# Juvenile Huntington Disease: towards better understanding its unique disease characteristics

Bakels, H.S.

# CHAPTER 1

## Introduction & Aims

_____

# INTRODUCTION

Huntington Disease is an autosomal dominant inherited brain disorder caused by a pathologically expanded Cytosine-Adenine-Guanine (CAG)-repeat (≥36) in the Huntingtin (HTT) gene on the short arm of chromosome 4 (4p16.3).[1] The expanded CAG-repeat codes for a polyglutamine (polyQ) stretch in exon 1 of the Huntingtin protein which causes the deposition of huntingtin protein (HTT) N-terminal fragments.[2] The repeat sequence is unstable and therefore prone to expansion, resulting in anticipation over subsequent generations.[3] This is particularly the case when the expanded gene is inherited via the paternal line.[4]

HD pathology is characterized by gradual atrophy, reactive changes and aggregates in the brain, most prominent in the neostriatum but subsequently evident in other deep brain structures, neocortex, brainstem, and cerebellum as well.[5,6] As in gametogenesis, somatic CAG-repeat instability is seen in all affected brain areas.[3,7,8]

HD is a rare disorder, with an estimated prevalence of 4-6 per 100,000 in the Caucasian population.[9,10] Clinically, patients present with a variety of neurological symptoms. These are mainly in motor, neurocognitive and psychiatric domains, but can also be experienced in autonomic and metabolic domains.[11,12] Being an inherited disorder, all HD-Expanded Gene Carriers (HDEGC) carry the expansion in the HTT gene ever since conception. Yet the mean age at which HDEGC become clinically manifest is between 30-50 years, with a wide range of 1.5 – 90 years.[11,13,14] The age at disease onset of HD is negatively correlated with the expanded HTT CAG-repeat. The mean survival after clinical onset is 17-20 years.[11] The most common cause of death is pneumonia, followed by suicide.[11] Apart from symptomatic treatments that may alleviate some of the symptoms that are seen in HD patients, there is currently no cure for the disease.[15]

## Juvenile-onset and Pediatric HD

Juvenile-onset Huntington Disease (JHD) is an arbitrarily defined term that represents a small and heterogeneous group of HD patients with motor disease onset ≤20 years of age, who are thought to represent approximately 1-5% of the total number of clinically manifest HD patients.[16,17] JHD patients can be grossly subdivided in childhood-onset JHD (cJHD; onset between 0-10 years of age) and adolescent-onset JHD (aJHD; onset between 11 and 20 years of age) based on differences

in developmental stage, clinical disease characteristics, disease progression and survival (Figure 1).[18]

In recent years, there has been debate concerning the definition and use of nomenclature for the JHD population, which was mainly driven by the presumed number of JHD patients and the forthwith need to come with a pediatric investigation plan for therapeutical trials in pediatric HD patients (≤17 years).[19] This led to the introduction of the new term 'Pediatric Huntington Disease '(PHD), which is used to refer to a proportion of JHD patients with clinically manifest disease and who are still under the age of 18 years (Figure 1).[19] The term PHD therefore excludes JHD patients with disease onset in the pediatric age range, but who have aged into adulthood. Up to now, it is unknown what proportion of JHD patients falls under the PHD category, but based on the prevalence estimates for the (J)HD population, it is expected to be low. In turn, this outcome largely influences the way investigational trials should be designed in both the JHD and PHD population.
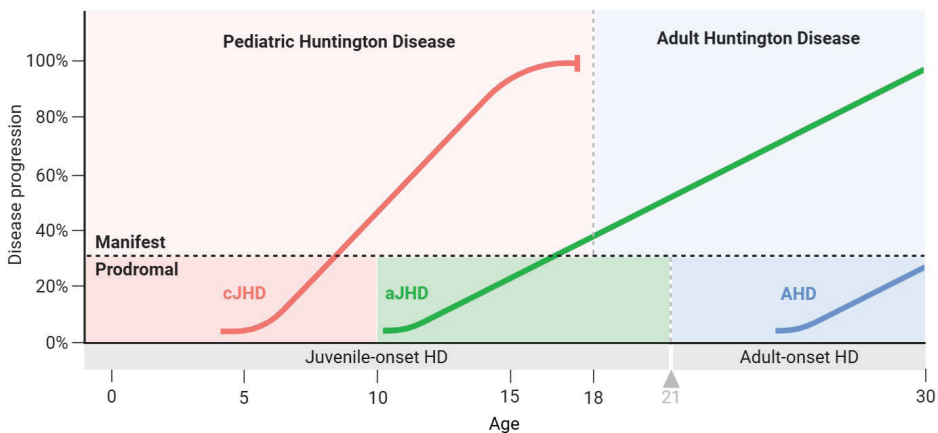


