

Huntington's Disease Like-2: Review and Update

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Abstract- Huntington's Disease-like 2 (HDL2), like Huntington's disease (HD), is an adult onset, progressive, neurodegenerative autosomal dominant disorder clinically characterized by abnormal movements, dementia, and psychiatric syndromes. Like HD, the neuropathology of HDL2 features prominent cortical and striatal atrophy and intranuclear inclusions. HDL2 is generally rare, accounting for only a few percent of HD-like cases in which the HD mutation has already been excluded. However, the rate is considerably higher among individuals of African ancestry, and is almost as common as HD in Black South Africans. The disorder is caused by a CTG/CAG expansion mutation on chromosome 16q24.3, with normal and expanded repeat ranges similar to HD, and a correlation between repeat length and onset age very similar to HD. Surprisingly, the available evidence suggests that HDL2 is not a polyglutamine disease. Rather, the repeat expansion is located within *Junctophilin-3* in the CTG orientation. The phenotypic similarities between HD and HDL2 suggest that understanding the pathobiology of HDL2 may shed new light on the pathogenesis of HD and other disorders of striatal neurodegeneration.

Key Words: Huntington's disease Like-2, Huntington disease, Neurodegeneration, Neurogenetic disease

Acta Neurol Taiwan 2005;14:1-8

INTRODUCTION

Huntington's disease, first described by George Huntington in 1872 is characterized by a triad of movement, emotional, and cognitive abnormalities. Clinically detectable signs or symptoms usually begin between the ages of 35 and 50, with death typically following 15-20 years later^(1,2). The most prominent neuropathological findings in HD are atrophy of the caudate and putamen, with a dorsal to ventral gradient of loss of medium spiny projection neurons and an accompanying reactive gliosis^(3,4).

Neuronal loss is also present in the cerebral cortex, particularly in layers III, V, and VI⁽⁵⁾ and to a milder degree in globus pallidus, thalamus, subthalamic nucleus, and substantia nigra. "Indirect pathway" medium spiny neurons, expressing preenkephalin/enkephalin and enriched in D2 receptors, may be preferentially lost relative to "direct" pathway medium spiny neurons expressing preprotachykinin/substance P and D1 receptors⁽⁶⁻⁸⁾, though neurons and their processes expressing the latter markers and projecting to the substantia nigra also appear to be selectively lost⁽⁹⁾. Intranuclear aggregations

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Received November 17, 2004.

Revised and Accepted January 19, 2005.

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of the abnormal huntingtin protein in association with a variety of other proteins are found in neurons of the striatum and cortex^(10,11) and axonal and dendritic abnormalities and inclusions have also been detected^(10,12,13). HD is caused by an expansion of a CAG repeat in the gene huntingtin located on chromosome 4p16.3^(14,15).

The predominant theory of HD pathogenesis is that the expanded polyglutamine tract encoded by the CAG repeat expansion is toxic. However, despite an intense effort, a complete understanding of the pathogenesis of this toxicity remains an unrealized goal. The selectivity of the loss of particular medium spiny neurons remains a central enigma⁽¹⁶⁻¹⁸⁾. Possible explanations for this phenomenon include selective vulnerability of these neurons to inhibition of mitochondrial respiratory processes⁽¹⁹⁾ or excitotoxic damage stemming from glutamatergic input⁽²⁰⁾. The role of intranuclear inclusions remains equally puzzling. It is possible that the inclusions themselves are not part of the pathogenic pathway, but that intermediate structures that form during the process of aggregation may be toxic⁽²¹⁻²³⁾.

We hypothesized that finding diseases with features similar to HD, yet arising from different mutations, would prove valuable in the understanding of HD pathogenesis. Indeed, about 1% of all cases of clinically or pathologically defined HD do not carry the HD mutation HD⁽²⁴⁻²⁷⁾. We therefore launched an effort to find the causative mutations in cases of HD-like disorders.

