Juvenile Huntington Disease: towards better understanding its unique disease characteristics

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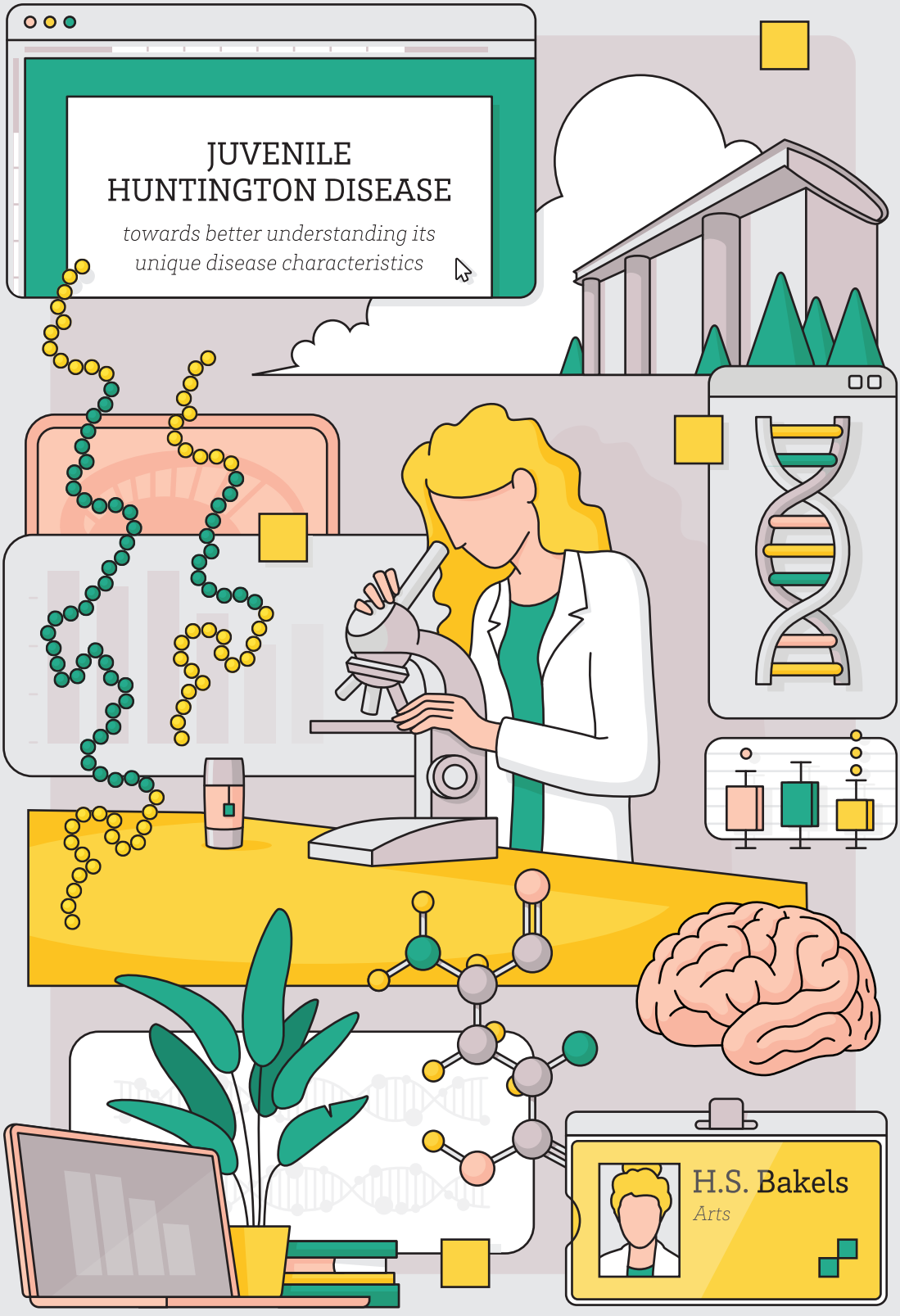
JUVENILE
HUNTINGTON DISEASE
towards better understanding its
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H.S. Bakels
Arts

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Hannah S. Bakels

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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.

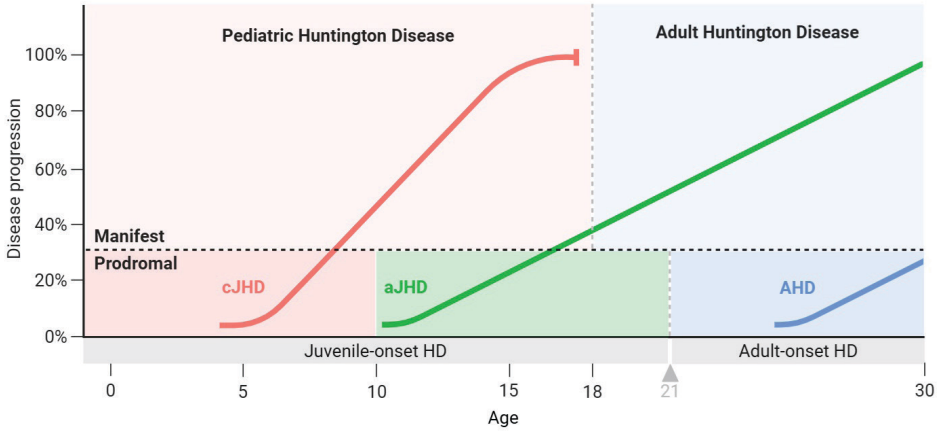

Figure 1. Graphic illustration for Pediatric and Age at Onset-defined HD subtypes.

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

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

CHAPTER 2

Prevalence of juvenile-onset and pediatric Huntington Disease epidemiology and competence for interventional trials: a Dutch population and Enroll-HD observational study

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ABSTRACT

Background: Juvenile-onset Huntington's disease (JHD) represents 1–5% of Huntington's disease (HD) patients, with onset before the age of 21. Pediatric HD (PHD) relates to a proportion of JHD patients that is still under 18 years of age. So far, both populations have been excluded from interventional trials.

Objective: Describe the prevalence and incidence of JHD and PHD in the Netherlands and explore their ability to participatein interventional trials.

Methods: The prevalence and incidence of PHD and JHD patients in the Netherlands were analyzed. In addition, we explored proportions of JHD patients diagnosed at pediatric versus adult age, their diagnostic delay, and functional and modelled (CAP100) disease stage in JHD and adult-onset HD patients at diagnosis.

Results: The prevalence of JHD and PHD relative to the total manifest HD population in January 2024 was between 0.84–1.25% and 0.09–0.14% respectively. The mean incidence of JHD patients being diagnosed was between 0.85–1.28 per 1000 patient years and of PHD 0.14 per 1.000.000 under-aged person years. 55% of JHD cases received a clinical diagnosis on adult age. At diagnosis, the majority of JHD patients was functionally compromised and adolescent-onset JHD patients were significantly less independent compared to adult-onset HD patients.

Conclusions: In the Netherlands, the epidemiology of JHD and PHD is lower than previously suggested. More than half of JHD cases are not eligible for trials in the PHD population. Furthermore, higher functional dependency in JHD patients influences their ability to participate in trials. Lastly, certain UHDRS functional assessments and the CAP100 score do not seem appropriate for this particular group.

INTRODUCTION

Juvenile-onset Huntington's disease (JHD) represents a small group of Huntington's disease (HD) patients with motor disease onset≤20 years of age. JHD patients can be subdivided in childhood-onset JHD (cJHD; onset between 0–10 years) and adolescent-onset JHD (aJHD; onset between 11 and 20 years).[1,2] The age at disease onset in HD is negatively correlated with the causal number of CAG-repeats in in the Huntingtin (HTT) gene (≥36), explaining approximately 60% of variability in adult-onset HD (AHD) and up to 84% in JHD.[3] Approximately 50% of JHD cases have a CAG≥60, even exceeding 80 CAGs in rare cJHD cases.[4] Although there are JHD cases reported with CAG-repeats in the lower abnormal CAG-range (CAG 40–50),[5] the likelihood of developing a juvenile phenotype exceeds 5% in case of a CAG≥51.[6] JHD patients are thought to represent approximately 1–5% of the total number of clinically manifest HD patients.[7,8] This, together with an estimated mean prevalence of 4–6 clinical HD patients per 100.000 in the Western European population,[9–11] indicates that the number of JHD patients is very low. The majority of JHD patients represents aJHD, with an estimated proportion of 4.4%, and as little as 1.3% represents cJHD patients.[7]

Over the years a variety of studies, reviews and meta-analyses reported the epidemiology of (J)HD, which is subject to constant change such as earlier recognition and diagnosis. One recent development that relies on the number of JHD patients is the removal of the European Medical Agency (EMA) class waiver for pediatric patients in the HD population, dictating a pediatric investigation plan for the study of new therapeutic strategies.[12] Pediatric HD (PHD) refers to a proportion of JHD patients that is below the age of 18 years, as opposed to JHD patients that became clinically manifest before that age but that have grown into adulthood (≥18 years).[12] Up to now there has been one study analyzing the number of PHD patients in the international ENROLL-HD dataset, which found proportional margins between 0.14–0.66% of the total number of manifest HD patients.[8] These numbers are even lower than may be expected from earlier epidemiology studies in the JHD population.[7] Accurate numbers of the current prevalence and incidence of the JHD population, as well as the proportion of JHD patients being diagnosed as a minor or adult, is important when it concerns the design of interventional trials in JHD or PHD patients.

Another important question is the ability of the JHD population to participate in interventional studies. Part of cJHD patients are known to have a faster disease

progression and with a shorter survival compared to AHD.[1] Together with the diagnostic delay in the pediatric population,[13–15] this may substantially influence the disease stage in which JHD patients reside when they are diagnosed and their availability to participate in interventional trials. Insight in the correlation between diagnosis on the one hand and disease stage markers, such as functional competence and by the normalized predictor CAG-Age-Product (CAP^{100}) score,[16] on the other hand, helps defining the ability of the JHD population to actually participate in interventional trials.

The aim of our study is to describe the current prevalence of PHD and JHD patients in the Netherlands relative to the entire manifest HD population and to determine 5-year incidences of the Dutch JHD and PHD population over the past 20 years. Furthermore, the availability and ability of the JHD population, to participate in interventional trials, is analyzed by 1) the proportion of JHD patients being diagnosed at a pediatric vs. adult age and 2) comparing disease stage markers at the time of diagnosis between JHD and AHD patients.

MATERIALS AND METHODS

Study design

Data from two (J)HD patient datasets was used to answer the different study goals and to enable comparison of JHD with prototypical disease onset in adulthood (AHD). The first, HD-JUNIOR, is a multi-source Dutch registry for JHD patients. HD-JUNIOR began in 2020 and consists of the following complementary datasets: 1) pseudonymized demographic and HTT genetic data from all HD expanded gene carriers with a CAG≥51 (*n*=121) that were tested in the Netherlands since 2000 and that is annually updated, combined with 2) retrospective clinical data from medical files of clinically diagnosed JHD patients (irrespective of HTT genetic status) that were derived from all HD care facilities in the Netherlands and additional medical sites by pearl-growing method (*n*=28). For this study, only cases were included where clinical data showed that the patient had a JHD phenotype. Written informed consent for the collection and use of pseudonymized clinical data was given by all living JHD patients or their caretakers. In the case of clinical data from deceased JHD patients, pseudonymized data was shared by the last treating physician. The second dataset, ENROLL-HD, is an international prospective longitudinal registry study in HD expanded gene carriers (≥36 CAG-repeats) and controls.[17] For the current study, the 5th periodic dataset (PDS5; release 18-DEC-2020; *n*=21,116 participants) was

used to retrieve genetic and clinical data from JHD and AHD patients, including a specified dataset with deaggregated data for age at enrolment below 17 years and number of CAG-repeats≥70. Data were generously provided by the participants in the Enroll-HD study and made available by CHDI Foundation, Inc. Core datasets were collected annually from all research participants as part of this multi-center longitudinal observational study. Data were monitored for quality and accuracy using a risk-based monitoring approach. All sites were required to obtain and maintain local ethical approval. In case of outcome measures with a similar assessment method in both datasets, the results for the JHD subtypes of the two different datasets were pooled provided that the baseline JHD sample characteristics of the two datasets were comparable (in total: cJHD *n*=44; aJHD *n*=120; AHD *n*=8808). Duplicate cases in the two different datasets were identified by the combination of CAG-repeat length and year of birth and corrected for in case of pooled analyses.

Study population

This study uses below defined age at onset-defined HD (AO-HD) subtypes, clinically manifest disease status and current age as grouping variables. Based on a lower prevalence of motor disease characteristics at onset in JHD patients,[18] we chose to define a JHD phenotype primarily on the basis of age at onset of any HD symptom or sign (e.g., psychiatric, neurocognitive, motor or neurodevelopmental) and subsequently on age at onset of motor symptoms, which had to occur within 5 years of first symptoms. Inclusion criteria for this study were as follows: 1) a clinical diagnosis of HD (Unified Huntington Disease Rating Scale – Total Motor Score: Disease Confidence Level of 4 →≥99% confidence motor abnormalities are unequivocal signs of disease) based on expert opinion and irrespective of CAG-repeat length, 2) onset of first symptom≤17 years of age, and 3) onset of motor symptoms≤22 years of age. Subsequently, JHD patients were subdivided in childhood-onset JHD (cJHD: primary onset≤10 and motor onset≤15 years of age) and adolescent-onset JHD phenotype (aJHD: primary onset between 11 and 17 and motor onset between 11 and 22 years of age, or a primary onset≤10 and motor onset between 16 and 22 years of age). Definition of PHD was 1) a clinical diagnosis of HD based on expert opinion and irrespective of CAG-repeat length, 2) onset of first and motor symptoms≤17 years of age, and 3) current age≤17 years. For the comparison of disease stage markers in JHD subtypes with prototypical disease onset in adulthood, eligibility criteria for an AHD phenotype in this study were based on an age at primary and or motor onset≥25 and≤60 (primary onset) /≤65 (motor

onset) years of age, to ensure that there is no overlap in disease phenotypes and to limit the influence of aging effects. See STROBE- flow diagrams for the number of eligible AO-HD defined cases in the HD-JUNIOR and ENROLL-HD datasets (Fig. 1).

