have been advances in identifying and characterising genetic modifiers which influence age at onset. This has led to the question of whether these data may be applied clinically to improve clinical practice. Here, on behalf of the EHDN Genetic Testing and EHDN Genetic Modifiers Working Groups, we review the current state of genetic testing for Huntington's disease and consider the personal impact that pre-symptomatic genetic testing has on those that undertake it. We then discuss how genetic information could be used to improve onset prediction clinically, and whether it could be applied in clinical trials stratification. We conclude by proposing short, medium and long-term recommendations to improve the use of genetic data to in clinical practice and clinical trials.

Plain language summary

Genetic testing for Huntington's disease enables not only people with symptoms of the condition be tested, but also enables people with a family history of the disease and no symptoms have a genetic test to determine whether they will develop symptoms of Huntington's disease in the future, known as pre-symptomatic testing. In this article we review the current state of genetic testing for Huntington's disease and consider the personal impact that pre-symptomatic genetic testing has on those that undertake it. The onset of Huntington's disease is influenced by the length of the CAG repeat inherited, and recent advances have found that other genetic factors also influence when

symptoms develop. We discuss whether genetic information could be used to improve the information that is shared with people undergoing pre-symptomatic testing, and whether it could be applied in clinical trial design. We conclude by proposing short, medium and long-term recommendations to improve the use of genetic data to in clinical practice and clinical trials.

Keywords

Huntington's disease, Genetic testing, Presymptomatic testing, Genetic Modifiers, Clinical Trials.

Introduction

Huntington's disease (HD) is a devastating neurodegenerative condition with an estimated prevalence of 12 – 15/100,000 in Caucasian populations (1-3). There is currently no disease-modifying treatment. HD is caused by a CAG repeat expansion of at least 36 trinucleotides in the huntingtin (*HTT*) gene. HD is inherited as an autosomal dominant trait, with a single allele with an expanded CAG being sufficient to cause disease; each child of an affected parent has a 50% risk of inheriting the expanded allele. A diagnosis of HD can be devastating not only for the individual; symptoms and caring responsibilities can impact whole families, and family members also have to come to terms with being at risk themselves. HD is characterised by progressive involuntary movements, neuropsychiatric difficulties and cognitive impairment. Despite the causative mutation being inherited and present from conception, symptoms of HD typically do not manifest until middle age (30 – 60 years), although there is wide variation with onset of symptoms described at all ages from infancy to over 80 years. The greatest influence on age at onset of HD is the inherited length of the pathogenic *HTT* CAG repeat(4). At the lower end of the pathological range, penetrance of the mutation is incomplete: those with 36 – 39 CAGs might or might not develop symptoms of HD in their lifetime. For fully penetrant alleles (CAG $\geq$ 40), longer repeat expansions are associated with

earlier age at onset of symptoms and signs of HD. The CAG repeat length inherited explains ~ 50 – 70% of the variance in age at motor onset observed in the HD population, with a 1 CAG change effecting the predicted age at onset by ~3 years in the 40-50 CAG range (4-7). After accounting for inherited CAG repeat length, the remaining variation in disease onset and progression is attributable to a combination of genetic and environmental factors (8-11). For example, in recent years it has been established that the exact sequence structure of the region of the *HTT* CAG repeat plays a significant role in both penetrance and age at onset, likely accounting for some of this variability (10, 12, 13, 14). The uncertainty created by the variability of the relationship between CAG repeat length and age at onset of disease symptoms impacts genetic counselling for at risk individuals (BOX1, BOX2).

Here, we reflect on current testing and counselling practise as well as recent genetic advances, including sequencing the CAG repeat region. We consider how these could be harnessed in the important population of HD mutation carriers wo do not yet have symptoms.

Box 1: A challenging personal journey through pre-symptomatic HD testing

"The day that I was born my mother was diagnosed with Huntington's disease (HD); she was 36. She bore her illness after that for 15 years.

I had always wanted to get tested from a young age. The 'not knowing' had always felt like a dark rain cloud hanging over me, wondering if and when the downpour would come. Once I had graduated from university in 2018 with a science degree, I decided that then was the right time to find out. I wanted to know so I could plan ahead, adapt my career, and speed up my travel plans and other life aspirations.