**Figure 1.** Graphic illustration for Pediatric and Age at Onset-defined HD subtypes.

The term JHD relates a certain age at onset of clinical disease characteristics. The JHD population can be subdivided in childhood-onset (red bar) and adolescent-onset (green bar) JHD patients. Note the steeper slope of disease progression and shorter survival in cJHD patients as compared to aJHD and AHD patients (blue bar). cJHD patients do not reach adulthood in many cases. The term Pediatric Huntington Disease (PHD) is only classified for clinically manifest JHD patients that are still in the pediatric age range. Note the trajectory of aJHD patients that can be referred to as a PHD patient at one point in time, and un adult having clinically manifest Huntington Disease in another point of time.

This figure has been created with Biorender.com (2023) by H. Bakels for the purpose of the current thesis.

## Genotype-Phenotype correlation

The age at disease onset and severity of HD is negatively correlated with the expanded HTT CAG-repeat, explaining approximately 60% of variability in age onset in Adult-onset HD (AHD) cohorts and up to 84% in a JHD cohort.[20] CAG-repeats ranging between 36 and 39 may give rise to an HD phenotype, generally on geriatric age, and are referred to as reduced-penetrance HD-causing alleles.[21] Assuming a normal life span, CAG-repeats ≥40 invariably lead to an HD phenotype. Approximately 50% of JHD cases have a CAG ≥60, even exceeding 80 CAGs in ultra-rare cJHD cases.[22] Although JHD cases with CAG-repeats in the lower abnormal CAG-range (CAG 40-50) have been described,[14] the likelihood of developing a JHD phenotype exceeds 5% in case of a CAG ≥51.[23]

Other genetic modifiers influencing age at onset and disease severity consist of cis-acting loss-of mHTT CAA-interruption,[24] and trans-acting single nucleotide polymorphisms (SNPs) in DNA-repair genes (e.g. FAN1, MLH1, MSH3) driving the rate of somatic CAG-repeat instability.[25] In addition, it has been suggested that the relative size of CAG-repeat length on the physiological and mutant HTT allele potentially causes dominant negative loss-of normal HTT function and is, therefore, another genetic factor affecting the clinical phenotype.[26]

## Problem definition

JHD is a rare subtype that represents one extreme end of the HD spectrum. As we have entered the era of investigational therapies aiming to modify disease progression in HD patients,[15] there are a number of open questions that require answering so that the JHD population is not left behind in the badly needed treatment options that are currently being investigated. From what is currently known largely based on JHD case series, disease characteristics in the JHD population do not always align with what is known in the prototypical adult-onset HD (AHD) form of the disease. However, structural comparison between these Age at Onset-defined HD (AO-HD) subtypes has been sparsely performed. This comparison is needed to better understand underlying causes for such differences, to investigate if (standardized) investigational methods are reliable in the JHD population and, subsequently, how to treat this particular population. Therefore, the main research question driving this thesis was: "How do the JHD subtypes relate to the continuum of HD disease characteristics and are there instances in which we should address it as a separate disease entity?" In the following two paragraphs we will address this research

question more specifically in relation to the phenotype of JHD and the function and pathomechanisms of the (mutant) Huntingtin gene.