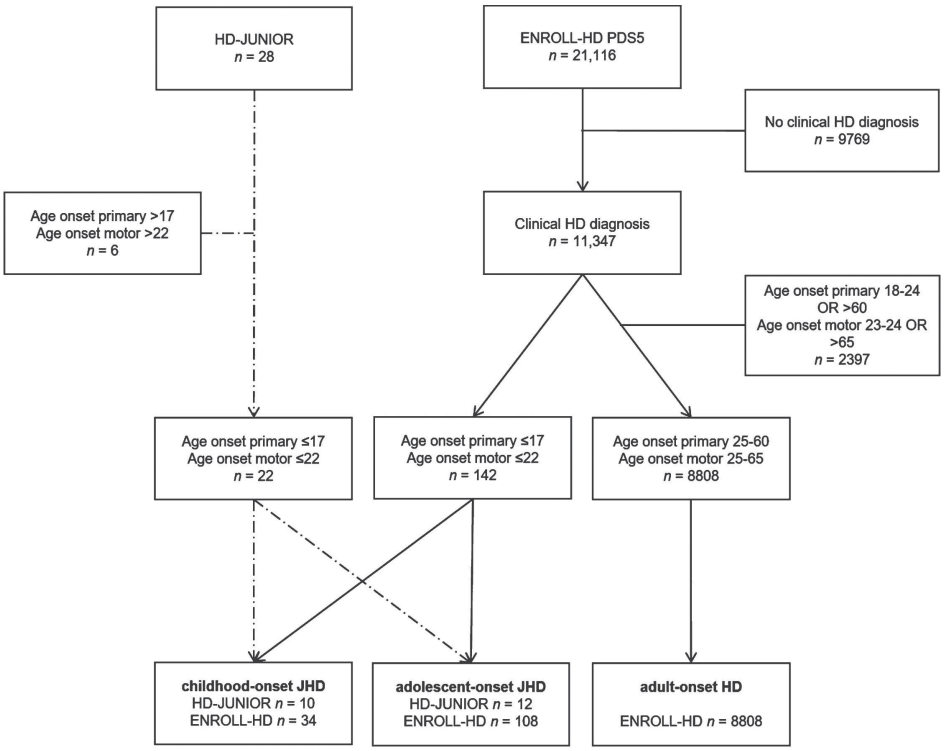

Figure 1. Patient selection of the HD-JUNIOR and ENROLL-HD PDS5 datasets.

STROBE flow diagram displaying patient selection of the HD-JUNIOR and ENROLL-HD PDS5 datasets based on the eligibility criteria for the current study and stratified by AO-HD subtype. Dashed lines represent patient selection from the HD-JUNIOR dataset into the two different JHD subtypes, straight lines represent patient selection from the ENROLL-HD PDS5 dataset into the three different AO-HD subtypes.

Outcome measures

To determine the point prevalence of the JHD and PHD population in the Netherlands, we defined the number of alive JHD and PHD individuals in the HD-JUNIOR registry at 1-JAN-2024. HD-JUNIOR is a national registry containing all genetic and demographic data (including survival) from HD expanded gene carriers with a CAG≥51 in the Netherlands, as well as verified JHD clinical data (irrespective of CAG-repeat length) retrieved from over 20 sources (find full credentials under

acknowledgements), including all specialized HD care facilities in the Netherlands, and general practitioners, revalidation institutions, regional and academic medical centers by Pearl-growing method. The non-invasive nature of HD-JUNIOR (retrospective data collection, no extra assessments, informed consent procedure via phone/e-mail) ensures a very low-threshold to participate, even for JHD patients in later disease stages. Therefore, this comprehensive multi-source dataset gives a reliable estimate of epidemiological counts of the JHD and PHD population in the Netherlands. To calculate the relative proportion of these prevalent JHD and PHD cases as part of the entire clinically manifest HD population in the Netherlands, an estimated mean prevalence of 4–6:100,000 in Western Europe,[9–11] and a total population of 17,947,684 in the Netherlands at 31-DEC-2023 were used. In addition, frequencies between 2000 and 2019 were determined for JHD patients 1) developing primary symptom onset, 2) developing motor symptom onset, 3) receiving a diagnosis, and 4) who deceased. Mean incidences of JHD diagnosis and JHD patient death were placed into perspective by the estimated total manifest HD population in the Netherlands in the same time period. In addition, the mean incidence rate of a PHD diagnosis was placed into perspective by the total under-aged (≤17 years) general population in the Netherlands in the same time period. Incidence rates were compared in 4 consecutive time intervals: from 2000 through 2004; from 2005 through 2009; from 2010 through 2014; and from 2015 through 2019.

For the definition of age and disease duration at diagnosis we used data from the HD-JUNIOR and ENROLL-HD dataset. Both datasets contain retrospectively collected data specifying the age or year at which a certain individual experienced the first symptom or received his/her clinical diagnosis based on expert opinion. We used age to dichotomize between patients receiving their diagnosis on pediatric age (≤17 years) and patients receiving their diagnosis on adult age (≥18 years). For individuals of the HD-JUNIOR dataset, the number of JHD patients that received their genetic status via preclinical genetic testing was additionally specified. Because ‘preclinical’ genetic testing in the Netherlands is only available for adult HD expanded-gene at risk individuals, these JHD cases received their genetic status prior to receiving a clinical diagnosis, but both on an adult age, and were labeled as JHD in retrospect. Data regarding preclinical genetic testing in the PDS5 of ENROLL-HD were not available. To compare AO-HD subtypes on diagnostic delay, we used disease duration between first symptom and clinical HD diagnosis, as captured retrospectively in both datasets.

To analyze the ability of JHD patients to participate in clinical trials, disease stage markers at the time of diagnosis were explored and, where possible, compared with those of AHD patients at diagnosis. The first, functional capacity, is a common measure to reflect the severity or disease stage in clinically manifest HD.[19–21] HD-JUNIOR data relating to functional capacity at diagnosis in JHD patients, were retrieved retrospectively from multi-source medical files carrying unspecified data from anamnesis or care taker reports. For the current study, we used all data that indicated a decline in skills, the need for help or the need to give up previously established activities such as education or work (cJHD *n*=5; aJHD *n*=8). To assess functional capacity in the ENROLL-HD dataset, prospective data from the Unified Huntington Disease Rating Scale– Independence Score (UHDRS-IS) [22] in aJHD (*n*=33) and AHD (*n*=3186) participants was used and compared, in case this data was captured within one year of receiving a clinical HD diagnosis. Because only four cJHD participants in ENROLL-HD had these data available within one year of diagnosis, these data were only described but not included in AO-HD subtype comparison. We deliberately chose not to include functional measures that were designed for adult participants. Particularly the UHDRS– Total Functional Capacity (UHDRS-TFC) and part of the UHDRS-Functional Assessment Scale (UHDRS-FAS) are not suited for pediatric participants as it focuses on outcomes that are generally not applicable to the pediatric population, such as working ability, finances and doing domestic chores.[22] A second disease stage measure used was the CAG-Age Product (CAP) formula that is commonly used as a predictor for HD disease progression and reflects the cumulative exposure to the effects of mutant huntingtin by the interaction of CAG-repeat length and age. For the current study we calculated the CAP^{100} score,[16] at 1) primary symptom onset, 2) motor symptom onset, and 3) HD clinical diagnosis by the formula: AGE * (CAG - 30) / 6.49. This CAP formula is normalized for CAG-repeat lengths up to 50, so that the CAP score approximates 100 when HD patients generally receive their clinical diagnosis, henceCAP.[100]

Statistics

IBM SPSS Statistics version 29.0.0.0 (241) was used for statistical analyses. Outcome measures and patient characteristics were described using mean and standard deviation if they were approximately normally distributed or median and interquartile range (IQR) otherwise. Prevalence frequencies and proportions were calculated and 95% Confidence Intervals (CI) for proportions were calculated.

Frequencies, mean and 95% CI were calculated to determine the incidence rate of JHD diagnosis and death in relation to the total clinically manifest HD population and the incidence rate of PHD diagnosis in relation to the total under-aged general population in the Netherlands (PHD). 95% CI for the means of normally distributed data was used in case of the CAP^{100} score.

For between group comparison of the incidence, disease duration, UHDRS-IS and CAP^{100} outcome measures one-way ANOVA was performed and p-values<0.05 were considered significant. In case of multiple testing, 95% CI and p-values were adjusted for multiple testing by Benjamini Hochberg method. In case of non-normal distribution, the outcome measure was log10-transformed. This was done for disease duration and UHDRS-IS score.

RESULTS

Patient characteristics

The number of eligible subjects in the HD-JUNIOR and ENROLL-HD dataset and stratified by AO-HD subtype are provided in Table 1. The included patient characteristics for sex, age at onset of primary and motor symptoms and CAG-repeat length did not significantly differ between the datasets. Therefore, results for JHD subtypes from the two different datasets were pooled in case of a comparable measurement method. These measures included disease duration at diagnosis and CAP^{100} score over time.

Table 1. Patient characteristics per AO-HD subtype and dataset

	Childhood-onset JHD			**Adolescent-onset JHD**			**Adult-onset HD**
	HD-JUNIOR (n=10)	**ENROLL-HD (n=34)**	**p**	**HD-JUNIOR (n=12)**	**ENROLL-HD (n=108)**	**p**	**ENROLL-HD (n=8808)**
Sex, M/F %	50/50	47/53	0.870	58/42	50/50	0.584	48/52
Age onset primary symptom, Mean±SD [Range]	6±2 [4–10]	6±2 [2–10]	0.835	15±2 [12–17]	15±2 [11–17]	0.421	44±9 [25–60]
Age onset motor symptom, Mean±SD [Range]	7±3 [4–11]	8±4 [1–15]	0.767	17±2 [13–20]	16±3 [11–22]	0.638	45±9 [25–65]
CAG-repeat, Mean±SD [Range]	74±12 [52–92]	75±17 [48–110]	0.787	59±5 [51–66]	60±8 [43–81]	0.579	44±3 [36–62]

n, number of patients; SD, standard deviation; M, male; F, female; CAG-repeat, Cytosine-Adenine-Guanine repeats in the Huntingtin gene.

Prevalence of JHD in the Netherlands

On January 1, 2024, there were 9 living JHD cases fulfilling the eligibility criteria for JHD in the HD-JUNIOR registry (Table 2). Six cases had an adolescent-onset JHD phenotype, 3 cases a childhood-onset JHD phenotype. Of these 9 cases, 1 was still under the age of 18 years, therefore referred to as PHD. Based on a clinically manifest HD prevalence estimate of 4–6:100,000 and a Dutch population of 17,947,684 on December 31, 2023, the estimated absolute number of the total clinically manifest HD population in the Netherlands was between 718 and 1077 cases. The prevalence of JHD as a percentage of the total clinically manifest HD population was between 0.84 to 1.25% (95% CI 0.29–2.07). For aJHD cases this was between 0.56 and 0.84% (95% CI 0.11–1.50), for cJHD between 0.28 and 0.42% (95% CI –0.04–0.89) and for PHD 0.09 to 0.14% (95% CI –0.09–0.41).

Table 2. Prevalence of JHD in the Netherlands

JHD subtype	Prevalence n (%)	Prevalence<18 y (PHD) n (%)	Prevalence 18–25 y n	Prevalence>25 y n
cJHD	3 (0.28–0.42%)	1	1	1
aJHD	6 (0.56–0.84%)	0	1	5
Total	**9 (0.84–1.25%)**	**1 (0.09–0.14%)**	**2**	**6**

Columns represent prevalence frequencies (1) in total, (2) of JHD patients that are currently<18 years of age, referred to as PHD, (3) of JHD patients that are currently between 18 and 25 years of age, and (4) of JHD patients that are currently older than 25 years of age. In addition, number of total cJHD, total aJHD, total JHD and PHD patients are given as a proportion of the total manifest HD population in the Netherlands based on an estimated prevalence of 6:100,000 (left percentage between brackets) or 4:100,000 (right percentage between brackets). n, number of patients; cJHD, childhood-onset JHD; aJHD, adolescent-onset JHD; PHD, pediatric Huntington's disease.

Incidence of JHD in the Netherlands

Between 2000 and 2019, a total of 19 JHD cases experienced onset of primary and onset of motor symptoms, 17 JHD cases received a clinical diagnosis and 10 JHD cases died (Fig. 2). In addition, a total of 10 JHD patients were clinically diagnosed≤17 years, therefore diagnosed as PHD patient. Based on this time period, the mean incidence for a clinical JHD diagnosis in relation to the total clinically manifest HD population in the Netherlands (4–6:100,000) was between 0.85 to 1.28 (95% CI –0.96–4.01) per 1000 HD patient years. The mean incidence of a JHD patient dying in the same patient population was between 0.50 to 0.74 (95% CI –0.89–2.85) per 1000 HD patient years. The mean incidence of a clinical diagnosis in a PHD patient, in relation to the general under-aged population in the Netherlands (≤17 years), was 0.14 (95% CI –0.25–0.54) per million person years. These rates

imply that, in the situation of the Netherlands, for every 1000 clinically manifest HD patients seen, 1 of them will have a JHD diagnosis and for every 1600, 1 of them dies with JHD. In addition, for every 7.000.000 under-aged individuals in the general population, approximately 1 of them will receive a PHD diagnosis. No statistically significant difference was observed between incidences of the consecutive 5-year time periods.

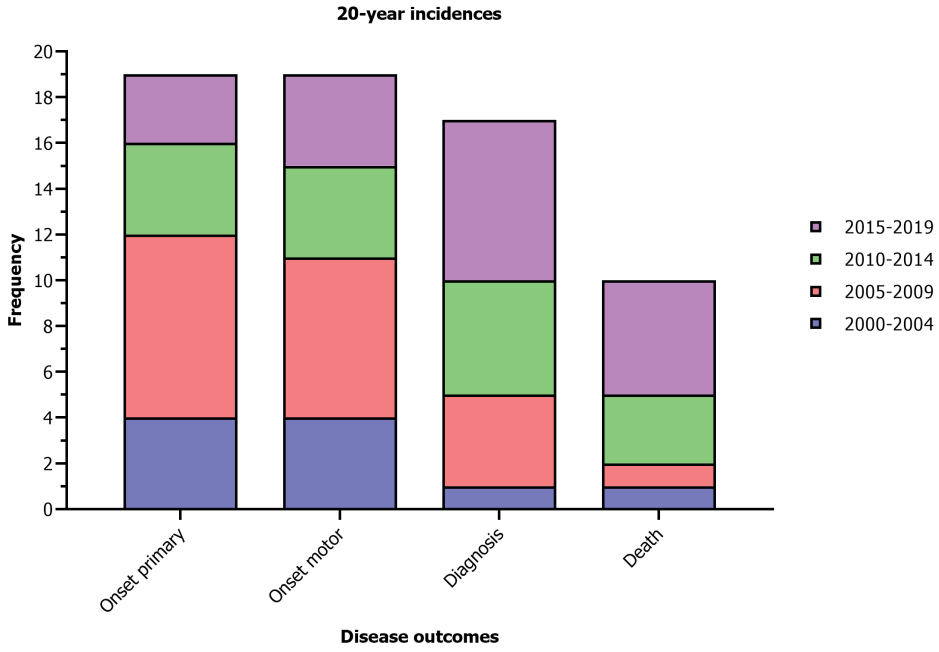

Figure 2. 20-year incidences of JHD in the Netherlands.