I had two genetic counselling consultations before the test, one for the test itself and then one for the result. Despite these sessions, when my result came back as gene-positive [showing an HTT CAG repeat expansion] I felt unsupported and there was no offer of further counselling. Additionally, the information provided around CAG length, symptoms and onset of symptoms was incorrect and outdated. But I didn't find this out until 3 months later at a local HD conference. I felt that no hope was offered at the time of my test result.

However, since then the genetic test result has become a great sense of motivation for me to achieve my life goals and tick off my travel destinations sooner. It has changed the path of my life, encouraging me to speed up my journey. I am engaged in the local HD clinic and take part in research studies where I can. Beyond that, I have found support in HD conferences and HD community groups like HD Youth Organization (HDYO). Overall, I'm pleased I got tested but I know that it wouldn't be the right choice for everyone."

Box 2: An experience of genetic testing to help plan the future with greater clarity.

"I am a 39 year-old man, and found out my maternal grandmother had had an observational diagnosis of Huntington's around four years ago, not long after she had passed away. My family and I chose to have Grandma's diagnosis confirmed a couple of years later through genetic testing, where it was discovered that she had had a CAG repeat level of 39, just inside the threshold of reduced penetrance, a level that was consistent with her experience of later onset, in her 60s, and relatively mild symptoms. Given that CAG repeat levels tend to stay consistent when passed down the female line, my understanding following the diagnosis was that if I had inherited the gene, it was likely that my CAG repeats would be at a similar level, and I could therefore expect a similar experience, in terms of symptoms, as a result. Given the incurable

nature of the disease, I decided not to have myself tested until the time came for family planning decisions.

Two years later, and earlier this year, my partner and I had decided we wanted to start a family, and this is when I was tested, and found to have inherited the gene, at a CAG repeat level of 40. Whilst this wasn't a shock in terms of it being unexpected, it has had a big impact on our lives, with my partner and I now pursuing IVF, with preimplantation genetic testing, in the immediate term.

Longer term, my expectations for my symptom development remain much as they were, but I have probably thought more about what the later years of my life might look like, in the last few months, than I had in the rest of my life previously. I would welcome any testing that would give me greater clarity on what I am likely to experience in the future, as it would help me to make more informed decisions around work, money and family, than I am able to do now. After all, the desire for greater certainty around my future is why I sought testing in the first place."

Clinical scenarios in presymptomatic genetic testing

Adults who are asymptomatic but at risk of HD because they have an affected relative face the difficult choice whether or not to have genetic testing for the disease-causing mutation. The majority (> 75%) of those at risk choose not to have presymptomatic genetic testing (15). Those that do should have a series of sessions with a specialist genetic counsellor to support them through the decision-making process. These sessions explore the medical, psychological and familial implications of predictive genetic testing, and support the autonomy of the individual to make an informed

choice (16). The way in which counselling is delivered and how test results are communicated can have a lasting impact (Box 1). Those who proceed with HD genetic testing often do so because finding out their genetic status would alleviate uncertainty, enabling them to make life plans, including around reproductive decisions (17). One further benefit of undergoing predictive testing is the opportunity, if carrying an *HTT* CAG repeat expansion, to take part in clinical trials of new treatments and observational studies (although some observational studies accept people without a genetic test, this is much less common than those requiring a genetic diagnosis). Future trials of potentially disease-modifying agents are likely to recruit those expansion-carriers who are early symptomatic or even those who are pre-symptomatic.

The uncertainty surrounding age of onset is challenging for those who are found to be expansion carriers and additional complexity in testing and counselling arises when the CAG repeat length is in the reduced penetrance range (36 – 39 CAGs) perpetuating uncertainty as illustrated in Box 3.