## Clinical phenotype

HD is characterized by motor, neurocognitive, psychiatric and behavioral symptoms, leading to loss of independence and eventually death.[11] JHD patients are not different from AHD patients in this perspective, but differences in the order and severity of symptoms and signs are eminent. In addition, certain atypical disease characteristics are specifically seen in JHD patients. In general, JHD patients have an early onset of hypokinetic-rigid syndrome including dystonia, neurocognitive - and behavioral changes.[27] In contrast, the prevalence of chorea is lower in the JHD population.[18] Yet from this clinical perspective, the distinction between the cJHD and aJHD subtype becomes more relevant. As said, there are clear differences between these JHD subtypes in relation to the developmental stage these patients are in, the appearance of clinical disease characteristics and the severity and progression of the phenotype. Whereas aJHD patients are thought to be in closer clinical resemblance with the AHD population, part of cJHD patients present with an atypical and more severe form of the disease in general. This is mirrored by an early onset of disease with neurodevelopmental delays or regression as presenting disease characteristic, more severe and faster progression of motor symptoms over time, epilepsy, and a resulting shorter survival with death often occurring before reaching adulthood.[14,18,27]

There is a lack of data comparing Age at Onset-defined Huntington's Disease (AO-HD) subtypes in terms of prevalence, severity, and progression of clinical features. Such comparisons are essential to understand the underlying causes of these differences, including developmental stage and CAG-repeat length-dependent pathomechanisms. These clinical differences have important implications for preparing future treatments aimed at modifying disease progression. Key questions remain regarding the ability of JHD and PHD populations to participate in therapeutic trials, as well as the applicability of prediction models, assessment tools, and biomarkers that are only validated for adult HD populations.

## Huntingtin

*HTT* is a highly conserved gene and the HTT protein has an important function in neurodevelopment. It has been reported to play a role in neuroectoderm formation,[28]

neurogenesis,[29] spindle orientation,[30,31] endocytosis,[32] transcriptional regulation,[33] functional circuitry orchestration[34] and maintenance of cell morphology.[35,36] A neurodevelopmental mechanism-of-interaction involving Brain-Derived Neurotrophic Factor (BDNF) has been proposed through the interaction of HTT with Huntingtin-associated protein 1.[37] BDNF is an important regulator of apoptosis and differentiation in neurons.[38] The CAG-repeat sequence in the *HTT* gene is located in exon1 and the N-terminus of the protein contains 3 domains. First there is a 17 amino acid tail H(HTT$^{NT}$) that is followed by the variable CAG$^n$-CAA-CAG-repeat sequence coding for the polyQ domain and thereafter a variably long proline-rich domain (PRD).[39] Functions thought to relate to HTT exon1 are membrane targeting,[40] chaperone binding,[41-43] nuclear export and trafficking,[44,45] regulatory post translational modifications,[46] serving as a structural base for oligomer formation,[39,47,48] and protein binding.[49] It has been hypothesized that increasing the *HTT* CAG-repeat in the physiological human range (13-35) exerts advantageous effects on gene and therefore brain function.[50-52]

A multitude of molecular mechanisms, through which mutant HTT (mHTT) causes HD pathogenesis, have been postulated over the years.[53] A dominant toxic gain-of-function hypothesis of mHTT has been the main line of reasoning and involves conformational mHTT protein changes causing the deposition of mHTT N-terminal fragments and protein aggregation.[2,54,55] This protein accumulation together with oxidative stress, inflammation and transcriptional deregulation are thought to be the most important mechanisms through which toxicity leads to regional cell dysfunction and subsequently loss and atrophy.[15,39] From what is known in relation to the JHD phenotype, neuropathological disease characteristics are generally more severe and widespread when compared to the AHD phenotype.[56] Questions remain, however, how this relates to clinical measures of disease progression, such as clinical disease burden and disease duration. Additionally, loss or modulation of physiological HTT function through dominant-negative loss-of-function effects is likely to contribute to the clinical picture of HD as well.[29,50,57] As described above, HTT function is essential for neurodevelopment and aberrations in this process can potentially cause a variety of clinical disease characteristics. More importantly, JHD patients not only experience clinical disease characteristics during postnatal brain development, they also more often experience clinical disease characteristics that relate to faulty neurodevelopment, such as developmental delay, epilepsy and behavioral disorders. This directly highlights the importance of a pathophysiological perspective to the JHD phenotype. This perspective raises questions as to (1) what pathomechanisms

