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Huntington's Disease: Relationship Between Phenotype and Genotype

Yi-Min Sun[1] · **Yan-Bin Zhang**[2,3] · **Zhi-Ying Wu**[2]

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Abstract Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease with the typical manifestations of involuntary movements, psychiatric and behavior disorders, and cognitive impairment. It is caused by the dynamic mutation in CAG triplet repeat number in exon 1 of *huntingtin* (*HTT*) gene. The symptoms of HD especially the age at onset are related to the genetic characteristics, both the CAG triplet repeat and the modified factors. Here, we reviewed the recent advancement on the genotype-phenotype relationship of HD, mainly focus on the characteristics of different expanded CAG repeat number, genetic modifiers, and CCG repeat number in the 3′ end of CAG triplet repeat and their effects on the phenotype. We also reviewed the special forms of HD (juvenile HD, atypical onset HD, and homozygous HD) and their phenotype-genotype correlations. The review will aid clinicians to predict the onset age and disease course of HD, give the genetic counseling, and accelerate research into the HD mechanism.

Keywords Huntington's disease · Genotype · Phenotype · *Huntingtin*

✉ Zhi-Ying Wu
zhiyingwu@zju.edu.cn

[1] Department of Neurology and Institute of Neurology, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China

[2] Department of Neurology and Research Center of Neurology in Second Affiliated Hospital, and the Collaborative Innovation Center for Brain Science, Zhejiang University School of Medicine, Hangzhou, China

[3] Department of Neurology and Institute of Neurology, First Affiliated Hospital, Fujian Medical University, Fuzhou, China

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease and generally begins insidiously in mid-adult life, usually 30–50 years. Its age at onset (AAO) ranges from 1.5–85 years [1–4]. Its typical manifestations include involuntary movements, psychiatric and behavior disorders, and cognitive impairment. The disease duration is generally 17–20 years [4]. The prevalence of HD is 5–17.2 cases per 100,000 in Caucasian population [5] and much lower in Japanese (0.5 per 100,000), Chinese, African, and Finnish populations [6, 7]. There is not an effective treatment to revert the disease course or delay its progression.

The causative gene, *huntingtin* (*HTT*), located on chromosome 4p16.3, was identified in 1993. The disease is associated with an unstable expansion of a CAG triplet repeat in exon 1 of *HTT*. The normal *HTT* allele contains a sequence of between 6 and 35 CAG triplet repeats. A CAG triplet expansion of 40 repeats or greater is abnormal and fully penetrant. An allele with 36–39 CAG repeats is considered to have a reduced penetrance. Although 27–35 CAG repeats are in the normal range, they are considered intermediate or unstable alleles and may extend or contract during reproduction [8].

In this review, we focused on the correlation between the phenotype and genotype of HD, particularly on the effects of the expanded CAG repeat number, genetic modifiers, and CCG repeat number in the 3′ end of CAG triplet repeats. And we also reviewed the special forms of HD (juvenile HD, atypical onset HD, and homozygous HD) and their phenotype-genotype correlations.

CAG Repeats Expansion and Phenotypes

The CAG repeat number in *HTT* plays a dominant role in HD phenotype. Generally, the CAG repeat number in the expanded allele is critical to AAO and contributes to approximately 70 % of the variation of AAO [9]. Increased numbers of CAG repeats were related to the reduced AAO [10, 11]. Many studies

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indicated that this relationship was stronger in early-onset patients with large CAG repeats and weaker in patients with moderate numbers of CAG repeats [9, 12]. Whether the CAG repeat number determines the rate of HD clinical progression has been investigated since the discovery of *HTT*. However, this relationship is controversial [13–20]. These paradoxical results might be attributed to methodological differences, such as the measurements that were used to assess progression and the statistical techniques that were used to detect an association.

In 1993, two sporadic HD patients from two Dutch families were reported [21]. The genetic testing revealed that the number of CAG repeat expanded into the HD threshold during paternal transmission. Then, intermediate alleles (IA) carrying 27–35 CAG repeats were termed [22], given their increased instability compared to alleles with fewer than 26 CAG repeats. IA was estimated to be high as 6 % in the general population and tended to expand or contract during transmission. Among the expansion, the risk of a new HD mutation increased along the IA CAG size, with the highest risk of the allele carrying 34 and 35 CAG repeats. As for the contracted alleles, 13 % contracted into the normal CAG range [23–25]. However, IA instability was not observed in 69 parent-child transmissions from a 10 generation Venezuelan kindred [26].