Box 3: Complex case of genetic testing in the context of a reduced penetrance range allele

a) Clinical history

***II,**3, aged 52, presented with a three-year history of choreiform movements and memory loss. HD was clinically suspected and genetic testing undertaken confirming the diagnosis (42 CAG repeats). There was no reported family history. Their mother was alive in her 80's and their father had died from a stroke in his 60's*

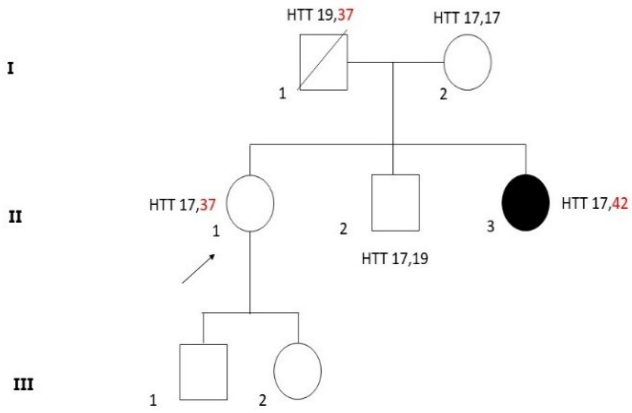
*The sister of **II,**3, **II,**1, aged 58 years, was referred for predictive testing. Her main reason for testing was to ascertain risks to her children.*

*Following genetic counselling, testing in **II,**1 demonstrated a reduced penetrance allele; 37 CAG repeats.*

*A stored DNA sample was available from their father; **I,**1 and a sample was obtained from their mother; **I,**2. Allele sizing and linked markers confirmed paternity for all children and that the 37 CAG repeat reduced penetrance allele was paternally inherited.*

***II**1's children were referred for genetic counselling.*

b) Pedigree diagram



The case presented in Box 3 highlights a number of challenges in presymptomatic counselling. Questions posed by the family included: what is the likelihood of **II**,1 developing HD and when might she be likely to develop symptoms? What is the risk to **III**,1 and **III**,2 of developing HD? What is the risk of expansion resulting in a full penetrance allele?

Current testing methods

An HD genetic test estimates the length of the inherited uninterrupted CAG trinucleotide repeat in exon 1 of the *HTT* gene: the probability of whether an individual will develop HD in a dominant fashion is dependent on the estimated size of the larger CAG allele (18) (Table 1).

***HTT* CAG repeat length**	**< 27**	**27 – 35**	**36 – 39**	**≥ 40**	**≥ 55**
Allele type	Non-disease associated	Intermediate	Variable penetrance	Full penetrance	Full penetrance
Clinical manifestation	Not associated with HD	Not considered pathogenic. May expand into disease range in future generations	Can be pathogenic and cause HD and at high risk of expanding into the fully penetrant range in future generations	Carrier will develop HD	Usually have juvenile onset HD (before age 20)

Table 1. Relationship between size of the *HTT* CAG repeat expansion and clinical outcome.

Approximately 20 HD predictive tests per million population are performed each year in the UK (15, 19). Current best practice in molecular genetic testing for HD follows guidelines from the European Molecular Genetic Quality Network (20) and American College of Medical Genetics (21, 22). Most laboratories use PCR followed by capillary electrophoresis to size repeats in bulk DNA samples obtained from thousands of blood cells in a standard venous blood draw; several sets of primers and conditions have been published, (23-27).

INSERT FIGURE 1 AROUND HERE (separate powerpoint file provided)

A widely used protocol for clinical CAG repeat length determination, particularly for the identification of very large expansions, and when confirming 'homozygous normal' genotypes, is the triplet primed PCR (RP-PCR) approach developed by Warner *et al* (Figure 1) (28). In this approach the

3’-end of the HD3 primer binds the CAG repeat tract at different points and can form PCR amplification products with the HD1 primer that binds outside the tract. The result is a ladder of PCR products, each separated by 1 CAG unit, from 5 CAGs (the minimum tract bound by HD3) up to and including the ‘tether’ product that represents the longest CAG repeat present and is amplified by full binding of HD3 both inside and outside the CAG tract. The PCR products can be separated by capillary electrophoresis and peaks counted until the ‘tether’ product is reached (Figure 1). Compared to standard PCR protocols in which the anomalous migration of CAG repeats can make conventional ladders unreliable and validation against material of known size essential, the tethered repeat primed-PCR method has the advantage of direct sizing and when optimised can provide a robust estimate of pure CAG length (20, 26). However, techniques based on PCR and capillary electrophoresis have drawbacks. For example, sizing the pure CAG repeat from estimated fragment lengths without sequencing assumes a canonical CAG repeat region sequence(29), an assumption that is erroneous in up to 5% of cases (as described below).