THE W PEDIGREE AND THE HDL2 PHENOTYPE

We ascertained a large family from the Southeastern United States with an autosomal dominant transmission of an HD-like disorder⁽²⁸⁾. Disease presentation and course in this family begin with weight loss and diminished coordination, and progress to include rigidity, dysarthria, hyperreflexia, bradykinesia, tremor, psychiatric syndromes, and dementia. Dystonia and chorea are frequently present, and the disorder culminates in a bedridden nonverbal state with profound dementia about 10-15 years after onset, with death following sometime thereafter. MRI findings, as in HD, show marked atro-

phy of the caudate and the cerebral cortex. The neuropathology of the first available case (Fig. 1) was also consistent with that seen in HD, with cortical gray matter atrophy, mild dilation of the ventricular system, and severe atrophy of the caudate and putamen on gross exam. Microscopically, severe neuronal degeneration and reactive astrocytosis, with vacuolation of the neuropil, was noted throughout the caudate, with more severe involvement of dorsal than ventral regions and a selective loss of medium sized neurons. The putamen showed a lesser degree of degeneration than the caudate, with the same dorsal to ventral gradient. There were no Lewy bodies, β amyloid deposits or neurofibrillary tangles. Immunohistochemically, the key finding was the presence of intranuclear aggregates that stained with 1C2 (considered relatively specific for expanded polyglutamine tracts) and anti-ubiquitin antibodies, but not anti-huntingtin antibodies.

Subsequent HDL2 pedigrees (see below) have suggested two variants of presentation. One, as seen in the index family, is characterized by more rigidity and parkinsonism, and may be associated with longer repeat lengths. This variant resembles juvenile onset HD. The other and more common variant resembles typical adult onset HD, with prominent chorea and perhaps a somewhat slower progression.

Cloning the HDL2 repeat expansion

DNA from the proband, and then from other family members, was tested for the presence of a CAG/CTG expansion mutation by the repeat expansion detection (RED) assay⁽²⁹⁾, previous used to find mutations in SCA7⁽³⁰⁾, SCA8⁽³¹⁾ and SCA12⁽³²⁾. All affected family members, and no unaffected family members, had a CAG repeat expansion of approximately 50 to 60 triplets, yielding a maximum lod score of 3.88 at $\theta = 0.00$. All known CAG repeat expansions were excluded, as was linkage to the 20p locus of the prion gene and the 4p locus of the HD gene. Using the same method that we had used to find the SCA12 gene⁽³²⁾, we were able to clone a 6 kb section of genomic DNA containing a CAG repeat expansion of 55 triplets from one of the family members. We used sequence flanking the repeat to

develop a PCR assay to test repeat length in genomic DNA. This test confirmed that all affected members of the family, and no unaffected family members, carried the repeat expansion.

HDL2 molecular genetics

We have used several approaches to confirm that the repeat expansion we identified was causative⁽³³⁾. First, we

have doubled the size of the original pedigree, and demonstrated that the mutation continues to completely segregate with the disease. Second, we established that the length of the repeat in the normal population (546 alleles tested) ranges from 7-27 triplets, which does not overlap with the size of the repeat in expanded individuals (40 to 55 triplets) (Fig. 2). This has been confirmed by several of our collaborators. Third, we and our col-