contribute to a certain disease characteristic, (2a) how differences between AO-HD phenotypes are caused by different contributions of pathomechanisms or (2b) by differences in the interaction of ongoing neurodevelopmental processes with concurrent pathomechanisms in pediatric HD cases.

## AIMS

This thesis focuses on the JHD and PHD population, using a translational approach to address questions regarding their epidemiology, clinical characteristics, neuropathology, and pathophysiology, in comparison to prototypical HD in adults. The epidemiology and competence of the JHD and PHD population to participate in therapeutical trials was explored (**Chapter 2**). The known clinical and neuropathological differences between JHD subtypes and AHD were reviewed and placed in a pathophysiological and neurodevelopmental perspective (**Chapter 3**). We performed comparative analyses on the occurrence, severity and progression of clinical characteristics between cJHD, aJHD and AHD cases (**Chapter 4**). We offer insight in the neuropathology of an aJHD brain donor who died mid-stage disease (**Chapter 5**). Subsequently, neuropathologic changes in the glucose transporter GLUT1 were found in the brains of cJHD donors, in contrast to findings in aJHD and AHD brain donors (**Chapter 6**). Finally, we discuss our study results in relation to the broader overarching perspective and offer future directions for JHD-related research (**Chapter 7**).

# REFERENCES

1. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell*. Mar 26 1993;72(6):971-83. doi:10.1016/0092-8674(93)90585-e

2. DiFiglia M, Sapp E, Chase KO, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*. Sep 26 1997;277(5334):1990-3. doi:10.1126/science.277.5334.1990

3. Telenius H, Kremer B, Goldberg YP, et al. Somatic and gonadal mosaicism of the Huntington disease gene CAG-repeat in brain and sperm. *Nat Genet*. Apr 1994;6(4):409-14. doi:10.1038/ng0494-409

4. Telenius H, Almqvist E, Kremer B, et al. Somatic mosaicism in sperm is associated with intergenerational (CAG)n changes in Huntington disease. *Hum Mol Genet*. Feb 1995;4(2):189-95. doi:10.1093/hmg/4.2.189

5. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol*. Nov 1985;44(6):559-77. doi:10.1097/00005072-198511000-00003

6. Rub U, Seidel K, Heinsen H, Vonsattel JP, den Dunnen WF, Korf HW. Huntington's disease (HD): the neuropathology of a multisystem neurodegenerative disorder of the human brain. *Brain Pathol*. Nov 2016;26(6):726-740. doi:10.1111/bpa.12426

7. Kacher R, Lejeune FX, Noel S, et al. Propensity for somatic expansion increases over the course of life in Huntington disease. *Elife*. May 13 2021;10doi:10.7554/eLife.64674

8. Mätlik K, Baffuto M, Kus L, et al. Cell-type-specific CAG-repeat expansions and toxicity of mutant Huntingtin in human striatum and cerebellum. *Nat Genet*. Mar 2024;56(3):383-394. doi:10.1038/s41588-024-01653-6

9. Baig SS, Strong M, Quarrell OW. The global prevalence of Huntington's disease: a systematic review and discussion. *Neurodegener Dis Manag*. Aug 2016;6(4):331-43. doi:10.2217/nmt-2016-0008