Newer methods that are now being employed in clinical practice include whole genome short-read sequencing (WGS) and then bioinformatic detection of an expansion and size estimation of the *HTT* CAG repeat (for example using Expansion Hunter)(30). This approach has the advantage of being able to determine the existence of non-canonical alleles, but low read depth and short read length (150 bp) currently limits the ability to accurately determine inherited CAG length for alleles > 35 CAG repeats. Furthermore, although WGS is increasingly used, including where the presentation is atypical for HD and the differential diagnosis wide, it is not yet universally available and validation with tethered repeat-primed PCR is still required.

It is best practice for individual laboratories to determine the error limits of their assays. According to the guidelines, acceptable error limits are ± 1 CAG at lengths of ≤ 42 and ± 3 repeats for alleles > 42 (20). Even with this error margin, genotyping results from the yearly European Molecular Genetics Quality Network scheme for molecular genetic testing of HD show that between 2008 – 2010, 3 – 9% of alleles fell outside the error limits set by the EMQN (at the time, these were

set at ±1 for alleles <40 repeats, and ±3 repeats for alleles >39 CAG repeats)(20). The European Huntington's Disease Network REGISTRY project centrally measures CAG repeat lengths (31). These data were used to compare 1,326 centrally generated CAG repeat lengths with local CAG reports generated from 121 laboratories across 15 countries: a discrepancy in the CAG size of the larger allele was found in 51% of cases, due to both under and over estimations of the CAG (32). Even when acceptable measurement errors proposed by the ACMG were applied the discrepancy rate remained at 13.3% (32). Such inconsistencies can have major ramifications for the individual undergoing a test and make genetic counselling extremely difficult and, potentially, inaccurate. For example, results for the larger allele changed from the reduced to full penetrance range in 36 cases (2.7%), whereas in 11 cases (0.8%) they moved from the full to the reduced penetrance range (32). In both these scenarios potentially devastating misinformation may have been given to the person undergoing testing. Therefore, it is vital that CAG repeat sizing is accurate- something that is frequently not achieved with current diagnostic methods. The major issue is not the method being error-prone, rather that an incorrect interpretation of the results, for example internal standards not being adapted to reference materials, leads to incorrect reporting of CAG lengths (20, 32).

Genetic counselling following a positive predictive HD gene test

A positive predictive test for HD is a life-changing event for that individual (Box 1). It is, therefore, imperative that the conveying of the test result is accurate, nuanced and tailored to the individual. Many people, having found out that they carry the disease-causing mutation, will have questions about the age that they will develop symptoms and how those symptoms might progress and impact their life. The major determinant (50 – 70%) of the age of motor onset of HD is the inherited *HTT* CAG repeat length (4-7). While it was previously standard not to share CAG repeat size information with patients, the updated 2013 'Recommendations for the predictive genetic test in HD' (16) suggest that the counsellor could share this and discuss the correlation between CAG repeat length

and mean age at onset. Not all individuals will want to know about CAG length and its interpretation; for those that do, discussions should stress that, on average, larger repeat expansions are associated with a younger age at onset of symptoms but that for a particular CAG repeat size there is a wide range in age at onset of symptoms and, as such, CAG length is of limited prognostic use for an individual.

However, for those carrying alleles of predicted reduced penetrance (36 – 39 CAGs) there is the question of whether they will develop HD at all: something that cannot currently be predicted for an individual carrier. Carriers can be advised that penetrance is length-dependent, so approximately 50% will develop symptoms by the age of 70 for 39 CAG repeats compared with 30% for 38 CAG repeats(33)(albeit these data did not account for CAG allele structure so over-estimate risk for a canonical HD allele). Recent studies have shown that premutation / reduced penetrance alleles have a carrier frequency of ~1 in 702 (34). This means that, with the rapid acceleration in whole genome sequencing in clinical medicine, asymptomatic individuals carrying alleles in the reduced penetrance range will be identified, and potentially reported as diagnostic or incidental findings. The ability to counsel these individuals effectively is of growing importance (33, 35).