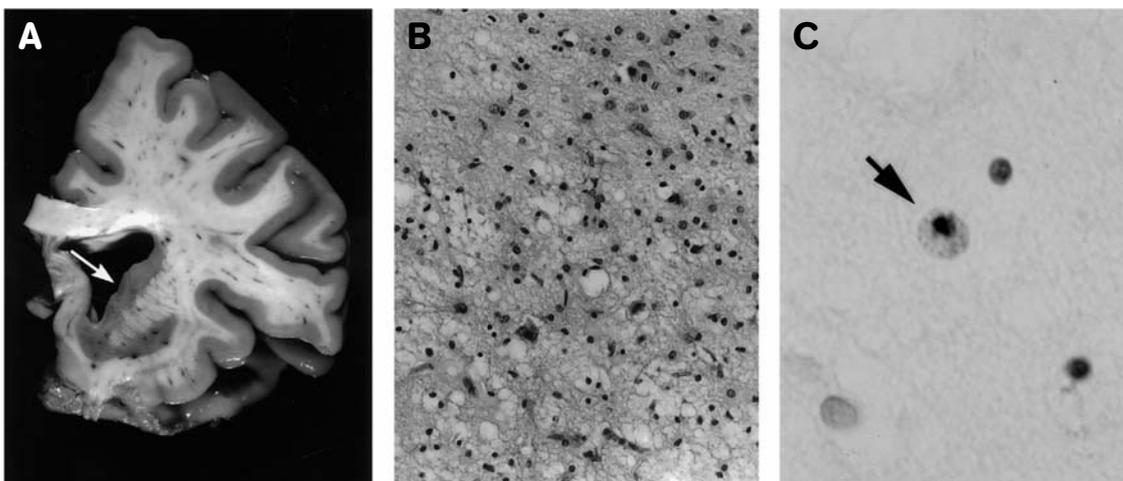


Figure 1. HDL2 Neuropathology. (A) Gross pathology, showing moderate cortical and prominent caudate atrophy. (B) Microscopic pathology of the caudate, showing neuronal degeneration, astrocytic gliosis, and vacuolization. (C) Staining with 1C2 antibody, showing intranuclear inclusion in cerebral cortex. Reprinted with permission from John Wiley and Sons⁽²⁸⁾.

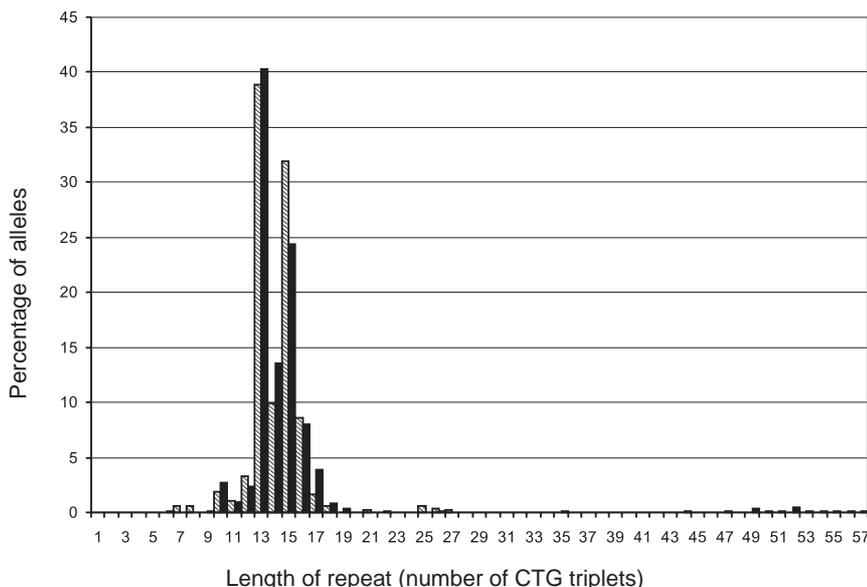


Figure 2. Distribution of HDL2 locus repeat lengths. Hatched bars, repeat length in 273 control individuals. Black bars, repeat length in 328 individuals with movement disorders. Note that there are no repeats between 35 and 43 triplets. All individuals with a repeat length greater than 43 have the HDL2 phenotype. An affected individual with 40 triplets has subsequently been ascertained. No alleles with repeat lengths greater than 35 or less than 40 triplets have been detected. Reprinted with permission of Nature Press⁽⁴⁸⁾.

laborators have now identified at least 23 unrelated pedigrees with the HDL2 mutation and an HD-like disease. The fourth line of evidence supporting a causative role for the HDL2 mutation is the strong correlation between younger age of disease onset and repeat length ($N = 24$, $R = -0.62$, $r^2 = 0.39$, $p = .0011$, slope = -1.24) (Fig. 3). The strength of the association and the slope are similar to the linear function relating repeat length and onset age in HD ($r^2 = .44$, -1.13).

The precise dividing line between normal and expanded repeat lengths remains uncertain⁽³³⁾. We have detected a mother and son with dissimilar and non-HD like neurological disorders with repeat lengths of 33 and 35 triplets. We suspect that their neurological findings are unrelated to the HDL2 repeat, though it is evidence of repeat length instability. An individual with typical genetically confirmed HD had an HDL2 repeat length of 34. An individual in an HDL2 pedigree with a repeat length of 44 triplets was apparently unaffected at the age of 65.