10. Medina A, Mahjoub Y, Shaver L, Pringsheim T. Prevalence and Incidence of Huntington's Disease: An Updated Systematic Review and Meta-Analysis. *Mov Disord*. Dec 2022;37(12):2327-2335. doi:10.1002/mds.29228

11. Roos RA. Huntington's disease: a clinical review. *Orphanet J Rare Dis*. Dec 20 2010;5:40. doi:10.1186/1750-1172-5-40

12. Aziz NA, Swaab DF, Pijl H, Roos RA. Hypothalamic dysfunction and neuroendocrine and metabolic alterations in Huntington's disease: clinical consequences and therapeutic implications. *Reviews in the neurosciences*. 2007;18(3-4):223-51. doi:10.1515/revneuro.2007.18.3-4.223

13. Moeller AA, Felker MV, Brault JA, Duncan LC, Hamid R, Golomb MR. Patients With Extreme Early Onset Juvenile Huntington Disease Can Have Delays in Diagnosis: A Case Report and Literature Review. *Child neurology open*. Jan-Dec 2021;8:2329048x211036137. doi:10.1177/2329048x211036137

14. Squitieri F, Frati L, Ciarmiello A, Lastoria S, Quarrell O. Juvenile Huntington's disease: does a dosage-effect pathogenic mechanism differ from the classical adult disease? *Mech Ageing Dev*. Feb 2006;127(2):208-12. doi:10.1016/j.mad.2005.09.012

15. Tabrizi SJ, Estevez-Fraga C, van Roon-Mom WMC, et al. Potential disease-modifying therapies for Huntington's disease: lessons learned and future opportunities. *Lancet Neurol*. Jul 2022;21(7):645-658. doi:10.1016/s1474-4422(22)00121-1

16. Quarrell O, O'Donovan KL, Bandmann O, Strong M. The Prevalence of Juvenile Huntington's Disease: A Review of the Literature and Meta-Analysis. *PLoS Curr*. Jul 20 2012;4:e4f8606b742ef3. doi:10.1371/4f8606b742ef3

17. Achenbach J, Saft C. Data from ENROLL-HD: Is the prevalence of juvenile and pediatric Huntington's disease overestimated? *Parkinsonism Relat Disord*. Jul 2021;88:1-2. doi:10.1016/j.parkreldis.2021.05.012

18. Fusilli C, Migliore S, Mazza T, et al. Biological and clinical manifestations of juvenile Huntington's disease: a retrospective analysis. *Lancet Neurol*. Nov 2018;17(11):986-993. doi:10.1016/S1474-4422(18)30294-1

19. Quarrell OWJ, Nance MA, Nopoulos P, et al. Defining pediatric huntington disease: Time to abandon the term Juvenile Huntington Disease? *Mov Disord*. Apr 2019;34(4):584-585. doi:10.1002/mds.27640

20. Schultz JL, Moser AD, Nopoulos PC. The Association between CAG-repeat Length and Age of Onset of juvenile-onset Huntington's Disease. *Brain Sci*. Aug 20 2020;10(9)doi:10.3390/brainsci10090575

21. Kay C, Collins JA, Miedzybrodzka Z, et al. Huntington disease reduced penetrance alleles occur at high frequency in the general population. *Neurology*. Jul 19 2016;87(3):282-8. doi:10.1212/wnl.0000000000002858

22. Quarrell OWJ, Nance M. The diagnostic challenge. *Juvenile Huntington's Disease*. Oxford University Press; 2009:136-149.

23. Brinkman RR, Mezei MM, Theilmann J, Almqvist E, Hayden MR. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. *Am J Hum Genet*. May 1997;60(5):1202-10.