Finally, accurate information regarding intergenerational transmission risks is also important to those carrying alleles in the intermediate (27 – 35 CAG) and reduced penetrance (36 – 39) ranges (36). Whilst intermediate alleles do not confer a lifetime risk of HD and reduced penetrance alleles do not always confer a lifetime risk of HD, there is, for some, a risk of expansion into the disease-causing range in subsequent generations (35, 37, 38). Other than being able to advise that there are important CAG length effects, further research is required to develop a greater understanding of genetic and other factors that affect the risk of expansion from an intermediate or reduced penetrance allele to fully penetrant allele in the next generation, as well as why large CAG size increases sometimes occur in the fully penetrant range. For men, in whom the risk of CAG size increase is higher than in women(39), direct sperm analysis to determine the CAG repeat size distribution may assist in predicting transmission risk in the future.

How could genetic information be used to improve age of onset prediction?

A greater understanding of the factors contributing to HD onset in individuals inheriting a disease-associated *HTT* CAG expansion could help drive improvements both in clinical counselling and management of patients (Table 2). Recent advances in genetic methodology and analysis have led to an explosion of data relating to the genetic risk factors for HD onset and progression, both at the *HTT* CAG locus and elsewhere in the genome (8-14, 40). Clinical application of these data is in its infancy and frameworks for translating population genetic risk into information applicable to individuals are required. Below we consider three areas where there is the potential to apply genetic data clinically to improve age at onset estimations for pre-symptomatic expansion carriers.

1. Accurate sizing of CAG repeat length

Given the inherent uncertainty of pure CAG repeat length that results from current standard PCR and capillary electrophoresis methods, it is critical that, as a minimum, local testing protocols should rigorously follow the European Quality Assurance or American College of Medical Genetics recommendations. This will ensure, as far as possible within testing limits, accurate and consistent reporting of the CAG repeat length for those alleles with canonical *HTT* repeats (Table 2)(20-22). Moving forward, next generation sequencing (NGS) methods will provide a more accurate approach, combining repeat length determination with sequencing of the repeat tract[1]. For example, short-read Illumina MiSeq sequencing and bespoke bioinformatic pipelines have been used in the research setting to call repeat lengths and sequences (41), and, as outlined above, whole genome sequencing has been introduced into clinical practice in some cases (30). One potential downside of using low-depth sequencing is that, particularly for larger alleles, somatic instability of the repeat in blood can add variation in CAG lengths and there are insufficient reads to accurately resolve these. NGS of the repeat with spanning reads of sufficient depth(minimum of hundreds of reads per sample) can mitigate this. However, short-read technologies such as MiSeq are limited in the repeat lengths they

can accurately size to. In the longer term, long-read sequencing using PacBio or Nanopore could provide a solution but these methods require further refinement of accuracy before they can be applied clinically.

2. Identification of *HTT* CAG repeat locus sequence variants

Next-generation sequencing of the *HTT* CAG repeat locus has revealed subtle but important differences in sequence that are associated with significant changes in the penetrance of the mutation and age at HD onset and progression, and also lead to inaccuracies of repeat sizing using standard repeat primed PCR protocols (10, 12, 29, 42, 43). The reference genome *HTT* CAG repeat tract is followed by CAACAG, also encoding glutamines, and then a further repetitive $CCGCCA(CCG)_n(CCT)_2$ sequence encoding polyproline: in over 95% of disease-associated *HTT* alleles in individuals of European ancestry, the CAG repeat is followed by the canonical CAACAGCCGCCA (10, 12, 13, 14). Several non-canonical *HTT* repeat structures have been identified, there may be a loss of CAACAG, of CAACAG and CCGCCA, of CCGCCA, or a duplication of CAACAG (12, 44-47). Recent data have shown that variations of the sequence arrangements are ancestry specific: they are present in up to 5% of disease-associated alleles in European populations, and more in African populations, (48, 49).