Individuals with IAs have been reported to exhibit HD clinical features [27–32]. The majority of these patients had late-onset HD (onset age beyond 60 years old) [33]. The lowest CAG repeat number that caused a HD phenotype was 29. The patient began involuntary movements at age 60, and it was confirmed as HD by autopsy [31]. Recently, a population-based study found that IA participants had significantly worse behavior of apathy and suicidal ideation compared with the controls or expanded participants. It was explained as a prodromal stage of HD or a phenotype of pathology independent of CAG expansion length [34].

Alleles with reduced penetrance contain 36–39 CAG repeats. The HD occurrence of this type may be accelerated by associated medical conditions and treatments as well as by environmental and modifying genetic factors [35]. The prevalence of such alleles was estimated as 0.1 % in the general population and over 2 % in patients with HD [25]. Penetrance estimates were that at least 40 % of these patients would be asymptomatic at age 65, with this probability dropping to 30 % at age 75 [29, 36]. The alleles with reduced penetrance could be expanded from an IA or contracted from a fully penetrant allele during transmission [26, 37]. Similarly, this type of allele could itself expand or contract during transmission [25, 26].

Genetic Modifiers and Phenotypes

The CAG repeat number accounted for at most 70 % of the AAO variability and the remaining portion was determined by other genetic modifiers and environmental factors [12].

Targeted candidate gene studies have identified certain modifier loci. In 2008, Htt-associated protein-1 (*HAP-1*) was identified as a modifier of AAO in HD. In an investigation of 980 European HD patients, p.T411M (rs4523977), a single nucleotide polymorphism (SNP) in *HAP-1*, was observed to modify the AAO of HD [38]. The patients who were homozygous for the p.M441 genotype exhibited a clearly delayed AAO, and functional experiments indicated that M441-HAP1 bound mutant Htt more tightly than T441-HAP1 did, reducing the levels of degraded soluble Htt products and protecting against Htt-mediated toxicity. However, the findings could not be replicated by two other groups, who also examined European populations [39, 40].

GluR6 is a subunit of kainate receptors, which took part in the striatal neuron cell death during HD [41, 42]. Investigations have determined that the polymorphic TAA repeat in the 3′ untranslated region of *GRIK2*, which encodes GluR6, is correlated with the AAO of HD. Patients carrying 155 TAA repeat allele (the genotypes 155/146, 155/149, and 155/152) were found to have lower AAOs than individuals with the other *GRIK2* genotypes [43, 44]. But the results were also controversial [11, 45].

ADORA2A encodes the adenosine A2A receptor and was also proposed to be a candidate gene for modifying AAO. A synonymous polymorphism (c. 1713T>C, rs5751876) in exon 3 of this gene was determined to contribute to the variability of AAO in HD patients. The T/T genotype of this SNP may advance the AAO of HD [46].

The ε4 allele of *APOE* has been proven to be a genetic risk factor of AD and advanced AAO in patients with AD [47]. Whether this allele affects the AAO in other neurodegenerative diseases, such as HD, has also been investigated; however, the results have been controversial. Certain authors have indicated that the ε2/ε3 genotype is associated with an earlier AAO [48] and that the ε4 allele with a higher AAO [49]; however, negative and even opposite results have been reported [45, 50–52].

Other genes may affect the AAO of HD, for example, *TCERG1* [53], which encodes protein that are involved in transcriptional regulation. Other potential AAO-affecting genes include autophagy-related 7 (*Atg7*), which is involved in autophagy [54], peroxisome proliferator-activated receptor C coactivator 1a (*PPARGC1A*), whose protein participates in peroxisomal function [55], *UCHL1* [56], and cannabinoid receptor 1 (*CNR1*) [57].

The first genome-wide association study (GWAS) for modifiers of AAO in HD was performed in 2003, in which 695 individuals were investigated. Suggestive evidence for linkage was observed in three loci: 4p16, 6p21-23, and 6q23-24 [58]. Subsequently, a replication study was performed by the same group in 102 newly recruited patients and the combined samples in 2006 [59]. 6q23-24 in the first GWAS study still exhibited evidence for linkage, and another non-significant

locus of 18q22 became significant in the replication study. G*RIK2* is located approximately 26 cm proximal to the peak of 6q23-24. A third GWAS study was performed in a subsample of 1332 Venezuelan HD families using a 5858 SNP marker panel [60]. Two novel loci on chromosome 2 were discovered. These findings indicated the locations of candidate genes that are capable of altering HD pathogenesis, and the genes at these loci should be further explored.