24. Wright GEB, Collins JA, Kay C, et al. Length of Uninterrupted CAG, Independent of Polyglutamine Size, Results in Increased Somatic Instability, Hastening Onset of Huntington Disease. *Am J Hum Genet*. Jun 6 2019;104(6):1116-1126. doi:10.1016/j.ajhg.2019.04.007

25. Moss DJH, Pardinas AF, Langbehn D, et al. Identification of genetic variants associated with Huntington's disease progression: a genome-wide association study. *Lancet Neurol*. Sep 2017;16(9):701-711. doi:10.1016/S1474-4422(17)30161-8

26. Aziz NA, Jurgens CK, Landwehrmeyer GB, et al. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. *Neurology*. Oct 20 2009;73(16):1280-5. doi:10.1212/WNL.0b013e3181bd1121

27. Cronin T, Rosser A, Massey T. Clinical Presentation and Features of juvenile-onset Huntington's Disease: A Systematic Review. *J Huntingtons Dis*. 2019;8(2):171-179. doi:10.3233/jhd-180339

28. Lo Sardo V, Zuccato C, Gaudenzi G, et al. An evolutionary recent neuroepithelial cell adhesion function of huntingtin implicates ADAM10-Ncadherin. *Nature Neuroscience*. 2012/05/01 2012;15(5):713-721. doi:10.1038/nn.3080

29. Barnat M, Capizzi M, Aparicio E, et al. Huntington's disease alters human neurodevelopment. *Science*. Aug 14 2020;369(6505):787-793. doi:10.1126/science.aax3338

30. Godin JD, Colombo K, Molina-Calavita M, et al. Huntingtin is required for mitotic spindle orientation and mammalian neurogenesis. *Neuron*. Aug 12 2010;67(3):392-406. doi:10.1016/j.neuron.2010.06.027

31. Ruzo A, Croft GF, Metzger JJ, et al. Chromosomal instability during neurogenesis in Huntington's disease. *Development (Cambridge, England)*. Jan 29 2018;145(2)doi:10.1242/dev.156844

32. Mackenzie KD, Lim Y, Duffield MD, Chataway T, Zhou XF, Keating DJ. Huntingtin-associated protein-1 (HAP1) regulates endocytosis and interacts with multiple trafficking-related proteins. *Cellular signalling*. Jul 2017;35:176-187. doi:10.1016/j.cellsig.2017.02.023

33. Thomas EA, Coppola G, Tang B, et al. In vivo cell-autonomous transcriptional abnormalities revealed in mice expressing mutant huntingtin in striatal but not cortical neurons. *Hum Mol Genet*. Mar 15 2011;20(6):1049-60. doi:10.1093/hmg/ddq548

34. McKinstry SU, Karadeniz YB, Worthington AK, et al. Huntingtin is required for normal excitatory synapse development in cortical and striatal circuits. *J Neurosci*. Jul 9 2014;34(28):9455-72. doi:10.1523/jneurosci.4699-13.2014

35. Nguyen GD, Gokhan S, Molero AE, Mehler MF. Selective roles of normal and mutant huntingtin in neural induction and early neurogenesis. *PLoS One*. 2013;8(5):e64368. doi:10.1371/journal.pone.0064368

36. Cepeda C, Oikonomou KD, Cummings D, et al. Developmental origins of cortical hyperexcitability in Huntington's disease: Review and new observations. *Journal of neuroscience research*. Dec 2019;97(12):1624-1635. doi:10.1002/jnr.24503

37. Gauthier LR, Charrin BC, Borrell-Pagès M, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell*. Jul 9 2004;118(1):127-38. doi:10.1016/j.cell.2004.06.018

38. Zuccato C, Ciammola A, Rigamonti D, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science*. Jul 20 2001;293(5529):493-8. doi:10.1126/science.1059581

39. Bates GP, Dorsey R, Gusella JF, et al. Huntington disease. *Nat Rev Dis Primers*. Apr 23 2015;1:15005. doi:10.1038/nrdp.2015.5

40. Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R. Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. *Hum Mol Genet*. Nov 1 2007;16(21):2600-15. doi:10.1093/hmg/ddm217