Stacked bar graphs shows the number of JHD cases 1) experiencing the onset of a primary symptom, 2) experiencing the onset of a first motor symptom, 3) receiving a clinical diagnosis of HD, and 4) dying, between 2000 and 2020 and color coded by time frames of 5 years. One-way ANOVA for between timeframe comparisons revealed no statistically significant results (p-values>0.05).

Age and disease duration at diagnosis

In the HD-JUNIOR registry, 10 of 22 (45%) JHD cases received a clinical diagnosis of HD before the age of 18 years and 12 of 22 (55%) JHD cases received a clinical diagnosis of HD in adulthood (defined as age≥18). Likewise, In the ENROLL-HD registry 61 of 142 (43%) JHD cases received a clinical diagnosis before the age of 18 years and the other 81 out of 142 (57%) from ENROLL-HD received their clinical

diagnosis of HD on adult age. In 5 of 12 JHD cases (42%) from HD-JUNIOR that received a clinical diagnosis at adult age, preclinical genetic testing was performed prior to receiving a clinical HD diagnosis.

The median disease duration between primary symptom and a clinical diagnosis of HD was, in cJHD (*n*=43) 4 years (IQR 1–7), in aJHD (*n*=119) 4 years (IQR 2–7) and in AHD patients (*n*=8808) 2 years (IQR 1–5) (Fig. 3). Between AO-HD subtype comparison of the log10-transformed disease duration at diagnosis revealed a statistically significant mean difference between aJHD and AHD patients (mean difference 0.17, 95% CI 0.08–0.25, *p*=<0.001).

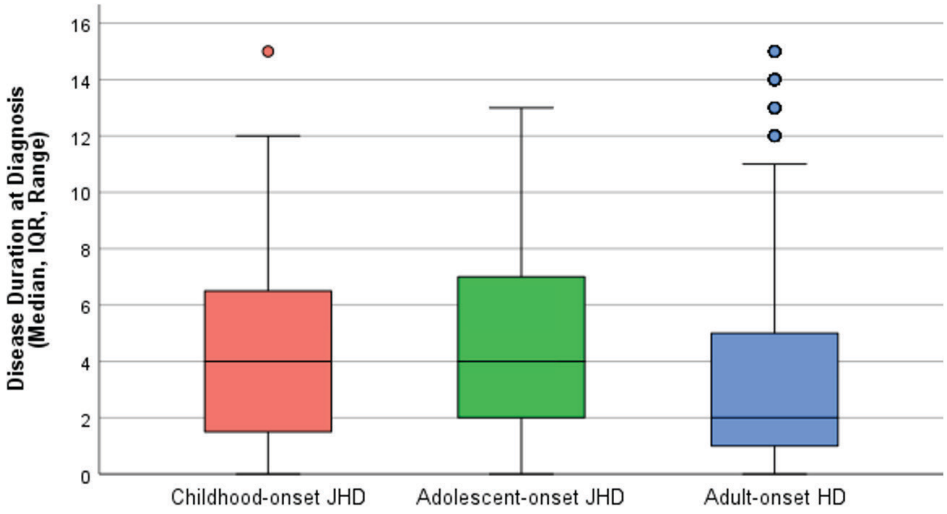

Figure 3. Disease duration at diagnosis in AO-HD subtypes

Boxplots showing the median, IQR, range and outliers of disease duration in years between primary symptom onset and clinical diagnosis of HD in the pooled cJHD (red; n=43), aJHD (green; n=119) and AHD (blue; n=8,808) patient samples. One-way ANOVA of the log10-transformed disease duration at diagnosis revealed a statistically significant mean difference between the aJHD and AHD patient samples (p=<0.001).

Disease stage markers

In the HD-JUNIOR registry, all cJHD cases (*n*=5) were functionally compromised at diagnosis (Table 3). Four out of five cases received special primary education and the other one received regular primary education, albeit with difficulty. In addition, chronic home care was needed for all and in one case temporary hospitalization

was needed. In aJHD cases of the HD-JUNIOR registry, their functioning at the time of clinical diagnosis was compromised in 7 out of 8 cases (Table 3). In 4 of these 7 compromised aJHD cases, secondary vocational education was discontinued at an early stage and in the other 3 secondary education was discontinued early. In addition, 4 cases received chronic nursery home care and 3 cases received partial care at home or in day care.

Table 3. Functional incapacities at diagnosis in JHD subtypes of the HD-JUNIOR registry

Patient	Age Dx (y)	Disease Duration Dx (y)	Education	Care level Dx	Functionally compromised Dx	Specified
JHDc-01-4	8	0.5	Special primary education	Home care (chronic)	Yes	- Needs tricycler
						- Needs help bathing/toileting
						- Needs help with transfers
JHDc-02-X	7	2.5	Primary education (with difficulty)	Home care (chronic)	Yes	- Needs help changing clothes
						- Gave up cycling
JHDc-03-3	10	4	Special primary education	Home care (chronic)	Yes	- Needs walking aids
						- Gave up cycling
						- Needs help maintaining personal hygiene
						- Needs help changing clothes
JHDc-04-4	11	5.5	Special primary education	Home care (chronic) and Inhospitalization (temporary)	Yes	- Change in independence with outdoor activities
						- Needs help changing clothes
JHDc-05-2	15	5.5	Special secondary education	Home care (chronic)	Yes	- Change in independence with outdoor activities
						- Needs walking aids
JHDa-01-1	18	2	Secondary vocational education drop-out	Day care (partial)	Yes	- Gave up cycling

Table 3. Continued

Patient	Age Dx (y)	Disease Duration Dx (y)	Education	Care level Dx	Functionally compromised Dx	Specified
JHDa-02-1	19	3	Secondary education drop-out	Nursery home care (chronic)	Yes	- Change in independence with outdoor activities
						- Gave up job
JHDa-03-X	20	3	Secondary education drop-out	Home care (partial)	Yes	- Cannot find job
						- Needs help with domestic chores
JHDa-04-1	21	3.5	Secondary vocational education drop-out	Home care (partial)	Yes	- Needs help with finances
						- Needs help with domestic chores
JHDa-05-1	21	3.5	Secondary vocational education finished	Independent	No	
JHDa-06-2	20	4.5	Secondary vocational education drop-out	Nursery home care (chronic)	Yes	- Difficulty writing
						- Cannot find job
JHDa-07-2	19	6	Secondary education drop out	Nursery home care (chronic)	Yes	- Gave up job
JHDa-08-X	23	11	Secondary vocational education drop-out	Nursery home care (chronic)	Yes	Unknown

Each row displays pseudonymized patient data from the HD-JUNIOR registry in relation to functional incapacities within one year of diagnosis. In the Patient column you find patient characteristics regarding: onset in childhood (JHDc) or in adolescence (JHDa) - case number - CAG-repeat length category ('1' for CAGs≥50 and<60, '2' for CAGs≥60 and<70, '3' for CAGs≥70 and<80, '4' for CAGs≥80, 'X' in case CAG was unknown). Age at clinical diagnosis and disease duration between primary symptom and clinical diagnosis are given in column 2 and 3. Latest or highest education received at clinical diagnosis are given in the 'education' column. Care level at clinical diagnosis in relation to place and partial (is partially independent) or chronic (is largely dependent) care are given in the 'care level' column. Dx, at diagnosis.

Functional capacity at clinical diagnosis in the ENROLL-HD registry was analyzed by means of the UHDRS-IS score. In four cJHD patients (in whom the UHDRS-IS was completed within one year of receiving a clinical diagnosis of HD), the UHDRS-IS score was twice 90% ('no physical care needed if difficult tasks are avoided'),

once 55% and once 50% ('24-hour supervision appropriate; assistance required for bathing, eating, toileting'). In aJHD patients (*n*=33), the median UHDRS-IS score at clinical diagnosis was 80% (IQR 70–90%), which refers to 'pre-disease level of employment/education changes or ends; cannot perform household chores to pre-disease level, may need help with finances' (Fig. 4). In AHD patients (*n*=3,186), the median UHDRS-IS at clinical diagnosis was 90% (IQR 80–100%), which refers to 'no physical care needed if difficult tasks are avoided' (Fig. 4). Between aJHD and AHD group comparison of the log10-transformed UHDRS-IS at diagnosis, revealed a statistically significant mean difference of –0.04 (95% CI –0.07—0.01, *p*=<0.001). This suggests a lower functional capacity at diagnosis in aJHD patients compared with AHD patients.

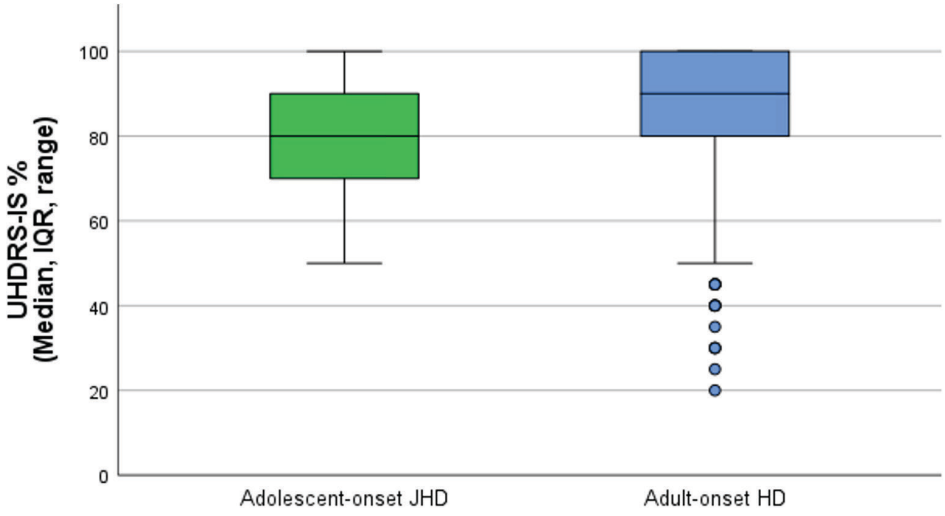

Figure 4. UHDRS-Independent Score at diagnosis in aJHD and AHD subtypes.

Boxplots showing the median, IQR, range and outliers of the UHDRS-IS score within one year of clinical diagnosis in the aJHD (green; n=33) and AHD (blue; n=3,186) patient samples of ENROLL-HD. One-way ANOVA of the log10-transformed UHDRS-IS score at diagnosis revealed a statistically significant mean difference between the aJHD and AHD patient samples (p=<0.001).

As an alternative measure for disease stage, taking into account CAG-repeat length, the CAP^{100} score at age of 1) primary symptom onset, 2) motor symptom onset, and 3) clinical diagnosis was analyzed and compared between AO-HD subtypes of pooled datasets (Fig. 5). The CAP^{100} score progressed from age at primary symptom onset, to age at motor symptom onset, to age at clinical diagnosis of HD in all 3 AO-HD subtypes. The mean CAP^{100} was lowest in the cJHD (*n*=43), followed by aJHD

(*n*=118) and then AHD (*n*=8,808) HD-subtype. Intergroup comparisons for the mean CAP^{100} score at the three different time points in AO-HD subtypes were significant by<0.001 for all comparisons. These outcomes would imply a lower cumulative exposure to the toxic effects of mHTT in cJHD and aJHD patients when compared to AHD patients, and therefore a less severe disease stage at these three fixed time points.

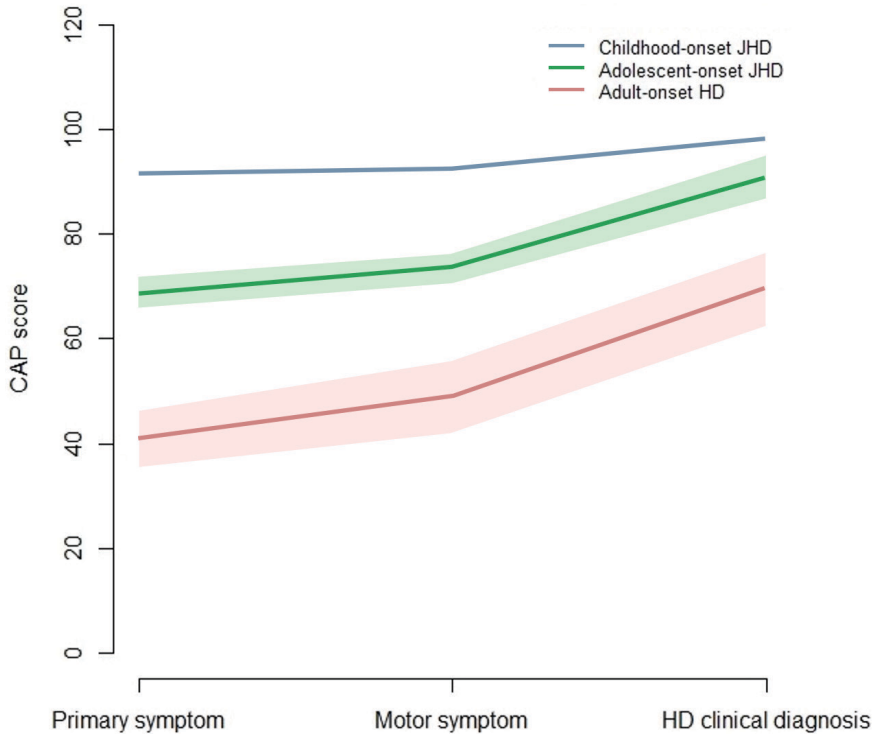

Figure 5. CAP^{100} score over time in AO-HD subtypes.

Line graphs showing the mean and 95% CI of the CAP^{100} score at time points: 1) primary symptom onset, 2) motor symptom onset, and 3) clinical diagnosis of HD, in the pooled cJHD (red; n=43), aJHD (green; n=118), and AHD (n=8,808) patient samples. One-way ANOVA for the comparison of the CAP^{100} score at the three different time points between AO-HD subtypes revealed statistically significant differences in means for all time points between all AO-HD subtypes (p=<0.001).

DISCUSSION

This study reveals the current low prevalence and incidence of JHD and PHD patients in the Netherlands and the limited availability and ability of this population to participate in interventional trials in the near future.

Based on a systematic review and meta-analysis of JHD epidemiology in 2012, the mean proportion of JHD patients as part of the total clinically manifest HD population has been estimated at 4.92% (95% CI 4.07–5.84%).[7] The last estimate of the proportional prevalence of JHD in the Netherlands, determined in 2002, was 3%.[23] Yet, one recent study analyzing JHD prevalence in the worldwide HD registry ENROLL-HD, found a substantial lower proportional margin of 1.44%.[8] Furthermore, the latter study was the first to specify the proportion of JHD patients still under the age of 18 years, referred to as PHD, which was 0.14–0.66%. The results of our study are in line with the latter study and reveal a significantly lower proportional prevalence, between 0.84 and 1.25%, for the JHD population and between 0.09 and 0.14% for the PHD population in the Netherlands, as compared to the estimates from 2002 and 2012. In addition, 20-year incidence rates reveal a stable number of JHD cases over time. This shows that factors such as recognition of the JHD phenotype, treatment options and birth control methods seem to have had no clear influence on the incidence of JHD cases between 2000 and 2020.