HDL2 epidemiology

Thus far, every case of HDL2 detected has been of African, or probable African, ethnic origin. HDL2 was not detected in either French or German populations of movement disorder patients^(34,35). The results imply that HDL2 is rare, if not nonexistent, in European popula-

tions, consistent with our initial observation. HDL2 has also not been detected in Japan⁽³³⁾. In a preliminary report of patients referred for HD testing in South Africa⁽³⁶⁾, 75% of white patients, but only 41% of black patients, had an HD expansion. Of those testing negative for the HD mutation, 7/20 of black patients had an HDL2 mutation (repeats of 40-49 triplets). None of the white HD-negative patients had an HDL2 expansion. Overall, it appears that HDL2 accounts for about 1% of all HD mutation negative cases with a very broadly defined HD-like phenotype. On the other hand, the rate of HDL2 is much higher in cases with an autosomal dominant disease with a very clear HD phenotype and African ethnic origins.

MOLECULAR PATHOGENESIS OF HDL2

The HDL2 repeat localized to 16q24.3. We initially suspected that HDL2 would be a polyglutamine disease, based on multiple lines of evidence: 1) autosomal dominant disorder with a phenotype resembling HD, 2) detection of a CAG/CTG expansion in the family by the RED assay, and 3) the presence of 1C2-positive intranuclear inclusions in patient brain. However, there is no gene or EST in GenBank or Celera databank in the CAG orientation. No exon corresponding to the repeat in the CAG orientation is predicted by any algorithm. Our efforts to

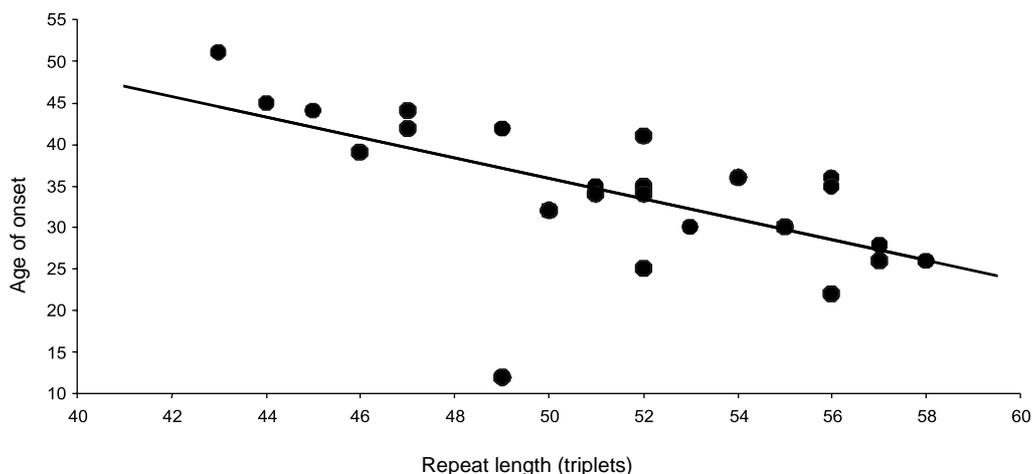


Figure 3. Relationship between HDL2 age of onset and repeat length. HDL2 repeat length is inversely correlated with age of onset. $N = 24$, $R = -0.62$, $r^2 = 0.39$, $p = .0011$. The relationship is similar to that observed in HD. Reprinted with permission from John Wiley and Sons⁽³³⁾.