After accounting for pure CAG length, absence of the CAA codon (CAACAG loss), leaving a pure CAG repeat followed by the polyproline-encoding section, is associated with significantly earlier onset disease and faster progression for repeat lengths of 36-55 ((12, 14, 43, 48, 50). The double CAACAG CCGCCA loss allele also hastens onset by 10 years in individuals with 40-55 CAG range(50), and was found to have a particularly notable effect in carriers of reduced penetrance alleles with CAG lengths of 36-39, making onset an average of 29.1 years earlier than predicted by CAG length alone(13). This variant is found at higher frequency in symptomatic than asymptomatic subjects in the reduced penetrance range, essentially dramatically increasing the penetrance of the CAG 36-39 alleles (43).

Extra 'interrupting' CAA (or other non-CAG(14)) codons in this region are associated with later onset disease, with the most recent GeM consortium analysis finding that the CAACAG duplication was associated with 3.7 years delayed onset (10, 12, 13, 43, 49, 50).

A recent study was able to differentiate canonical from non-canonical CAG repeat region sequences with tethered repeat primed-PCR based on differential binding of the reverse primer to different alleles: if replicated, this method could be used to identify subjects requiring further sequence confirmation (51).

Although high-depth *HTT* repeat tract sequencing could provide highly accurate measures of CAG length and locus sequence, it may also come with added costs and bioinformatic requirements for genetic testing services. To balance accuracy of information, time-to-results and cost effectiveness in a real-world clinical setting, we propose the medium-term development of a pragmatic two-step approach to predictive testing (Table 2), building in the appropriate counselling and consent processes. Initial PCR and electrophoresis based fragment analysis will give a sufficiently accurate CAG length in ~90% of cases and can be reported back to individuals within a few weeks. For those with 35-42 CAGs by fragment analysis, we propose a secondary level of analysis based on short-read next generation sequencing (for example MiSeq) in order to accurately determine CAG length and to identify subjects with sequence variants which affect onset and penetrance. Protocols that are being developed to genotype repeat sequences from long-read or whole genome sequencing data may be options to identify sequence variants in the future (30, 52-54) (30, 41, 55).

3. Application of *trans*-acting variants: genetic information away from the *huntingtin* CAG repeat which may influence HD onset

A series of genetic studies have identified variants away from the *HTT* gene which are associated with variation in onset, progression and other phenotypes in HD ((8, 10, 11, 14, 50, 56)). Many of these variants occur at loci containing DNA repair genes such as *FAN1, MSH3, MLH1, PMS2, PMS1* and *LIG1*.

At least some of these (e.g. *MSH3, MLH3, PMS2, FAN1*) modify the rate of expansion of the *HTT* CAG repeat tract in somatic cells over a person's lifetime: more somatic expansion being associated with earlier onset and faster disease progression ((12, 50, 57, 58)). There is now interest in these DNA repair proteins as therapeutic targets for HD. Other association signals were found such as loci containing *TCERG1, RRM2B, CCDC82* and *MED15* that may be related to other mechanisms, or more indirectly involved in DNA maintenance. These genetic modifier variants have been identified from large-scale studies, with most being common in the population but having individually small effects on HD onset.

One way to try to link population variant data to individualised risk is to generate polygenic risk scores (PRS). PRS combine the effect sizes of many SNPs, derived from a population, to predict the genetic risk of a disease or trait in an individual. PRS are used in other areas of medicine to aid clinical decision making such as disease prediction and risk stratification. For example, in oncology, PRS have been developed for breast cancer screening ((59-61)), and PRS forms part of CanRisk, an interactive tool which is used by clinicians to calculate an individual's risk of developing breast and ovarian cancer based on genetic and environmental risk factors and family history (61-63). In cardiovascular disease, PRS have been found similar or superior to traditional risk factors in clinical risk models of cardiometabolic disease (64), and it has been found that disclosing a polygenic risk score to individuals may reduce cardiovascular events in those at intermediate risk (65). In neurodegenerative disease the use of various PRS have been explored, for example they can be used to identify people at greater risk of developing Alzheimer's disease(66).