Apart from the prevalence and incidence of JHD patients in the Dutch population, our study reveals that JHD patients have a median diagnostic delay of 4 years, and less than half of JHD cases are clinically diagnosed on pediatric age. These JHD cases are labeled as 'JHD' in retrospect, already at adult age, and are therefore not available for interventional trials in the PHD population. As has been mentioned before, the design of interventional trials in a PHD population is unrealistic with these small numbers.[12] Although there are ongoing international efforts to identify as many JHD and PHD cases as possible, it makes us strongly doubt if EMA class waiver removal for pediatric investigation plan in the PHD population outweighs its purpose. In our opinion, the possibility to start compassionate use programs with medical agents tested in the AHD population, should be considered for these rare PHD cases. Moreover, 8 of 9 prevalent JHD cases in the Netherlands are currently above 17 years of age and therefore potentially eligible to participate in interventional trials designed for adult HD cases. Although such JHD cases are often severely affected by the disease (and therefore not comparable to AHD cases), it does make us wonder if alternative trial designs, like for example multiple crossover n-of-1 studies, should be considered.

In the Netherlands, approximately 40% of JHD cases who received a clinical diagnosis on adult age, received their genetic status prior to a clinical diagnosis. This shows that a substantial portion of JHD cases, while experiencing yet unrecognized

disease characteristics, are mistakenly counselled for presymptomatic genetic testing by clinical geneticists rather than diagnostic testing by a neurologist. Optimized collaboration and consultation of clinical geneticists and neurologists, in particular in expanded gene risk carriers with a medical history in psychiatric or neurocognitive disease domains, should allow for appropriate counselling for all HD cases in the future.

Part of JHD cases are known to have faster disease progression and a shorter survival when compared to prototypical disease onset in adulthood.[1] This extremely vulnerable patient population is likely to have a lower ability to participate in the heavy interventional studies that are currently ongoing in the AHD population.[24] Our study shows that all cJHD patients and most aJHD patients in the Netherlands had severe functional incapacities (HD-ISS stage 3)[19] when they were clinically diagnosed. This was also reflected by the significantly lower independent scores at diagnosis in aJHD when compared to the adult-onset group in the international Enroll-HD database. This has an impact on the possibility of JHD patients to participate in interventional studies that requires informed consent, long clinical/research visits every few weeks or months and invasive procedures such as venipuncture, lumbar punctures, MRI and other assessments. Furthermore, many UHDRS assessments, like the motor and functional measures, are less suited for the PHD and JHD population.[25,26] Our study reveals another interesting finding in that respect, by the invalidity of the CAP^{100} score as a predictor of disease progression for the JHD population. The CAP^{100} score is a formula that considers age and CAG-repeat length to predict disease stage and is normalized so that the outcome approximates a score of 100 when an HD patient enters clinical HD-ISS stage 2.[16] This is grossly in line with the mean CAP^{100} score at diagnosis of 98 that was found in the AHD population of ENROLL-HD. In contrast, the mean CAP^{100} score at diagnosis of 70 in cJHD and 91 in aJHD patients would suggest a lower accumulative exposure to the toxic effects of mHTT in the JHD population, which is highly unlikely given the faster disease progression and shorter survival in this particular population. Lack of fit of this model for CAGs$\geq$50 has already been noted in the original article.[16] An explanation for these findings could be the non-linear relationship between CAG and age at clinical phenotype that has been found to influence specifically the JHD population, but not AHD population.[3] Another explanation could be the greater effect of shorter and mutant HTT allele interaction in the JHD population, influencing loss-of-function pathomechanisms.[27] These outcomes signify the need for adjusted measures to predict disease stage and progression in the JHD population.

Our study has its limitations. In particular the definition of 'what is a juvenile HD phenotype' is still under debate and may have had its effects on our prevalence and incidence estimates. Clear international eligibility criteria for a JHD phenotype are needed to ensure consensus in future interventional trials. In addition, it is possible we have missed true JHD cases in our registry due to unrecognized cases (e.g., diagnostic delay; diagnostics that were performed at sites/departments that are unfamiliar with specialized HD care facilities or Enroll-HD) or unwillingness to share medical records for research purposes. Yet, by the synergistic effect of combining genetic and clinical data from multiple sources, our registry is likely to be very conclusive with regard to numbers of cases. Furthermore, due to the limited sample sizes particularly in the functional assessment in the cJHD subtype, the generalizability of these results are limited. However, by working with two different JHD cohorts, these sample sizes are the best that can actually be established in such a rare phenotype. The use of adjusted prospective functional measures as part of standard clinical practice in the JHD population, could help in overcoming the limited generalizability of our study results in the near future.

JHD and PHD are extremely rare and vulnerable HD patient populations, requiring a tailored approach when participating in future interventional trials. Compassionate use programs in PHD cases, alternative trial designs, like multiple cross-over designs, including JHD patients who are aged≥18 in AHD trials, should also be considered. Furthermore, adjusted and validated measures for disease progression in the JHD and PHD population are urgently needed.

REFERENCES

1. Fusilli C, Migliore S, Mazza T, et al. Biological and clinical manifestations of juvenile Huntington's disease: a retrospective analysis. Lancet Neurol 2018; 17: 986–993.
2. Bakels HS, Roos RAC, van Roon-Mom WMC, et al. juvenile-onset Huntington disease pathophysiology and neurodevelopment: a review. Mov Disord 2022; 37: 16–24.
3. Schultz JL, Moser ADandNopoulos PC. The association between CAG-repeat length and age of onset of juvenile-onset Huntington's disease. Brain Sci 2020; 10: 575.
4. Quarrell OWJandNance MA. The diagnostic challenge. In: Quarrell OWJ, Brewer HM, Squitieri F, et al. (eds) Juvenile Huntington's Disease and Other Trinucleotide Repeat Disorders. New York: Oxford University Press; 2009.
5. Squitieri F, Frati L, Ciarmiello A, et al. Juvenile Huntington'sdisease: does a dosage-effect pathogenic mechanism differ from the classical adult disease? Mech Ageing Dev 2006; 127: 208–212.
6. Brinkman RR, Mezei MM, Theilmann J, et al. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. Am J Hum Genet 1997; 60: 1202–1210.
7. Quarrell O, O'Donovan KL, Bandmann O, et al. The prevalence of juvenile Huntington's disease: a review of the literature and meta-analysis. PLoS Curr 2012; 4: e4f8606b742ef3.
8. Achenbach JandSaft C. Data from ENROLL-HD: Is the prevalence of juvenile and pediatric Huntington's disease overestimated? Parkinsonism Relat Disord 2021; 88: 1–2.
9. Rawlins MD, Wexler NS, Wexler AR, et al. The prevalence of Huntington's disease. Neuroepidemiology 2016; 46: 144–153.
10. Baig SS, Strong MandQuarrell OW. The global prevalence of Huntington's disease: a systematic review and discussion. Neurodegener Dis Manag 2016; 6: 331–343.
11. Medina A, Mahjoub Y, Shaver L, et al. Prevalence and incidence of Huntington's disease: an updated systematic review and meta-analysis. Mov Disord 2022; 37: 2327–2335.
12. Quarrell OWJ, Nance MA, Nopoulos P, et al. Defining pediatric huntington disease: Time to abandon the term juvenile Huntington disease? Mov Disord 2019; 34: 584–585.
13. Gonzalez-Alegre PandAfifi AK. Clinical characteristics of childhood-onset (juvenile) Huntington disease: report of 12 patients and review of the literature. J Child Neurol 2006; 21: 223–239.
14. Ribai P, Nguyen K, Hahn-Barma V, et al. Psychiatric and cognitive difficulties as indicators of juvenile huntington disease onset in 29 patients. Arch Neurol 2007; 64: 813–819.
15. Oosterloo M, Bijlsma EK, Die-Smulders C, et al. Diagnosing juvenile Huntington's disease: an explorative study among caregivers of affected children. Brain Sci 2020; 10: 155.
16. Warner JH, Long JD, Mills JA, et al. Standardizing the CAP score in Huntington's disease by predicting age-at-onset. J Huntingtons Dis 2022; 11: 153–171.
17. Sathe S, Ware J, Levey J, et al. Enroll-HD: an integrated clinical research platform and worldwide observational study for Huntington's disease. Front Neurol 2021; 12: 667420.
18. Cronin T, Rosser AandMassey T. Clinical presentation and features of juvenile-onset Huntington's disease: a systematic review. J Huntingtons Dis 2019; 8: 171–179.
19. Tabrizi SJ, Schobel S, Gantman EC, et al. A biological classification of Huntington's disease: the Integrated Staging System. Lancet Neurol 2022; 21: 632–644.
20. Shoulson IandFahn S. Huntington disease: clinical care and evaluation. Neurology 1979; 29: 1–3.

21. Paulsen JS, Zhao H, Stout JC, et al. Clinical markers of early disease in persons near onset of Huntington's disease. Neurology 2001; 57: 658–662.
22. Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. Mov Disord 1996; 11: 136–142.
23. Maat-Kievit A, Losekoot M, Zwinderman K, et al. Predictability ofage at onset in Huntington disease in the Dutch population. Medicine (Baltimore) 2002; 81: 251–259.
24. Sampaio C. Huntington disease - Update on ongoing therapeutic developments and a look toward the future. Parkinsonism Relat Disord 2024; 122: 106049.
25. Horton MC, Nopoulos P, Nance M, et al. Assessment of the performance of a modified motor scale as applied to juvenile-onset Huntington's disease. J Huntingtons Dis 2019; 8: 181–193.
26. Brewer HM, Barker, Roger A, et al. Challenges in assessment. In: Quarrell OWJ, Brewer HM, Squitieri F, et al. (eds) Juvenile Huntington's Disease and Other Trinucleotide Repeat Disorders. New York: Oxford University Press, 2009, p. 181–188.
27. Aziz NA, Jurgens CK, Landwehrmeyer GB, et al. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. Neurology 2009; 73: 1280–1285.

CHAPTER 3

Juvenile-onset Huntington Disease pathophysiology and neurodevelopment: a review

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ABSTRACT

Huntington disease is an autosomal dominant inherited brain disorder that typically becomes manifest in adulthood. Juvenile-onset Huntington disease refers to approximately 5% of patients with symptom onset before the age of 21 years. The causal factor is a pathologically expanded CAG-repeat in the Huntingtin gene. Age at onset is inversely correlated with CAG-repeat length. Juvenile-onset patients have distinct symptoms and signs with more severe pathology of involved brain structures in comparison with disease onset in adulthood. The aim of this review is to compare clinical and pathological features in juvenile and adult-onset Huntington disease and to explore which processes potentially contribute to the observed differences. A specific focus is placed on molecular mechanisms of mutant huntingtin in early neurodevelopment and the interaction of a neurodegenerative disease and postnatal brain maturation. The importance of a better understanding of pathophysiological differences between juvenile and adult-onset Huntington disease lies in development and implementation of new therapeutic strategies.

INTRODUCTION

Huntington disease (HD) is an autosomal dominant progressive brain disorder caused by a pathological CAG-repeat expansion coding for huntingtin (HTT gene), with an elongated polyglutamine tract.[1] The length of the CAG-repeat shows an inverse correlation with the age at onset.[2] Symptoms become manifest at a mean age of 45 (range 2–87) years.[3,4,5] All patients with symptom onset before the age of 21 years, irrespective of their present age, are referred to as juvenile-onset Huntington disease (JHD), which is seen in 4% to 10% of all HD cases.[6,7] The term pediatric Huntington disease (PHD) is reserved for all patients with manifest disease who are still below the age of 18 years.[7] Based on clinical signs, further distinction is made between childhood (<10 years) and adolescent-onset (10−18 years).[2,8] In approximately 50% of JHD cases the CAG expansion is ≥60, exceeding 80 repeats in childhood onset.[8,9] About 80% of JHD patients inherit the repeat expansion via paternal transmission.[8,10]

JHD patients are often difficult to diagnose.[11,12] This is mainly due to psychiatric and cognitive complaints that are easily misdiagnosed.[9,11,12,13] Apart from the atypical clinical presentation, disease progression in childhood-onset HD patients is faster and survival shorter compared to adult-onset HD (AHD).[8] Furthermore, morphological changes in JHD brains are generally more severe than in AHD brains.[14] These phenotypical and pathological differences raise the hypothesis of aberrant pathomechanisms. Detangling pathophysiological differences between JHD and AHD is important for the successful treatment of pediatric patients. Various treatments are currently under investigation in AHD patients, yet JHD patients are excluded from most therapeutic trials.

This review aims to highlight differences in clinical, neuropathological, and imaging features between JHD and AHD. Suggestions for further studies are made by explaining these differences in the light of HD pathophysiology and brain development.

Clinical features

HD is characterized by motor and cognitive dysfunction and by psychiatric and behavioral changes, leading to loss of independence and eventually death. The median disease duration after motor onset in childhood JHD is 9 years, compared to 18 years in adolescent and adult HD.[5,8]

Motor Symptoms

Between 42% and 94% of JHD patients develop postural instability, bradykinesia, and rigidity, in combination with dystonia in the initial stages of motor onset.[8,12,15,16,17,18] In contrast, motor onset with bradykinesia and rigidity is seen in only 20% of AHD patients, yet most AHD patients may become hypokinetic and rigid at the end of their disease.[19,20] Chorea, which is the initial motor sign in 80% of AHD cases, is rarely seen in early JHD, but gradually evolves with disease progression in a subset of adolescent HD.[8,18,21,22] Motor signs more often seen as initial signs in JHD include dysarthria and loss of dexterity, such as writing.[8,12,21] Oral dyskinesias,[8,11,18] tics,[8,21,22] and myoclonus[8,12,15,21] are more frequently seen in later stages of JHD. Of note are conflicting reports on the appearance of ataxia,[8,15,21] which is probably a definition problem. Ataxia, imbalance, incoordination, and unsteady walking are words probably that refer to the same common early signs in JHD, which can also be seen in early stages of AHD.[23]

Cognitive Symptoms

Cognitive deficits are reported as initial disease signs in 30%–83% of JHD patients, before motor onset is apparent.[8,21,24,25] This wide range might be attributed to differences in description but emphasizes the notion that cognitive deficits are prominent in the initial stage of JHD. Similarly, in about onethird of AHD cases, early cognitive disturbance, typically related to psychomotor speed and deterioration of executive functions such as attention, planning, and flexibility of mind, are present years before the first motor symptoms appear.[25,26] More specific for JHD is the prevalence of developmental delay. This feature is used to describe delays in cognitive, motor, language, and social development, thus referring to a broader range of neurological features than just cognition. An early cohort study reported that 6 of 33 JHD cases present with some form of developmental delay.[18]An additional description of three unrelated JHD patients (CAG-repeats of 93,100, and 120) mentioned a developmental delay in speech and language, followed by a delay in social and motor skills.[27]Several other publications confirmed the presence of developmental delay in the JHD population and showed that these delays are particularly seen in patients with childhood disease onset.[8,21] Of note is the lack of data delineating the neurological base of the observed delays (eg, cognition, motor, and/or social) as well as comparative data with AHD.