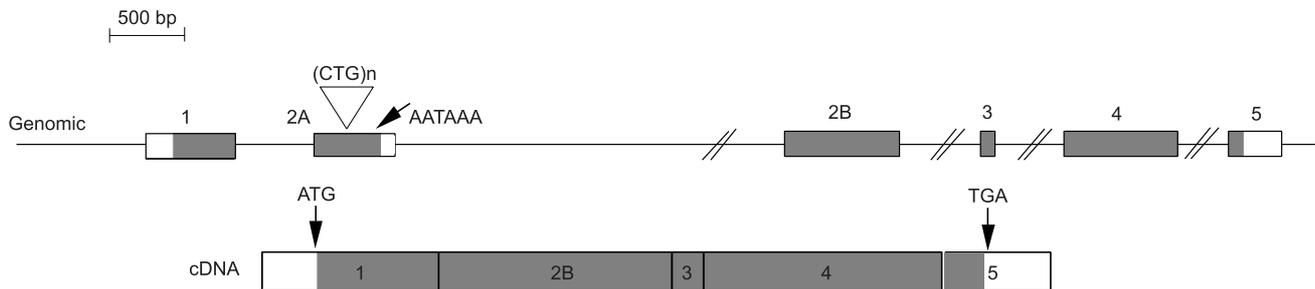


Figure 4. Genomic structure of *JPH3*. The repeat, in the CTG orientation is located within a variable spliced exon, termed 2A, of *JPH3*. Exon 2A is not included in the full length *JPH3* transcript. Reprinted with permission of Nature Press⁽⁴⁸⁾.

identify a transcript containing the repeat in the CAG orientation using RT-PCR, Northern analysis, and cDNA library screening were unsuccessful.

On the opposite strand, the HDL2 repeat, in the CTG orientation, is located 760 nucleotides downstream of the 3' end of exon 1 of the gene junctophilin-3 (*JPH3*) (Fig. 4). The genomic structure of this gene had been previously determined⁽³⁷⁾, and our genomic contig enabled us to place the CTG repeat 760 nt 3' to the end of *JP-3* exon 1. Exon originally termed "exon 2" is more than 36 kb downstream from exon 1.

It appears that a previously undetected *JPH3* exon, which we termed 2A, contains the HDL2 repeat and forms (with exon 1) an alternative *JPH3*. First, a canonical polyadenylation signal (AATAAA) is located 281 nt 3' to the end of the repeat. Second, GENSCAN predicts a transcript in which exon 1 of *JP-3* is spliced to an exon containing the CTG repeat. Third, multiple ESTs contain the exon 1-exon 2A transcript. Multiple different canonical splice acceptor sites are used in these transcripts, leading to three different reading frames for the repeat-containing exon. We subsequently confirmed the presence of these transcripts in human cerebral cortex. The conclusion we drew from these bioinformatic and experimental approaches is that exon 2A, containing the HDL2 repeat, is spliced such that the repeat may encode polyalanine (the predominant form of the exon1-exon 2A transcript) or polyleucine, or fall outside of the reading frame in the 3' untranslated region (Fig. 5).

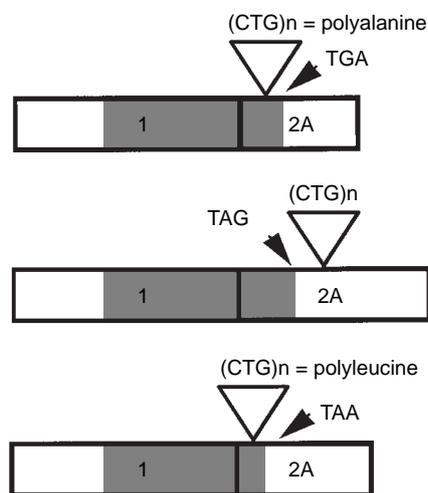


Figure 5. Alternative splice variants of *JPH3* that contain exon 2A and the CTG repeat. Each splice variant uses a different splice acceptor site, such that the CTG repeat is in-frame to encode polyalanine, in-frame to encode polyleucine, or in 3' UTR. Reprinted with permission of Nature Press⁽⁴⁸⁾.

JUNCTOPHILIN-3

The first three of the four known members of the junctophilin gene family were first described in 2000⁽³⁷⁻³⁹⁾. *JPH1* is expressed in skeletal muscle, *JPH2* is expressed in cardiac and skeletal muscle, and *JPH3*, the family member associated with HDL2, is expressed primarily in brain and to a much lesser extent in testes. The

most recently identified family member, *JPH4*, is also expressed in brain. The members of this protein family each have eight repeated N-terminal units of 14 amino acids termed MORN (membrane orientation and recognition nexus) motifs which serve to anchor the protein to the plasma membrane, and a C-terminal endoplasmic/sarcoplasmic reticulum transmembrane domain. This structure, immunohistochemical analyses, and analysis of muscle function and morphology in *junctophilin-1* knockout mice⁽⁴⁰⁾ strongly support the hypothesis that junctophilins serve to link plasma membrane voltage sensors with intracellular ion channels, particularly Ca⁺⁺ channels, especially in the SR/ER.