A recent GWAS study of European HD patients containing 40–55 CAG repeats found that two independent effects on a chromosome 15 locus accelerate or delay onset age of HD by 6.1 and 1.4 years, respectively, and a chromosome 8 locus increases onset age by 1.6 years [61]. The discrepancy between this study and two former studies might be caused by the different populations (Venezuelan vs European population) [60] and the different methods of association or linkage [59].

Most candidate gene studies focus on the genetic modifiers of AAO, and few focus on different phenotypes like disease progression [62]. The genetic modifier researches on HD progression need good HD cohort, good tools of assessments, and prospective observation. The results might be affected by different genetic backgrounds of population and different stages of disease. These problems should be solved in the future studies.

These modified loci interact with each other and contributed to the phenotypes and might affect differently in different populations. Also, the discoveries of genetic modifiers in patients with HD could improve the exploration of the involved pathological pathways and ultimately identify therapeutic targets [63, 64].

CCG Polymorphism and Phenotypes

A CCG polymorphism is located on 3′ of the CAG triplet repeats in *HTT*. This region consists of a series of CCG triplet repeats and encodes a proline-rich region adjacent to the polyQ tract. This proline-rich region is involved in the cytoplasmic localization of *HTT* exon 1 protein (Httex1p) and may be related to the stability and aggregation of Htt [65, 66]. Alleles carrying 7 or 10 CCG repeats are predominant in most populations compared with repeats of other sizes (i.e., 6, 8, 9, or 11 repeats). CCG polymorphisms were reported not to affect AAO or neurological dysfunction [51, 67–69], but affect the CAG repeat length. In Western populations, linkage disequilibrium was identified between CCG 7 and larger CAG repeat length, both in normal and HD alleles [6, 70]. This result was explained by the hypothesis that in a CCG 7 background on a normal allele, a higher CAG repeat number was more unstable and tended to expand into the penetrant range [6].

In contrast, in Japanese and Chinese populations, the association of larger CAG repeat numbers was with CCG 10 [6, 67, 69, 71]. Similarly, the CAG repeat number in the normal allele in a Chinese population was higher in a CCG 10 than in a CCG 7 background, which was in accordance with the explanation of development into the HD allele in the examined Western population. However, other investigations did not detect an association between CCG polymorphisms and CAG number in Chinese or other populations [51, 67–69, 72, 73]. These results may in part explain the differences between populations, in which other modifiers and the haplotype may be involved in the CAG repeat number [7].

A specific set of 22 tagging SNPs in *HTT* related to the CAG instability of expansion was reported in Caucasian population but not in Chinese, Japanese, and Nigerian populations [7]. These polymorphisms indicated the presence of other genetic factors in the occurrence of HD and could also give clues as to the difference in prevalence between different populations.

Phenotype-Genotype Correlations of Juvenile HD, Atypical Onset HD, and Homozygous HD

Juvenile HD (JHD) is a variant form of HD and is characterized by disease onset at 20 years of age or younger, accounting for approximately 3–10 % of all HD patients [1, 25, 74]. The clinical manifestations of patients with JHD are usual atypical. The symptoms of rigidity, bradykinesia, dystonia, dysarthria, seizures, ataxia, behavior disturbance, and cognitive decline are prevalent, and chorea may develop later. JHD is observed more frequently with a paternal inheritance pattern and is generally characterized by a large number of CAG repeats. JHD progresses more rapidly than adult HD does. The mean survival time is 8–9.3 years, with a range of 2–38 years [1, 75]. Earlier AAO is associated with a shorter survival time. The underlying pathogenesis of JHD was the differences from adult-onset HD in both biochemistry and ultrastructure, like increased caspase-3 activity, reduced mitochondrial function, and increased cytoplasmic autophagosomes [76, 77].

According to the inverse relationship between AAO and the CAG repeat number, the latter generally exceeds 60 [9, 78]. The largest CAG repeat reported contained approximately 250 trinucleotides [79], followed by 214 and 180 copies [80, 81]. There have also been JHD cases with CAG repeats <60, or even as low as 42 [32]. Paternal transmission is responsible for an estimated 70–90 % of JHD cases [82], whereas certain cases with greatly increased CAG repeat number were due to maternal transmission [3]. Larger alleles (>100 CAGs) are inherited from mothers with large expansions (>60 CAGs) but can be inherited from fathers with expansions below 60 CAGs [3]. The CAG expansion between generations is observed in both sons and daughters, so the sex of the parent is

a critical factor [83]. The intergeneration expansion may cause the "absence" of family history in the affected family and mislead the clinicians to making a diagnosis of JHD in the early disease stage.