Psychiatric Symptoms

About 30% of JHD patients present with some form of psychiatric or behavioral disturbances before motor onset, which increases to 75% during disease progression.[8,12,15,21,22,25] These numbers largely resemble those in adult-onset disease.[25,26,28] However, the nature of psychiatric complaints in JHD patients differs from those seen in AHD patients. Obsessive–compulsive behaviors are more common in adolescent-onset (50%–73%) compared to AHD (26%). The prevalence of behavioral deficits is higher in adolescent-onset HD patients compared with childhood-onset HD.[8,28,29] Psychotic symptoms are reported in 17% to 39% of JHD patients[8,22] and only in about 4% of AHD cases.[28]

Other Symptoms

The most remarkable difference in clinical appearance between JHD and AHD is the higher prevalence of epileptic seizures in the JHD population, estimated to be 30% to 35%. Observational studies show it is far more common in childhood-onset HD patients than in adolescent-onset HD.[8,13,30] In AHD, the prevalence of epileptic seizures is comparable to population risk. Furthermore, sleep disturbance, pain, and itching are explicitly or more commonly mentioned in the juvenile population.[22] Unintended weight loss and hypermetabolic state is seen in both AHD and JHD cases, yet its severity correlates with an increase in CAG-repeat length and thus is more severe in JHD patients.[31,32]

Neuropathology and Imaging

In HD pathology, various cell types and brain regions are affected, and although there are shared characteristics between JHD and AHD, subtle pathological differences between the two forms exist. There is a lack of systematic assessments of differences between JHD and AHD brains. What is known, mainly based on small sample sizes from either pathology or imaging studies, will be discussed here and illustrated in Fig. 1.

Subcortical Structures

In HD, progressive neostriatal (eg, putamen and caudate nucleus) loss of medium spiny projection neurons and concomitant reactive increase of astroglial cells (eg, astrogliosis) are the most prominent neuropathological changes observed, and determine mainly the neuropathological grading (see Box 1).[33] Neostriatal volume

loss is generally more severe in JHD brains when compared with AHD brains and follows a linear correlation with CAG-repeat length (see Table 1and Fig. 1).[14,33,34,35,36]

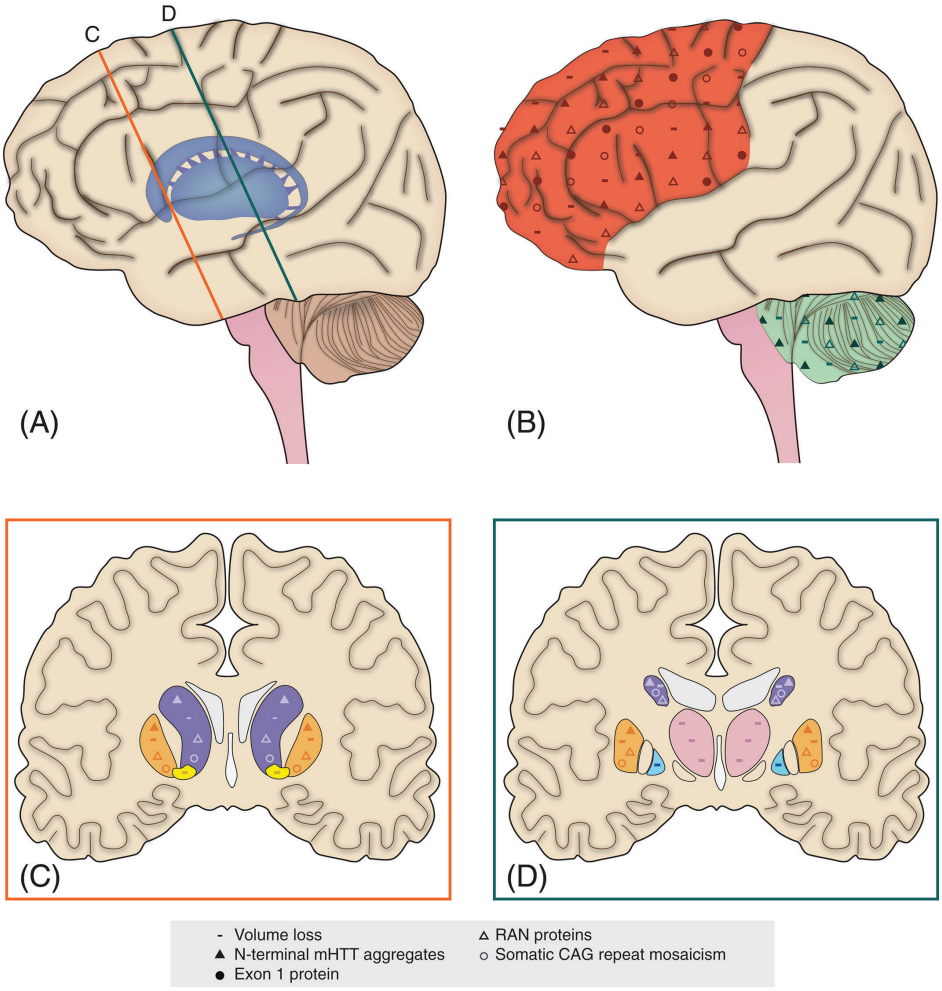


Figure 1. Schematic illustration showing differences in juvenile-onset Huntington disease (JHD) neuropathology in comparison with adult-onset Huntington disease (AHD) neuropathology.

More severe pathological hallmarks of JHD brains are seen in **A**, inset **C** and **D**: the subcortical grey matter structures and **B**: the frontoparietal cortex and, to a lesser extent, the cerebellum. Volume loss (–) is more pronounced in the frontoparietal cortex (red), cerebellum (green), caudate nucleus (purple), putamen (orange), nucleus accumbens (yellow), internal segment of the globus pallidus (blue), and the thalamus (pink). Nterminal mutant huntingtin (mHTT) aggregates (▲) are more abundant in the frontal cortex, caudate nucleus, putamen, and, to a lesser extent, cerebellum of JHD brains. Exon 1 protein (●) is more abundant in the frontoparietal cortex and hippocampus (not displayed) of JHD brains. Repeatassociated nonATG nuclear (RAN) proteins (△) are more abundant in the striatum, frontal cortex, and cerebellum of JHD brains. Somatic CAG-repeat mosaicism (◦) is greater in the neocortex, caudate nucleus, and putamen of JHD brains.

Box 1. Vonsattel Grading System.

This is a fivescale neuropathological classification (0–4) based on the sequential pathology of striatal regions.[33] The assignment of a grade is based on macroscopic and microscopic findings, the latter on three standardized coronal sections of the striatum.

Grade 0: no macro and microscopic alterations are seen, yet subtle neuronal cell loss of the head of the caudate nucleus can be quantified when compared with nonneurological control brains; **grade 1**: there are no major macroscopic changes, but neuronal loss and astrogliosis can be reliably observed microscopically in the caudate nucleus and to a smaller extent in the putamen; **grade 2**: caudate nucleus volume loss can be observed macroscopically (medial outline into the lateral ventricle slightly convex); **grade 3**: gross caudate nucleus volume loss extents in a straight or concave line with the lateral ventricle; and **grade 4**: outline of caudate nucleus is concave, as is the anterior limb of the internal capsule, and 95% of neostriatal neurons are lost microscopically.

Of note is the importance of disease severity and duration of illness at autopsy in the interpretation of volume loss between the two forms, which is only sporadically taken into account in pathological comparisons. Magnetic resonance imaging (MRI) studies confirm these findings by comparing neostriatal volume loss and disease severity between patient groups with varying CAG-repeat lengths.[37] Furthermore, other pathological hallmarks like mutant huntingtin (mHTT) aggregates and somatic CAG-repeat mosaicism (see Box 2) are more severe in the neostriatum of JHD brains when compared to AHD brains (Fig. 1).[38,39,40,41] In addition to neostriatal pathology, other subcortical regions, such as the internal segment of the globus pallidus, the nucleus accumbens, and thalamus, are more often affected in postmortem JHD brains when compared to AHD brains (see Fig. 1).[34] Thalamic and pallidal volume loss in JHD patients was also observed in a recent age-matched crosssectional MRI study (N=19).[42] An inverse relationship between CAG-repeat length and thalamic volume loss was found within this same cohort. Taken together, these findings show more severe pathology of subcortical structures in JHD patients.

Table 1. Neuropathological severity of the striatum in Huntington disease brains[14,33]

Vonsattel grading system	JHD brains (N = 50)	AHD brains (N = 1300)
Grade 0	Not reported	1%
Grade 1	10% a	4%
Grade 2	Not reported	16%
Grade 3	26%	53%
Grade 4	64%	28%

[a] Ninety percent of grade 1 juvenile-onset Huntington disease (JHD) brains were from JHD patients who committed suicide and therefore do not reflect endstage disease. For the adult-onset Huntington disease (AHD) brains, these percentages are unknown/not provided.

Box 2. Pathological hallmarks

Aberrant protein expression and aggregates are well known pathological hallmarks in neurodegenerative disease and can be found in both intra and extracellular compartments. In HD, Nterminal mHTT aggregates are found in neurons of the neocortex, neostriatum, hippocampal area, and brainstem and, to a lesser extent, in glial cells.[39, 40, 41, 43, 44]_Also, other mHTT protein species are selectively expressed in HD brains, such as aberrantly spliced exon 1 protein and repeatassociated nonATG (RAN) proteins.[39,45,46] As to the relative toxicity of mHTT protein species, HD cell and animal models show that, in particular, exon 1 and certain RAN protein species have more detrimental effects on cell function and death than fulllength mHTT protein.[46,47] Another pathological hallmark in HD is the degree of somatic CAG-repeat mosaicism. With increasing CAG-repeat length, the occurrence of both germline and somatic expansion of the trinucleotide length increases. HD pathology is positively correlated with the extent of CAG-repeat mosaicism.[38,48,49]

Neocortex

Neocortical volume and pyramidal neuron loss is also found in AHD and PHD brains. In general, atrophy is most pronounced in frontal and parietal regions and is most often seen in Vonsattel grade 3 and 4 brains.[33] Based on macroscopy, frontal and parietal atrophy is more commonly observed in postmortem JHD brains when compared with AHD brains (see Fig. 1), like the higher Vonsattel grades in JHD brains.[34] More widespread cortical volume loss and faster volume loss over time has also been observed in a longitudinal MRI analysis of 2 JHD patients who carried CAGs higher then 55, when compared with 34 AHD patients with repeat lengths between 40 and 55.[50,51] However, crosssectional analysis of cortical volume loss and CAG-repeat length in the same cohort failed to replicate this result. [52] In addition, structural brain MRI in 19 JHD patients showed relatively preserved cortical volumes when compared with age-matched controls,[42] suggesting that cortical volume loss in JHD brains is related to endstage disease. Other pathological hallmarks such as Nterminal mHTT , exon 1 , and RAN protein aggregates and somatic CAG-repeat mosaicism, are more highly expressed in the neocortex of JHD brains when compared with AHD brains, as shown in Fig. 1.[39,40,41,45,46] The need for more refined comparison of JHD and AHD postmortem brains, with extensive longitudinal MRI data, analyzing and comparing JHD and AHD patients, will be essential to clarify the relative involvement of cortical pathology in the two forms.

Cerebellum

The hypothesis of more pronounced cerebellar pathology in JHD brains remains a matter of controversy. Severe macroscopic cerebellar atrophy is described in a subset of neuropathological and MRI studies of JHD brains, all of them from childhood-onset cases (see Fig. 1).[53,54,55,56,57,58] However, of concern is the cause of cerebellar atrophy

in these JHD brains, since most of the examined cases (11 out of 13) were known to have epilepsy, which could potentially cause hypoxic–ischemic events in this area. In both JHD and AHD brains, subtle macroscopic cerebellar atrophy is seen together with extensive striatal degeneration (Vonsattel grades 3 and 4)[33] Crosssectional imaging studies of cerebellar volume loss in both JHD and AHD patients have failed to replicate group differences in the amount of cerebellar atrophy[51,52] Of particular interest are two recent in vivo MRI studies that revealed relative enlargement of anterior cerebellar compartments in AHD and JHD subjects when compared with age-matched controls.[42,59] Additional functional investigations have suggested the cerebellum is a compensatory brain structure for pathological basal ganglia changes in early HD disease stages.[60,61] Studies of the presence of mHTT and RAN protein aggregates in HD cerebelli found evidence that such pathological hallmarks are selectively or more prominently found in JHD cerebelli (Fig. 1).[45,46,56] In particular, the higher expression of RAN protein species suggests that toxicological processes are more likely to take place in JHD cerebelli when compared to AHD cerebelli. Additional research into the role of the cerebellum in HD pathophysiology is needed to further clarify discrepant in vivo and postmortem findings and to address its relation to JHD and AHD.

White Matter

While substantial evidence suggests (microstructural) white matter changes in HD, the relative involvement of white matter pathology in JHD as compared to AHD is as yet unknown.[62] In JHD patients, scarce quantitative data of white matter involvement are available and diffusion tensor imaging (DTI) measures have never been published. Hedjoudje and others showed subtle cerebral white matter volume decrease in three JHD siblings with childhood onset of disease, carrying CAG-repeats >120, when compared to age-matched controls.[63] Tereshchenko and others replicated this finding in a sample of 19 JHD patients (CAG-repeat range 54 to 96) and revealed more pronounced white matter volume decrease in JHD patients with longer repeats, suggesting a CAG-dependent relation.[42] More comparative imaging data in both JHD and AHD patients is needed to reliably interpret possible differences in white matter involvement between the two forms.

Differences in Clinicopathology and Causal Factors

Summarizing the previous paragraphs, JHD shows a faster disease progression and a shorter survival, as compared to AHD, with some distinct clinical symptoms and

signs, like hypokinetic and rigid syndrome, developmental delay, behavioral disorder, epilepsy, and psychosis. This is accompanied by increased pathological hallmarks, such as subcortical volume loss and gliosis and selective or higher number of mHTT aggregates, exon 1 and RAN proteins, and somatic CAG-repeat mosaicism (Fig. 1). Differences in these clinicopathological measures can be explained by environmental, biological, and pathophysiological factors. Although the upbringing of JHD patients in a family with an affected HD parent might well affect certain cognitive and psychiatric measures, differences in biological and pathophysiological factors are important to consider in the light of treatment opportunities.