These developments in the clinical application of PRS raise the question of whether a HD genetic modifier PRS could be used in HD alongside CAG length to improve the accuracy and clinical utility of age at onset predictions. In HD, the total effect of all genotyped SNPs in an unselected population of ~9,000 individuals has been estimated to explain ~ 25% of the residual age at onset of HD after accounting for CAG length – this is the SNP-heritability of residual age at onset (14). So if CAG length accounts for ~60% of the variance in age at onset, all SNPs combined could explain a maximum of an additional ~10% of the absolute variance in age at onset (25% of remaining 40% variance). Thus,

theoretically, PRS could add a small amount to onset prediction over CAG length alone(10, 14). In practice, the predictive power of PRS in an individual will likely be significantly less than the SNP-heritability as causal variants and effect sizes are inferred from GWAS data(67). This leads to uncertainty at the variant level in terms of causal associations which translates into even larger uncertainties in polygenic risk score estimates at the individual level (67). Therefore, while PRS could explain variation at a group level, and have been useful for showing genetic overlap between psychiatric disease risk and psychiatric symptoms in HD patients(68), they are currently not sufficiently predictive to give any particular individual refinement of expected age at developing symptoms of HD. Future work in this area, combining greater understanding of common and rare variants that impact HD onset and their interaction with CAG length, the dominant predictor of onset, could lead to PRS of clinical utility.

Could genetic data be used to stratify populations in HD clinical trials?

Incorporation of genetic modifier data for clinical trials risk stratification has been deployed prior to incorporation in clinical practice in other disease areas and could be considered in HD, particularly the use of accurate repeat structure sequences. The US Food and Drug Administration outlined two approaches for the enrichment of clinical trials(69). 'Prognostic enrichment' aims to increase statistical power (and thus decrease sample size and cost) by increasing the proportion of patients likely to demonstrate disease onset or progression. 'Predictive enrichment' aims to enrol participants who are more likely to have an increased benefit to the trial intervention. Post-hoc analyses of clinical trials of statins and cardiovascular events suggest that enrolling only people in the top quintile of polygenic risk score may have required 90% fewer participants and demonstrate a greater relative risk reduction compared with the overall trial population(70), leading PRS to be explored for the trial design of various conditions(71, 72).

In Parkinson's disease (PD), the impact of *not* considering the genetic make-up of participants in clinical trials has also been considered. The PD genetic risk score can predict PD progression(73): in a simulation study it was demonstrated that if patients are randomly allocated into clinical trial arms and the sample size is small, then there is a high chance of PD genetic risk score differences between groups(74). Thus, classic randomisation will create differences in genetic risk score between trial arms, which could lead to false positive and false negative results(73).

In HD, developing PRS for clinical trial enrichment could be considered, particularly for *HTT* CAG sequence variants having a large impact on AAO in the context of a desire for trials targeting presymptomatic or early symptomatic groups(75). In addition, taking into account the genetic variation in DNA repair genes of trial participants may be particularly relevant for drugs targeting DNA repair pathways, if existing genetic variation might influence drug efficacy.

Limitations of the use of PRS in clinical trials include ancestry (most GWAS thus far have focused on European ancestries, albeit so do many trials to date), and a requirement for regulatory approval of PRS use.

Recommendations for the clinical application of HD genetic data to improve genetic counselling and clinical trials.