CAG expansion in different tissues of HD patients was identified by repeat mosaicism in all tissues, with a greater instability in the brain and sperm [84]. Other studies reported the propensity for substantial repeat size increases to occur more in the course of spermatogenesis than oogenesis [78, 85]. This effect may contribute to the CAG repeat size expansion during paternal transmission. Another possibility is that a massive expansion of CAG may be particularly detrimental to the oocyte and therefore result in impaired fertilization [85].

In addition to the CAG repeat number in JHD cases, intergenerational changes of *HTT* CAG repeat number in lower or normal ranges were investigated [21, 78, 85]. Two hundred fifty-four affected parent-child pairs with HD and 440 parent-child pairs with normal CAG repeat number ranges were enrolled to investigate intergenerational CAG changes in HD [85]. It was determined that normal-range CAG triplet numbers (10–28 repeats) rarely changed between generations (0.68 %), whereas expanded alleles changed in length in 70 % of meioses, with expansions accounting for 73 % of such changes. Increases of CAG triplet repeat number ranged from +1 to +74, and decreases in triplet repeat size ranged from −1 to −4. Larger intergenerational expansions (>7 CAG repeats) were influenced by larger CAG repeat sizes in the father (in paternal transmission); however, the likelihood of smaller expansions (≤7 CAG repeats) or contractions was not influenced by CAG size. Large expansions (>7 CAG repeats) occurred nearly exclusively in paternal transmission, whereas the offsprings of affected mothers were more likely to exhibit no changes or even reduced CAG repeat size [3].

Like JHD, atypical onset manifestations may also appear in adult-onset HD. In a previous study, parkinsonism, ataxia, dystonia, and tics were reported as the atypical onset movement disorders [86]. We retrospectively examined clinical data in our 82 HD probands and identified seven who presented with an atypical onset of ataxia [87].

Homozygosity in HD, indicating a CAG repeat expansion on both alleles, rarely occurs [88–90]. The frequency ranged from 0.1 [91] to 0.4 % [92]. In investigations from 1987 until the present, homozygosity has not been reported to influence the AAO compared with heterozygosity. The impact of HD homozygosity on disease severity has been controversial [92, 93]. However, in other dynamic mutation diseases of CAG triplets, such as certain subtypes of spinocerebellar ataxia (SCA) and dentatorubro-pallidoluysian atrophy (DRPLA) [94–97], homozygotes tend to present with an earlier onset and a more severe evolution than do heterozygotes. This difference may mainly be attributed to the mechanisms of the mutations. The pathogenesis of adult HD was not *HTT* dosage dependent, and the mutation might reflect the dominant effect of a single mutant allele, which acts as a gain of function mutation rather than a dominant negative mutation, as in SCA or DRPLA [61, 76]. We first reported an individual who was homozygous for CAG repeats in the Chinese Han population [98]. This individual was a premanifest. She was 38 years old with CAG repeat numbers of 37 and 42. Her both parents were heterozygous, with CAG repeat numbers of 17/37 and 20/42, respectively.

Genetic counseling with respect to the patients homozygous for *HTT* mutation is very important. First, the offspring, whose parents are both heterozygous for *HTT* mutation, has an increased risk of inheriting the *HTT* mutation in either a homozygous (25 %) or heterozygous (50 %) form. Second, the offspring of a homozygous patient will certainly inherit the *HTT* mutation (100 %). Additional counseling information and more psychiatric services have been suggested [92, 93].

Conclusion

The main clinical symptoms and the causative gene of HD are quite clear. As a major role, the CAG triplet expansion together with many modifier genes contributes to the occurrence and clinical manifestations of HD. Since the carrying status of CAG expansion could be detected by the genetic testing of *HTT*, observations have been made in the manifestations especially the development from preclinical to the clinical stage. Moreover, the variation of expanded CAG repeat number was also investigated during transmission. With the development of next generation sequence, the modifier loci were scanned in a genomic range, the results of which might give a clearer picture of the phenotype-genotype correlation, and it could also indicate the pathogenesis of HD and help to identify the therapeutic targets of the disease.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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