General HD pathophysiology involves both cell dysfunction and cell loss, with clinical symptoms and signs as a result. Contributing factors to cell dysfunction and loss are a gain of toxic mHTT protein function and a loss of normal huntingtin protein function, and include RNA toxicity, transcriptional dysregulation, mitochondrial dysfunction, excitotoxicity, and inflammation.[64] Neurodegenerative pathomechanisms in HD follow a linear correlation with CAG-repeat length and age at disease onset and are an important contributor to the clinicopathological differences between JHD and AHD patients. Apart from neurodegeneration, aberrant neurodevelopment is also thought to contribute to HD pathophysiology as studies in diverse HD models and postmortem HD brain material have shown defects in cell differentiation, migration, and maturation.[65,66] A recent in vivo study in children carrying a HTT gene expansion – whom will develop HD clinical characteristics later in life – substantiates these preclinical data and showed structural and functional changes in the striatum and cerebellum as young as 6 years.[37,60] As to how these pathological changes during brain development relate to clinical measures later in life (eg, AHD) is unknown. In this regard, JHD patients not only have clinical symptoms during brain maturation, they also have a higher incidence of clinical characteristics that relate to abnormal neurodevelopment. It is therefore likely that postnatal brain maturation and neurodevelopmental pathomechanisms also contribute to the clinicopathological differences between AHD and JHD patients. One could argue that neurodevelopmental defects exceed a certain threshold for normal brain function in JHD patients, whereas these defects only act on a subclinical level in AHD patients. In the following paragraph we will highlight certain neurodevelopmental pathomechanisms and postnatal brain maturation processes and relate these to distinct clinical characteristics in JHD patients.

Neurodevelopmental Defects

Brain development involves overlapping processes of: (1) neurogenesis and cell differentiation; (2) neuronal migration; (3) synaptogenesis; (4) neural circuitry formation;

and (5) synaptic pruning and myelination (see Fig. 2). The first two processes are mainly established before birth (prenatal neurodevelopment), and the latter three continue to change well into early adulthood (postnatal brain maturation).[67] As mentioned earlier, various preclinical studies have revealed aberrant neurogenesis and cell differentiation in relation to pathologically expanded CAG-repeat lengths, as is nicely reviewed by Wiatr and others.[65] Two such studies, using HD stem cells with increasing CAG-repeat lengths, have revealed defective progenitor cell differentiation, abnormal multinucleated neuron morphology, chromosomal instability, and changes in cytokinesis in a CAG-dependent manner.[68,69] This suggests greater neurogenesis and cell differentiation defects in JHD when compared with AHD. Greater defects in early developmental processes can cause distinct clinical characteristics in the JHD population, such as developmental delay (see pink, green, and blue lines in Fig. 2), as is similarly seen in a large heterogeneous group of neurodevelopmental disorders.[70] Another study in R6/2 mice highlighted changes in neuronal migration and arborization (see green and blue lines in Fig. 2) that are similar to those found in focal cortical dysplasia (FCD) type 1.[71] Changes in cortical development are a common etiology of developmental delay and epileptic seizures in a variety of neurodevelopmental disorders. Future studies should determine if there are FCDlike changes in JHD and AHD patient material.

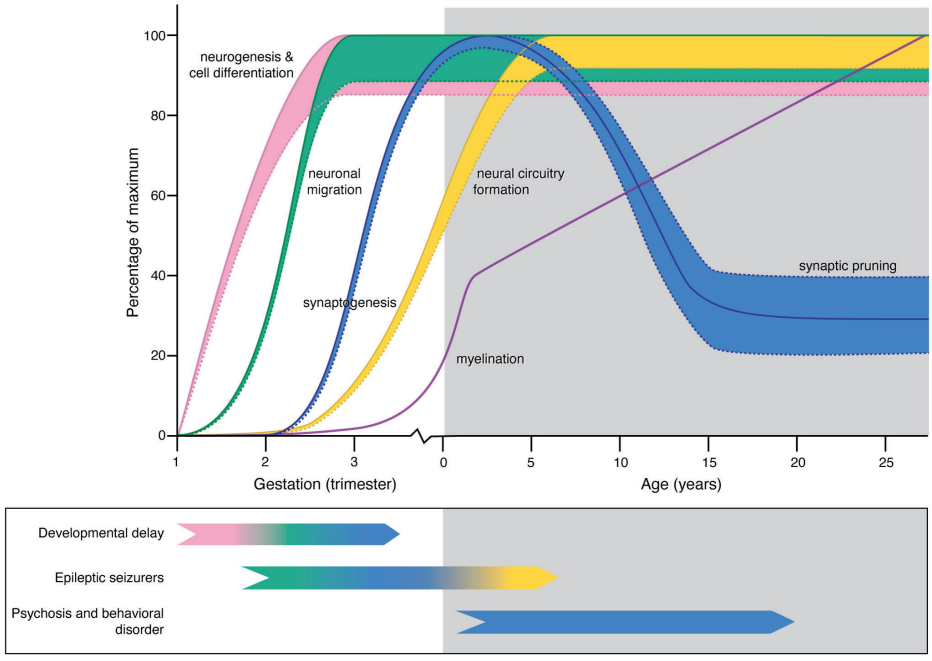


Figure 2. Legend next page

▲**Figure 2.** Model for potential effects of mutant huntingtin (mHTT) in the developing brain of juvenile-onset Huntington disease (JHD) patients.

Normal brain development involves overlapping processes of neurogenesis and cell differentiation (pink line), neuronal migration (green line), neural circuitry formation by dendrite branching (yellow line), and synaptogenesis (first part of blue line), followed by selective synaptic pruning (second part of blue line) and myelination (purple line). Straight lines represent physiological brain development, dotted lines potentially altered neurodevelopment due to a dosage effect of CAG-repeat length or interplay with brain maturation in JHD patients. Distinct clinical characteristics in JHD patients include developmental delay, epileptic seizures, psychosis, and behavioral disorders that could relate to various defects in neurodevelopment or brain maturation (see color code of the representative lines). Note the potential bidirectional effect on synapse abundance (blue dotted lines) on developmental delay (associated with impaired prenatal synaptogenesis) and psychosis and behavioral disorders (associated with impaired postnatal synaptic pruning).

Changes in postnatal brain maturation could also contribute to the clinical picture in JHD. Neural circuitry formation by dendrite branching and formation of new synapses starts during prenatal neurodevelopment but continues to expand into childhood (see yellow line in Fig. 2). A longitudinal imaging study in asymptomatic children carrying an HTT gene expansion showed an incremental effect of CAG-repeat length on functional circuitry adaptations.[37] Resilience of brain regions such as the cerebellum is thought to functionally compensate for early developmental changes, with symptoms occurring as soon as this resilience cannot overcome the accumulating toxic effects of mHTT.[61] A higher burden of toxic RAN and mHTT protein species in JHD cerebelli could explain early loss of resilience and symptom onset, as well as the early occurrence of clinical characteristics, such as postural instability, ataxia, dysarthria, hand dexterity, and dystonia. Furthermore, significant changes in neurotransmitter systems and ion channels during neural circuitry formation render the immature brain more prone to an imbalance between neuronal (GABAergic) inhibition and (glutamergic) excitation, in favor of the latter.[72] This imbalance is an important contributor to the higher incidence of epileptic seizures in childhood neurodevelopmental disorders. The simultaneous dysfunction and loss of GABAergic medium spiny neurons of the striatum as well as prefrontal neurons in JHD patients with childhood-onset of disease renders these patients prone to such an imbalance.

This could provide another explanation for selective occurrence of epileptic seizures in JHD patients. Changes in synapse abundance and pruning could also be involved in JHD pathophysiology (blue line in Fig. 2). By the age of 2 years, infant brain contains about 150% of synaptic connections compared to adult brain. During adolescence, a steep decline in synaptic connections is determined by the amount and timing of neural activity, which is further regulated by elements of the immune system such as microglial and complement function.[73,74] Changes in synapse abundance play an important role in the pathogenesis of neurodevelopmental disorders such as schizophrenia, obsessive–

compulsive disorder, and autism.[73,75] As mentioned earlier, functional circuitries are known to be altered in HD patients. Furthermore, reactive and cellautonomous effects of HD microglia induce a proinflammatory transcription profile and reduced fractalkine expression.[76,77] The latter protein plays an important role in microglial capacity for synaptic pruning. Since psychosis and obsessive–compulsive disorder are far more prevalent in adolescent-onset HD, aberrant synaptic pruning is an interesting target for future studies.

DISCUSSION AND FUTURE DIRECTIONS

Clinicopathological differences between JHD and AHD exist and can be explained by pathological, biological, and environmental factors. A dosage effect of CAG-repeat length on neurodegenerative and neurodevelopmental defects, as well as interaction of pathology with ongoing brain maturational processes in JHD patients, may be responsible for the observed difference. Due to the low prevalence of JHD there is still a huge lack of data linking CAG-repeat length or age at disease onset with the underlying pathophysiology. In addition, many (pre)clinical studies focus on JHD or AHD and fail to structurally compare the two forms. Unraveling possible pathophysiological differences between JHD and AHD is important for the development of therapeutics designed to reduce symptoms or alter disease progression. For instance, due to differences in symptoms that affect brain areas and pathomechanisms, JHD patients might benefit from therapeutics which have been shown to be ineffective in adult patients. Conversely, therapeutics that target mutant huntingtin might be disproportionally damaging in JHD patients due to its effect on concurrent brain maturation. Furthermore, neurodevelopmental pathomechanisms largely take place in utero and are more difficult to influence once symptoms present themselves. Patients with a complex disorder such as HD might therefore benefit from a combination of therapies in a personalized way rather than a generalized one. Although the severity of JHD will lead clinicians to treat JHD patients as soon as viable therapeutic options are identified in the adult population, lack of wellestablished and reliable outcome measures in the pediatric population will further complicate successful implementation of therapeutic strategies. Therefore, future studies should focus on inclusion of both JHD and AHD patients, longitudinal study designs, structural comparison of CAG-repeat lengths, or age at disease onset, the specification of JHD readout parameters and possible interactions with postnatal brain development. Furthermore, international collaboration is necessary due to the rarity of JHD, and ethical and legal issues in pediatric studies must be overcome. Finally, the implementation of highly standard translational research methods will greatly enhance our knowledge of JHD.

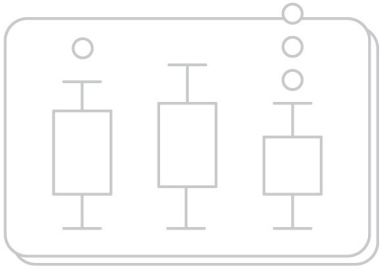
REFERENCES

1. The Huntington Disease Collaborative Research Group . A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington disease chromosomes. Cell 1993;72(6):971–983.
2. Squitieri F, Frati L, Ciarmiello A, Lastoria S, Quarrell O. Juvenile Huntington disease: does a dosageeffect pathogenic mechanism differ from the classical adult disease? Mech Ageing Dev 2006;127(2):208–212.
3. Milunsky JM, Maher TA, Loose BA, Darras BT, Ito M. XL PCR for the detection of large trinucleotide expansions in juvenile Huntington disease. Clin Genet 2003;64(1):70–73.
4. Warren JD, Firgaira F, Thompson EM, Kneebone CS, Blumbergs PC, Thompson PD. The causes of sporadic and 'senile' chorea. Aust N Z J Med 1998;28(4):429–431.
5. Roos RA. Huntington disease: a clinical review. Orphanet J Rare Dis 2010;5:40.
6. Quarrell O, O'Donovan KL, Bandmann O, Strong M. The prevalence of juvenile Huntington disease: a review of the literature and metaanalysis. PLoS Curr 2012;4:e4f8606b8742ef8603.
7. Quarrell OWJ, Nance MA, Nopoulos P, et al. Defining pediatric Huntington disease: time to abandon the term juvenile Huntington disease? Mov Disord 2019;4:584–585.
8. Fusilli C, Migliore S, Mazza T, et al. Biological and clinical manifestations of juvenile Huntington disease: a retrospective analysis. Lancet Neurol 2018;17(11):986–993.
9. Quarrell OWJ, Nance M. Juvenile Huntington Disease and Other Trinucleotide Repeat Disorders. The Diagnostic Challenge. Oxford: Oxford University Press; 2012.
10. Went LN, Vegtervan der Vlis M, Bruyn GW. Parental transmission in Huntington disease. Lancet 1984;1(8386):1100–1102.
11. GonzalezAlegre P, Afifi AK. Clinical characteristics of childhood-onset (juvenile) Huntington disease: report of 12 patients and review of the literature. J Child Neurol 2006;21(3):223–229.
12. Ribai P, Nguyen K, HahnBarma V, et al. Psychiatric and cognitive difficulties as indicators of juvenile Huntington disease onset in 29 patients. Arch Neurol 2007;64(6):813–819.
13. Achenbach J, Thiels C, Lücke T, Saft C. Clinical manifestation of juvenile and pediatric HD patients: a retrospective case series. Brain Sci 2020;10(6):340–357.
14. Vonsattel JPG, Cortes EP, Keller CE. Juvenile Huntington Disease and Other Trinucleotide Repeat Disorders. Juvenile Huntington Disease: Neuropathology. Oxford: Oxford University Press; 2012.
15. Siesling S, Vegtervan der Vlis M, Roos RA. Juvenile Huntington disease in The Netherlands. Pediatr Neurol 1997;17(1):37–43.
16. Brackenridge CJ. Factors influencing dementia and epilepsy in Huntington disease of early onset. Acta Neurol Scand 1980;62(5):305–311.
17. van Dijk JG, van der Velde EA, Roos RA, Bruyn GW. Juvenile Huntington disease. Hum Genet 1986;73(3):235–239.
18. Nance MA. Genetic testing of children at risk for Huntington disease. US Huntington disease genetic testing group. Neurology 1997;49(4):1048–1053.
19. Jacobs M, Hart EP, van Zwet EW, et al. Progression of motor subtypes in Huntington disease: a 6year followup study. J Neurol 2016;263(10):2080–2085.
20. van de Zande NA, Massey TH, McLauchlan D, et al. Clinical characterization of dystonia in adult patients with Huntington disease. Eur J Neurol 2017;24(9):1140–1147.
21. Cronin T, Rosser A, Massey T. Clinical presentation and features of juvenile-onset Huntington disease: a systematic review. J Huntington Dis 2019;8(2):171–179.
22. Moser AD, Epping E, EspePfeifer P, et al. A surveybased study identifies common but unrecognized symptoms in a large series of juvenile Huntington disease. Neurodegener Dis Manag 2017;7(5):307–315.