41. Aiken CT, Steffan JS, Guerrero CM, et al. Phosphorylation of threonine 3: implications for Huntingtin aggregation and neurotoxicity. *The Journal of biological chemistry*. Oct 23 2009;284(43):29427-36. doi:10.1074/jbc.M109.013193

42. Tam S, Spiess C, Auyeung W, et al. The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. *Nature structural & molecular biology*. Dec 2009;16(12):1279-85. doi:10.1038/nsmb.1700

43. Kuiper EF, de Mattos EP, Jardim LB, Kampinga HH, Bergink S. Chaperones in Polyglutamine Aggregation: Beyond the Q-Stretch. *Front Neurosci*. 2017;11:145. doi:10.3389/fnins.2017.00145

44. Cornett J, Cao F, Wang CE, et al. Polyglutamine expansion of huntingtin impairs its nuclear export. *Nat Genet*. Feb 2005;37(2):198-204. doi:10.1038/ng1503

45. Rockabrand E, Slepko N, Pantalone A, et al. The first 17 amino acids of Huntingtin modulate its sub-cellular localization, aggregation and effects on calcium homeostasis. *Hum Mol Genet*. Jan 1 2007;16(1):61-77. doi:10.1093/hmg/ddl440

46. Steffan JS, Agrawal N, Pallos J, et al. SUMO modification of Huntingtin and Huntington's disease pathology. *Science*. Apr 2 2004;304(5667):100-4. doi:10.1126/science.1092194

47. Jayaraman M, Kodali R, Sahoo B, et al. Slow amyloid nucleation via α-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. *J Mol Biol*. Feb 3 2012;415(5):881-99. doi:10.1016/j.jmb.2011.12.010

48. Thakur AK, Jayaraman M, Mishra R, et al. Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nature structural & molecular biology*. Apr 2009;16(4):380-9. doi:10.1038/nsmb.1570

49. Faber PW, Barnes GT, Srinidhi J, Chen J, Gusella JF, MacDonald ME. Huntingtin interacts with a family of WW domain proteins. *Hum Mol Genet*. Sep 1998;7(9):1463-74. doi:10.1093/hmg/7.9.1463

50. van der Plas E, Schultz JL, Nopoulos PC. The Neurodevelopmental Hypothesis of Huntington's Disease. *J Huntingtons Dis*. 2020;9(3):217-229. doi:10.3233/jhd-200394

51. Caron NS, Desmond CR, Xia J, Truant R. Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin. *Proc Natl Acad Sci U S A*. Sep 3 2013;110(36):14610-5. doi:10.1073/pnas.1301342110

1

52. Lee JK, Ding Y, Conrad AL, et al. Sex-specific effects of the Huntington gene on normal neurodevelopment. *Journal of neuroscience research*. Jan 2 2017;95(1-2):398-408. doi:10.1002/jnr.23980

53. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol*. Jan 2011;10(1):83-98. doi:10.1016/s1474-4422(10)70245-3

54. Hickman RA, Faust PL, Marder K, Yamamoto A, Vonsattel JP. The distribution and density of Huntingtin inclusions across the Huntington disease neocortex: regional correlations with Huntingtin repeat expansion independent of pathologic grade. *Acta Neuropathol Commun*. Apr 19 2022;10(1):55. doi:10.1186/s40478-022-01364-1

55. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature*. Oct 14 2004;431(7010):805-10. doi:10.1038/nature02998

56. Vonsattel JPG, Etty P. Cortes, Keller CE. Juvenile Huntington's Diseaseand other trinucleotide repeat disorders. *Juvenile Huntington's disease: neuropathology*. Oxford University Press; 2012.

57. Wiatr K, Szlachcic WJ, Trzeciak M, Figlerowicz M, Figiel M. Huntington Disease as a Neurodevelopmental Disorder and Early Signs of the Disease in Stem Cells. *Mol Neurobiol*. Apr 2018;55(4):3351-3371. doi:10.1007/s12035-017-0477-7