Short term recommendations	• Ensure accurate reporting of CAG repeat length through adherence to current best practice guidelines • Focus groups/further research with patients and clinicians to explore understanding and acceptability of incorporating genetic data into routine clinical practice (CAG size, repeat sequence)

	• Focus groups/ further research to explore understanding in relation to use of genetic data (repeat sequence, polygenic modification scores) in HD clinical trials • Develop educational tools for incorporating genetic data into clinical practise
Medium term recommendations	• Two-step predictive testing CAG repeat sizing: 1. Existing PCR/capillary electrophoresis method 2. For those with 35-42 CAG repeats recommend additional testing to incorporate accurate sequencing of *HTT* CAG repeat locus sequence variants using next generation sequencing technologies (• Ensure that external quality assessment (EQA) programs integrate new sequencing techniques and bioinformatic tools • Validate mathematical models of age of onset prediction for incorporation into clinical practise • Establish best practice in communication of genetic modifiers and age of onset and incorporate into predictive test recommendations • Develop guidelines for the use of genetic modifier data in HD clinical trials
Long term recommendations	• Accurate sequencing of *HTT* CAG repeat to be incorporated for predictive and diagnostic testing: based on the outcomes from the short and medium term recommendations • Development of clinically useful predictive models for HD onset incorporating CAG length and sequence, *trans* modifiers and phenotypic data • Explore how short-read and long-read whole genome sequencing data, which is increasingly available, may be used to identify repeat

	sequence, along with presence of common and rare modifier variants, while acknowledging that, for short-read WGS, read depth and read length are likely to preclude its use as a definitive diagnostic test for the CAG repeat.

Table 2: Summary of recommendations to improve the use of genetic data to in clinical practice and clinical trials.

CONCLUSION

The last decade has seen significant advances in our understanding of genetic factors which influence the development of various diseases. Whilst topical, translating these genetic risk factors identified in research studies into clinical use on an individual basis poses considerable statistical, technological and counselling challenges. HD, a paradigm for a fully penetrant autosomal dominant neurodegenerative disease, is now well established as being strongly influenced by other genetic variants and provides a good example of these translational challenges. Although the results from GWAS are highly statistically significant, and have increased understanding of disease mechanism, the overall contribution of population-derived variants to an individual's age at onset is small, and dwarfed by the effect of CAG length. Thus, we caution against premature clinical incorporation of PRS in onset prediction and genetic counselling.

The priority should be to employ existing technologies appropriately, particularly to ensure accurate CAG repeat sizing and effective communication of the results to patients. Next, the development of clinical testing pathways incorporating technologies which enable both accurate sizing of the CAG repeat and identification of *HTT* sequence variants should be developed. It is imperative that family and clinician engagement and education occurs in parallel to ensure accurate communication of these genomic advances and incorporation into predictive testing recommendations. The long-term aspiration is for the clinical application of *HTT* and genetic modifier variant sequencing with the

development of a clinically useful individualised PRS to be offered within predictive and diagnostic testing pathways.

Datasets/Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analysed during this study.

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Consent for publication:

We hereby confirm that we have written consent to publish clinical scenarios which are based on the lived experience of people's journal through genetic testing. Some of the cases are based on more than one clinical scenario.

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Conflict of interest statement:

T.M., P.H., D.H.M. are on the Scientific and Bioethics Advisory Board of the European Huntington's Disease Network (EHDN). N.L. is on the Executive Committee of the EHDN. T.M. and D.H.M. are lead facilitators of the EHDN Genetic Modifiers Working Group. N.L. and R.McL. are lead facilitators of the EHDN Genetic Testing and Counselling Working Group. T.H.M. is a member of the scientific advisory boards of Harness Therapeutics Ltd and LoQus23 Therapeutics Ltd. Within the past 36

months D.G.M. has been a scientific consultant and/or received an honoraria/research contract from AMO Pharma, Dyne, F. Hoffman-La Roche, Function Rx, LoQus23, MOMA Therapeutics, Novartis, Ono Pharmaceuticals, Pfizer Pharmaceuticals, PTC Therapeutics, Rgenta Therapeutics, Sanofi, Sarepta Therapeutics Inc, Script Biosciences, Skyhawk Therapeutics, Triplet Therapeutics, and Vertex Pharmaceuticals. D.G.M. also had research contracts with AMO Pharma and Vertex Pharmaceuticals. D.G.M. is on the Scientific Advisory Board of the Myotonic Dystrophy Foundation and EuroDyMA (European Dystrophia Myotonica Association), is a scientific advisor to the Myotonic Dystrophy Support Group, and is a vice president for research of Muscular Dystrophy UK.

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