23. Franklin GL, Camargo CHF, Meira AT, et al. Is ataxia an underestimated symptom of Huntington disease? Front Neurol 2020;11:571843.
24. Poniatowska R, Habib N, Krawczyk R, Rakowicz M. Correlation between magnetic resonance and genetic, clinical, neurophysiological and neuropsychological studies of patients with juvenile form of Huntington disease. Case Rep Clin Pract Rev 2001;146:1633–1645.
25. McAllister B, Gusella JF, Landwehrmeyer GB, et al. Timing and impact of psychiatric, cognitive, and motor abnormalities in Huntington disease. Neurology 2021;96(19):e2395–e2406.
26. Craufurd D, Snowden J. Neuropsychiatry and neuropsychology. Huntington Dis 2014;4:36–65.
27. Yoon G, Kramer J, Zanko A, et al. Speech and language delay are early manifestations of juvenile-onset Huntington disease. Neurology 2006;67(7):1265–1267.
28. van Duijn E, Craufurd D, Hubers AA, et al. Neuropsychiatric symptoms in a European Huntington disease cohort (REGISTRY). J Neurol Neurosurg Psychiatry 2014;85(12):1411–1418.
29. Langbehn KE, Cochran AM, van der Plas E, et al. Behavioral deficits in juvenile-onset Huntington disease. Brain Sci 2020;10(8):543–554.
30. Cloud LJ, Rosenblatt A, Margolis RL, et al. Seizures in juvenile Huntington disease: frequency and characterization in a multicenter cohort. Mov Disord 2012;27(14):1797–1800.
31. Aziz NA, van der Burg JM, Landwehrmeyer GB, et al. Weight loss in Huntington disease increases with higher CAG-repeat number. Neurology 2008;71(19):1506–1513.
32. Tereshchenko A, McHugh M, Lee JK, et al. Abnormal weight and body mass index in children with juvenile Huntington disease. J Huntington Dis 2015;4(3):231–238.
33. Vonsattel JP, Keller C, Cortes Ramirez EP. Huntington disease neuropathology. Handb Clin Neurol 2011;100:83–100.
34. Vonsattel J, Cortes EP, Keller CE. Juvenile Huntington Disease: Neuropathology. New York, NY: Oxford University Press; 2009.
35. Rosenblatt A, Margolis RL, Becher MW, et al. Does CAG-repeat number predict the rate of pathological changes in Huntington disease? Ann Neurol 1998;44(4):708–709.
36. Penney JB Jr, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH. CAG-repeat number governs the development rate of pathology in Huntington disease. Ann Neurol 1997;41(5):689–692.
37. van der Plas E, Langbehn DR, Conrad AL, et al. Abnormal brain development in child and adolescent carriers of mutant huntingtin. Neurology 2019;93(10):e1021–e1030.
38. Kennedy L, Evans E, Chen CM, et al. Dramatic tissuespecific mutation length increases are an early molecular event in Huntington disease pathogenesis. Hum Mol Genet 2003;12(24):3359–3367.
39. DiFiglia M, Sapp E, Chase KO, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997;277(5334):1990–1993.
40. MaatSchieman ML, Dorsman JC, Smoor MA, et al. Distribution of inclusions in neuronal nuclei and dystrophic neurites in Huntington disease brain. J Neuropathol Exp Neurol 1999;58(2):129–137.
41. Becher MW, Kotzuk JA, Sharp AH, et al. Intranuclear neuronal inclusions in Huntington disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol Dis 1998;4(6):387–397.
42. Tereshchenko A, Magnotta V, Epping E, et al. Brain structure in juvenile-onset Huntington disease. Neurology 2019;92(17):e1939–e1947.

43. Rüb U, Hentschel M, Stratmann K, et al. Huntington disease (HD): degeneration of select nuclei, widespread occurrence of neuronal nuclear and axonal inclusions in the brainstem. Brain Pathol 2014;24(3):247–260.
44. Jansen AH, van Hal M, Op den Kelder IC, et al. Frequency of nuclear mutant huntingtin inclusion formation in neurons and glia is celltypespecific. Glia 2017;65(1):50–61.
45. Neueder A, Landles C, Ghosh R, et al. The pathogenic exon 1 HTT protein is produced by incomplete splicing in Huntington disease patients. Sci Rep 2017;7(1):1307.
46. BañezCoronel M, Ayhan F, Tarabochia AD, et al. RAN translation in Huntington disease. Neuron 2015;88(4):667–677.
47. Benn CL, Landles C, Li H, et al. Contribution of nuclear and extranuclear polyQ to neurological phenotypes in mouse models of Huntington disease. Hum Mol Genet 2005;14(20):3065–3078.
48. 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 1994;6(4):409–414.
49. de Rooij KE, de Koning Gans PA, Roos RA, van Ommen GJ, den Dunnen JT. Somatic expansion of the (CAG)n repeat in Huntington disease brains. Hum Genet 1995;95(3):270–274.
50. Ruocco HH, Bonilha L, Li LM, LopesCendes I, Cendes F. Longitudinal analysis of regional grey matter loss in Huntington disease: effects of the length of the expanded CAG-repeat. J Neurol Neurosurg Psychiatry 2008;79(2):130–135.
51. Ruocco HH, LopesCendes I, Laurito TL, Li LM, Cendes F. Clinical presentation of juvenile Huntington disease. Arq Neuropsiquiatr 2006;64(1):5–9.
52. Ruocco HH, LopesCendes I, Li LM, SantosSilva M, Cendes F. Striatal and extrastriatal atrophy in Huntington disease and its relationship with length of the CAG-repeat. Braz J Med Biol Res 2006;39(8):1129–1136.
53. Jervis GA. Huntington chorea in childhood. Arch Neurol 1963;9(3):244–257.
54. Markham CH, Knox JW. Observations on Huntington chorea in childhood. J Pediatr 1965;67(1):46–57.
55. Byers RK, Gilles FH, Fung CJN. Huntington disease in children: neuropathologic study of four cases. Neurology 1973;23(6):561–561.
56. Latimer CS, Flanagan ME, Cimino PJ, et al. Neuropathological comparison of adult-onset and juvenile Huntington disease with cerebellar atrophy: a report of a father and son. J Huntington Dis 2017;6(4):337–348.
57. Nicolas G, Devys D, Goldenberg A, et al. Juvenile Huntington disease in an 18monthold boy revealed by global developmental delay and reduced cerebellar volume. Am J Med Genet A 2011;155a(4):815–818.
58. Sakazume S, Yoshinari S, Oguma E, et al. A patient with early onset Huntington disease and severe cerebellar atrophy. Am J Med Genet A 2009;149a(4):598–601.
59. de Azevedo PC, Guimarães RP, Piccinin CC, et al. Cerebellar gray matter alterations in Huntington disease: a voxelbased Morphometry study. Cerebellum 2017;16(5–6):923–928.
60. Tereshchenko AV, Schultz JL, Bruss JE, Magnotta VA, Epping EA, Nopoulos PC. Abnormal development of cerebellarstriatal circuitry in Huntington disease. Neurology 2020;94(18):e1908–e1915.
61. van der Plas E, Schultz JL, Nopoulos PC. The neurodevelopmental hypothesis of Huntington disease. J Huntington Dis 2020;9(3):217–229.
62. Casella C, Lipp I, Rosser A, Jones DK, MetzlerBaddeley C. A critical review of white matter changes in Huntington disease. Mov Disord 2020;35(8):1302–1311.
63. Hedjoudje A, Nicolas G, Goldenberg A, et al. Morphological features in juvenile Huntington disease associated with cerebellar atrophy magnetic resonance imaging morphometric analysis. Pediatr Radiol 2018;48(10):1463–1471.

64. Ross CA, Tabrizi SJ. Huntington disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011;10(1):83–98.
65. 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 2018;55(4):3351–3371.
66. Barnat M, Capizzi M, Aparicio E, et al. Huntington disease alters human neurodevelopment. Science 2020;369(6505):787–793.
67. Lee Gregory M, Burton VJ, Shapiro BK. Chapter 3 developmental disabilities and metabolic disorders. In: Zigmond MJ, Rowland LP, Coyle JT, eds. Neurobiology of Brain Disorders. San Diego, CA: Academic Press; 2015:18–41.
68. Conforti P, Besusso D, Bocchi VD, et al. Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes. Proc Natl Acad Sci U S A 2018;115(4):E762–e771.
69. Ruzo A, Croft GF, Metzger JJ, et al. Chromosomal instability during neurogenesis in Huntington disease. Development 2018;145(2).
70. van Bokhoven H. Genetic and epigenetic networks in intellectual disabilities. Annu Rev Genet 2011;45:81–104.
71. Cepeda C, Oikonomou KD, Cummings D, et al. Developmental origins of cortical hyperexcitability in Huntington disease: review and new observations. J Neurosci Res 2019;97(12):1624–1635.
72. Wong M. Advances in the pathophysiology of developmental epilepsies. Semin Pediatr Neurol 2005;12(2):72–87.
73. Sakai J. Core concept: how synaptic pruning shapes neural wiring during development and, possibly, in disease. Proc Natl Acad Sci U S A 2020;117(28):16096–16099.
74. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complementdependent manner. Neuron 2012;74(4):691–705.
75. Paolicelli RC, Ferretti MT. Function and dysfunction of microglia during brain development: consequences for synapses and neural circuits. Front Synap Neurosci 2017;9:9.
76. CreusMuncunill J, Ehrlich ME. Cellautonomous and noncellautonomous pathogenic mechanisms in Huntington disease: insights from in vitro and in vivo models. Neurotherapeutics 2019;16(4):957–978.
77. Kim A, GarcíaGarcía E, Straccia M, et al. Reduced fractalkine levels lead to striatal synaptic plasticity deficits in Huntington disease. Front Cell Neurosci 2020;14:163.



CHAPTER 4

Comparison of the clinical spectrum of juvenile- and adult-onset Huntington Disease: a national cohort and Enroll-HD observational study

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ABSTRACT

Background and Objectives: Differences in clinical characteristics between juvenile-onset Huntington Disease (JHD) and adult-onset HD (AHD) are hypothesized but not directly compared. This study compares clinical characteristics occurrence and severity across the age-at-onset (AO) subtypes.

Methods: Using the national juvenile-onset HD patient cohort and the international Enroll-HD registry (NCT01574053), we compared childhood-onset JHD (cJHD; AO 0-10), adolescent-onset JHD (aJHD; AO 11-20), and adult-onset HD (AHD; AO 21-65) on proportions of clinical characteristics at onset and psychiatric characteristics in pooled datasets. Additionally, mixed models were applied to longitudinal data from ENROLL-HD to compare fitted severity and annual progression in motor and neurocognitive domains 5 years post-onset across the 3 AO-HD subtypes.

Results: The combined datasets provided clinical data from 46 patients with cJHD (mean AO 6.7, 45% female), 243 patients with aJHD (mean AO 16.7, 46% female), and 9 504 patients with AHD (mean AO 44.7, 51% female). At onset, neurocognitive symptoms occurred in 47.5% of patients with cJHD (n=46; 95% CI 31.8-63.7%), significantly more often compared to 24.9% of patients with aJHD (n=209; 19.3-31.4%) and 15% of patients with AHD (n=8 177; 14.3-15.8%). Psychiatric symptoms occurred in 47.1% of patients with aJHD (95% CI 40.2-54.1%), significantly more compared to 31% of patients with AHD (30.1-32%). Throughout the disease, aggressive behavior occurred in 73.9% of patients with cJHD (n=46; 95% CI 58.6-85.2%) and 55.9% of patients with aJHD (n=238; 49.3-62.3%), significantly more compared with 40.7% of patients with AHD (n=9 501; 39.7-41.7%). Psychosis occurred in 23.5% of patients with aJHD (95% CI 18.4-29.5%), significantly more compared with 12.8% of those with AHD (12.1-13.5%). Linear regression revealed significantly higher predicted mean UHDRS-TMS scores for dysarthria (1.38, 95% CI 1.08-1.69), parkinsonism (9.85, 8.43-11.27), dystonia (6.47, 4.98-7.96), and oculomotor disturbances (9.86, 7.75-11.97), along with higher predicted annual changes in dysarthria (0.25, 0.16-0.34), oculomotor (1.53, 0.99-2.07) and gait and balance (0.79, 0.55-1.03) in earlier onset phenotypes.

Discussion: This study highlights distinct clinical patterns in JHD subtypes compared with AHD. Stratification by age at onset–defined HD subtypes is needed in future studies. Our use of regression models should not be interpreted as prediction model or to infer causality.

INTRODUCTION

Huntington Disease (HD) is an autosomal dominant brain disorder caused by a pathologically-expanded CAG-repeat (≥36) in the Huntingtin gene.[1]Age at clinical onset is inversely correlated with CAG-repeat length, explaining up to 84% of variability.[2] The mean age at symptom onset is between 30-50 years (range 1.5-87).[3] The term juvenile-onset HD (JHD) is arbitrarily defined for HD patients with symptom onset <21 years, which is seen in approximately 0.5-5% of HD patients.[4,5] Importantly, clinical differences exist between JHD patients with disease onset in childhood (cJHD; onset ≤10 years) and in adolescence (aJHD; onset between 11-20 years).[6] cJHD, mostly associated with CAG-repeats ≥80, represents a different and more aggressive HD subtype.[7]

Over the years, various retrospective JHD case reports and series aided our understanding of this subtype of HD.[6] Patients with JHD often present with a combination of neurocognitive impairments (decline in attention, memory or school performance), psychiatric (e.g. irritability and depression) and behavioral disturbances, early onset of gait, speech and swallowing disturbances, and a hypokinetic-rigid syndrome. In addition, other HD symptoms such as sleep disturbances, epileptic seizures, pain and weight loss are commonly described. Furthermore, systemic disease manifestations in cardiovascular, respiratory and gastrointestinal domains are frequently observed in HD and in some instances correlate with age at disease onset or CAG-repeat length.[8-11] The comparison of clinical characteristics between patients with cJHD, aJHD and adult-onset HD (AHD) becomes more relevant when considering pathophysiologic differences between these Age at Onset-defined HD (AO-HD) subtypes.[12] However, comparative studies between patiens with JHD subtypes and AHD are rarely performed. One such study revealed faster progression of motor symptoms and shorter survival of patients with cJHD patients compared to those with aJHD and AHD.[7] Other studies highlighted differences between patients with JHD and AHD regarding neurocognitive and psychiatric changes at the onset of disease,[13,14] and epilepsy,[15] however, they did not differentiate between childhood-onset and adolescent-onset of disease.

To address JHD subtype differences and to ensure participation of patients with JHD in future interventional studies, quantification of expected clinical differences between AO-HD subtypes is essential.

The major limitation of studying the JHD phenotype is its low prevalence. To allocate as much JHD cases as possible, we started in 2020 a national Dutch registry for

juvenile-onset HD patients (HD-JUNIOR). By the combined use of HD-JUNIOR and the international Enroll-HD platform,[16] the objective of this study was to describe and compare JHD subtypes with AHD in the occurrence of clinical characteristics at onset and during the disease course. In addition, by use of linear mixed models we aimed to compare the fitted severity and annual change for 3 reference patients (cJHD, aJHD and AHD) based on longitudinal clinical data of Enroll-HD. Compared with AHD, we hypothesize that JHD subtypes will have a higher proportion of psychiatric and neurocognitive disease characteristics at onset and a higher occurrence of behavioral changes, epilepsy and pain during the course of the disease. We also hypothesize that cJHD will have more severe and faster progression of motor disease characteristics related to hypokinetic-rigid syndrome, dystonia and dysarthria and less severe chorea compared with aJHD and AHD.