Interestingly, *junctophilin-3* knockout mice assessed at 1.5 to 3 months of age showed problems with balance on the rotarod test, but no evidence of other behavioral abnormalities, impaired health, neuropathology, or electrophysiological impairment. However, this analysis focused on Purkinje cells, and evidence from other mouse models of neurodegenerative diseases suggests that abnormalities may only occur in much older mice. Our preliminary data, based on observations of *junctophilin-3* knockout mice for over 18 months, suggest that the mice continue to display a slowly progressive motor disease, with eventual weight loss and early mortality. Heterozygote mice show a similar but milder pattern of deficits. It is possible that *junctophilin-4* can partially compensate for the loss of *junctophilin-3*, modulating the phenotype of *junctophilin-3* knockouts.

HDL2 PATHOGENESIS AND JUNCTOPHILIN

What is the pathogenesis of HDL2 ? The first, and as yet unanswered, question is what effect the mutation has on gene expression or protein function. One possibility, given the existence of *JPH3* exon 1-exon 2A splice variants in which the repeat is transcribed, is that the repeat expansion is expressed, and is toxic at the RNA or protein level. At the level of RNA, as in myotonic dystrophy type 1 and 2, an expanded CUG repeat at the level of RNA could exert a toxic effect⁽⁴¹⁾. This seems less likely since the HDL2 repeat expansion is considerably

shorter than either form of myotonic dystrophy. At the protein level, long polyalanine repeats aggregate and are toxic in cell culture^(42,43). HDL2 intranuclear inclusions might arise from expanded polyalanine or poly-leucine repeats, which could adopt a conformation detectable by the 1C2 antibody.

Another possibility is that the repeat expansion might alter *JPH3* expression, either by influencing transcription or translation (see, for instance, the effect of the intronic GAA repeat on gene expression in Friedreich's ataxia⁽⁴⁴⁾, or by altering the normal pattern of splice variants⁽⁴⁵⁾. It seems possible that changes in *JPH3* expression could alter calcium flux, raising the possibility of an excitotoxic mechanism of cell death, and potentially other mechanisms linked to metabolic instability.

If HDL2 is a result of a change in *JPH3* expression, how is it possible to explain inclusions that stain with an antibody supposedly specific for polyglutamine expansions ? Part of the explanation derives from recognition that 1C2 stains inclusions in other diseases that are not caused by polyglutamine expansions, including hyaline inclusion disease⁽⁴⁶⁾ and oculopharyngeal muscular dystrophy⁽⁴⁷⁾. Inclusions are found in many neurodegenerative disorders; it may be that certain types of inclusions contain non-specific epitopes which resemble expanded polyglutamine. Alternatively, 1C2-positive inclusions may reflect the specific recruitment of normal proteins that contain relatively long stretches of polyglutamine, such as TBP. A better understanding of the nature of HDL2 inclusions might shed additional light on HDL2 inclusions.

CONCLUSION

Much remains to be learned about HDL2. It is now clear that HDL2 is rare, primarily (if not exclusively) a disease of individuals of African descent, and clinically and neuropathologically nearly identical to HD. The disease is caused by a CAG/CTG repeat expansion in an alternatively spliced exon of *JPH3* on chromosome 16q24.3. The length of repeat is associated with onset age and potentially with clinical presentation. The mechanism by which the repeat expansion causes disease is

unknown, but it now seems very unlikely that HDL2 results from polyglutamine expansion. A loss of function mechanism is speculative, but remains of interest due to the putative role of *JPH3* in modulating calcium flux.

ACKNOWLEDGMENTS

The authors would like to thank Ms. Amira Pavlovich and Drs. Elizabeth O'Hearn, Adam Rosenblatt, and Christopher A. Ross for essential contributions to the study of HDL2. We would also like to express our appreciation to the families who have volunteered to participate in our research studies. This work was funded by a Lieberman Award from the Hereditary Disease Foundation to R.L.M., and NIH NS16375.

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