METHODS

Study Design and Population

To analyze JHD patient data from as many patients as possible and to allow for the comparison of JHD with typical disease onset in adulthood, data from 2 (J) HD datasets were used: the HD-JUNIOR and Enroll-HD registries. HD-JUNIOR was started in 2020 and retrospectively collects clinical data of both alive and deceased patients with JHD in the Netherlands (n=28). Enroll-HD[16] is an international (183 sites in 23 countries) prospective observational study since 2012 in which clinical data from (J)HD gene carriers, - patients and controls are gathered. Core Unified Huntington's Disease Rating Scale (UHDRS) datasets were collected annually from all research participants as part of this multicenter longitudinal observational study. Data were monitored for quality and accuracy using a risk-based monitoring approach. Data were generously provided by the participants in the Enroll-HD study and made available by Cure Huntington's Disease Initiative (CHDI) Foundation, Inc. For this study, the 5th periodic dataset was used (PDS5; release 18-DEC-2020; n=21 116 participants), including a specified dataset with deaggregated data for AO and enrolment younger than 17 years and CAG-repeat length of 70 and higher.

Selection and stratification criteria for this study consisted of a clinical diagnosis of HD and AO-HD subtypes (as defined below). Based on a higher suggested occurrence of psychiatric and neurocognitive disease characteristics in with JHD

at onset,[6] a JHD phenotype was primarily defined by any HD-related first symptom below 21 years of age and, subsequently, occurrence of motor symptoms within 15 years of first symptoms. Subsequently, patients with JHD were subdivided into childhood-onset JHD (cJHD: primary onset ≤10 years) and adolescent-onset JHD phenotype (aJHD: primary onset between 11 and 20 years). For the comparison of clinical characteristics in JHD subtypes with typical disease-onset in adulthood, inclusion criteria for an AHD phenotype were any HD-related first symptom between age 21 and 65 years and a CAG-repeat ≥40. In HD-JUNIOR, primary assessment of eligibility was performed by H.S.B and T.A.K. based on all available information in the medical records. In case of a questionable relationship of first symptom with JHD phenotype, S.T.B was consulted for confirmation or withdrawal of the patient with JHD in the registry. In Enroll-HD, participants were selected from the PDS5 by using of the retrospective HD Clinical Characteristics (HDCC) questionnaire, including: raters' estimate of age at first symptom onset; age at first motor symptom onset; and clinical HD diagnosis. Based on this selection H.S.B., R.A.C.R and S.T.B. then further analyzed clinical outliers of the JHD groups based on raters' confidence of AO estimation and time between age onset and enrolment. Patients who we classified as outliers were removed from further analyses and are listed in Supplementary Table 1. See the STROBE- flow diagram for the number of eligible AO-HD defined patients in the HD-JUNIOR and ENROLL-HD datasets (Figure 1). Five patients with aJHD were part of both datasets and therefore excluded from the HD-JUNIOR dataset in case of pooled analysis.

Standard Protocol Approvals, Registrations, and Patient Consents

Local ethical approval for the conduct of assessments on human participants (Enroll-HD, NCT01574053) and use of pseudonymized clinical data (HD-JUNIOR) was provided by the medical research ethical committee of Leiden-The Hague-Delft (MREC-LDD). In addition, all participating sites in Enroll-HD were required to obtain and maintain local ethical approval. Written informed consent was obtained from all participants (or guardians of participants) in the Enroll-HD registry and from all alive participants in the HD-JUNIOR registry. In the case of clinical data from deceased patients with JHD in the HD-JUNIOR registry, the MREC-LDD determined that consent was not required and pseudonymized data were shared by the last treating physician.

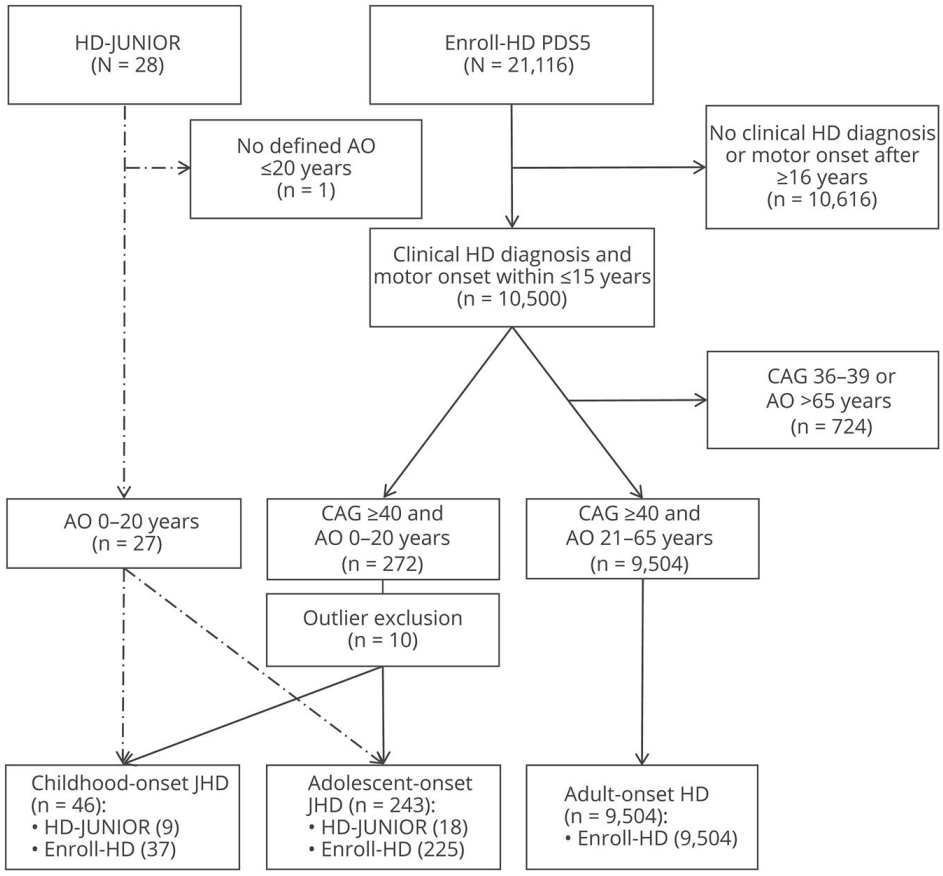


Figure 1. Participant Selection in HD-JUNIOR and Enroll-HD PDS5.

The STROBE flow diagram illustrates the selection and stratification criteria for participants from the HD-JUNIOR (long dashed dot line) and Enroll-HD (solid line) datasets, detailing the number of patients who contributed data for 1 or more outcome measures in this study. Selected patients were stratified into 3 AO-HD subtypes: childhood-onset JHD, adolescent-onset JHD, and adult-onset HD.

AO = age at onset; CAG = cytosine-adenine-guanine repeats in the Huntingtin gene; HD = Huntington disease; n = number of participants; PDS5 = periodic dataset 5; STROBE = Strengthening the Reporting of Observational Studies in Epidemiology.

Outcome Variables

Our aim was to analyze and compare clinical characteristics at disease onset and throughout the disease course across the 3 key neurologic domains in HD: (1) motor, (2) neurocognitive, and (3) psychiatric, as well as (4) other domains.

For the cross-sectional analysis of the prevalence of HD clinical characteristics at onset and occurrence of psychiatric characteristics during the disease course,

retrospective data of both datasets were pooled (HD-JUNIOR: patient/caretaker answer retrieved from medical records; Enroll-HD: patient answer in the HDCC questionnaire) because of comparable outcome and assessment method. For the analysis of disease characteristics at onset, we defined 3 outcome variables:(1) (mixed) motor onset, (2) (mixed) neurocognitive onset, and (3) (mixed) psychiatric onset. For the occurrence of psychiatric disease characteristics, we specified 6 subclusters of which comparable data were available in both datasets: (1) irritability, (2) violent/aggressive behavior, (3) depression, (4) apathy, (5) perseverative and obsessive-compulsive behavior and (6) psychosis. For both outcomes, patients were omitted from analyses in case of missing data.

To perform a cross-sectional analysis of the occurrence of HD motor characteristics during the disease course we used retrospective data from HD-JUNIOR alone (patient/caretaker answer and neurological examination).

To assess the severity of neurocognitive disease characteristics during the disease course in the HD-JUNIOR dataset we used neurocognitive measures (full, verbal and performance IQ) based on neuropsychological assessments obtained from 7 patients with cJHD and 9 patients with aJHD.

In the 'other' domain we performed cross-sectional analysis on the occurrence of epilepsy and pain during the disease course. Regarding epilepsy, in HD-JUNIOR epileptic seizures were recorded in case it was mentioned by the patient, caretaker or medical expert and confirmatory EEG data or summary were available. In the Enroll-HD dataset, we used the comorbidity information to select all patients with an ICD-10 registration "G40": epilepsy and recurrent seizures. For both datasets, the occurrence of epilepsy was recorded as not present in case of no specified data on the outcome. The occurrence of epilepsy was pooled between datasets because the assessment method for epilepsy in both relate to a clinical diagnosis of epileptic seizures. Regarding the assessment of pain, we specified the occurrence of pain in case it was mentioned by the patient or caretaker in the medical file. The occurrence of pain was recorded as not present in case of no specified data on the outcome. Different from the retrospective assessment of pain in the HD-JUNIOR dataset, we used the prospective Short Form health-survey (SF12) at baseline from the Enroll-HD dataset to assess pain interference during daily activities in the past week and to compare it between AO-HD subtypes.[17]

Prospective measurements from the UHDRS-Total-Motor-Score (UHDRS-TMS; motor symptoms), the Symbol-Digit-Modalities-Test (UHDRS-SDMT; psychomotor processing speed) and Stroop-Interference-Test (UHDRS-SIT; executive functioning) of the Enroll-HD dataset were used to predict the severity and annual progression of motor and neurocognitive symptoms for 3 hypothetical patients referring to the AO-HD subtypes as measured 5 years after onset. Regarding the UHDRS-TMS, 6 outcome subclusters were defined based on neuroanatomical and - physiological origin: (1) oculomotor, (2) dysarthria, (3) chorea, (4) dystonia, (5) parkinsonism (hypo- , bradykinesia and rigidity), and (6) gait and balance (see legend Figure 3 for more details). For the assessment of severity (mean score), data from all available visits of the participants in the defined AO-HD subgroups was used (cJHD *n*=60 visits from *n*=37 participants, aJHD *n*=465 visits from *n*=225 participants, AHD *n*=23 225 visits from *n*=9 504 participants). For the assessment of annual progression (mean annual change), data from all participants in the defined AO-HD subgroups, with more than 1 visit, was used (cJHD *n*=35 visits from *n*=13 participants, aJHD *n*=265 from *n*=125 participants, AHD *n*=14 291 visits from *n*=6 075 participants).

Statistics

All analyses were performed using R Statistical Software (v4.3.2; R Core Team 2020).[18] The tidyverse (v2.0.0; 2019) package was used for statistical analyses.[19]

Pairwise comparisons for proportions (Z-test) were performed to compare AO-HD subtypes of the pooled datasets on the occurrence(yes/no) of (1) motor, neurocognitive or psychiatric disease features at onset, (2) specified psychiatric disease characteristics during the disease course (3) epileptic seizures during the disease course and (4) pain interference during the disease course. Adjustment of p-values for 3x multiple testing (1: cJHD vs aJHD, 2: cJHD vs AHD and 3: aJHD vs AHD) was done by Holm's method. Fisher's exact test was performed to compare JHD subtypes of the HD-JUNIOR dataset (too small sample size to assume normality) on (1) the occurrence of specified disease characteristics at onset and (2) on specified motor disease characteristics during the disease course. P-values $<.05$ (2-tailed) were considered statistically significant.

We want to compare the progression of motor and neurocognitive symptoms after onset between the AO-HD subtypes. This is particularly challenging because age at measurement is an important determinant, but there is very little age overlap between the subtypes. To overcome this challenge, we fitted multivariable linear

mixed regression models to the observed motor and neurocognitive symptoms taking into account the patients' sex, age at onset and age at measurement for the 3 different AO-HD subtypes. We do not intend to use the models to predict the disease course for a new patient. Rather, we use the fitted values from the model to describe the "typical" disease progression among the patients from the 3 subtypes, and to make tentative comparisons. To allow for a flexible description of disease severity and progression, we included the following independent variables: sex, age at onset (AO), AO^2, age at measurement (AM), AM^2 and the interactions between (1) AO and AM, (2) AO^2 and AM, (3) AO and AM^2 and (4) AO^2 and AM^2. Finally, we used a random intercept per patient to account for the correlation between repeated measurements on the same individual. This model has 10 regression coefficients which are difficult to interpret in isolation. Therefore, we graph the fitted severity scores during the first 20 years after disease onset for 3 reference patients representing the AO-HD subtypes: A female with AO=6 (cJHD), a female with AO=17 (aJHD) and a female with AO=45 (AHD). We then compare the fitted severity scores of the different outcomes at 5 years since the primary onset of the disease. To compare the annual rate of progression (mean annual change) at 5 years after disease onset, we simplified our model to include only sex, AO, AM and their interaction. In this model, the rate of annual progression is a simple slope. P-values were adjusted by Tukey's method to account for the 3 comparisons of AHD versus aJHD, AHD versus cJHD and cJHD versus aJHD.

Data availability

Enroll-HD anonymized data are available upon request through the CHDI Foundation, Inc. For additional information regarding HD-JUNIOR data for research purposes, the principal investigator S.T. de Bot MD PhD may be contacted.

RESULTS

1.0 Demographic characteristics of the AO-HD subtypes per dataset.

The number of included patients of the HD-JUNIOR and Enroll-HD datasets and stratified by AO-HD subtype are provided in Table 1. No clinically meaningful difference was observed between the JHD samples of the 2 different datasets regarding CAG-repeat length and age at primary onset. Owing to missing values and made selections (as described in methods), the number of participants for the specified outcome measures may slightly differ from the number given in Table 1 and is therefore explicitly stated per outcome measure.

Table 1. Patient Sample Characteristics per AO-HD Subtype and Dataset

	Childhood-onset JHD		Adolescent-onset JHD		Adult-onset HD
	HD-JUNIOR (n = 9)	**Enroll-HD (n = 37)**	**HD-JUNIOR (n = 18)**	**Enroll-HD (n = 225)**	**Enroll-HD (n = 9,504)**
Age at primary onset, mean ± SD	6.70 ± 2.10	6.50 ± 2.60	16.60 ± 2.40	16.80 ± 2.50	44.70 ± 10.40
Age at enrollment, mean ± SD (range)	17.90 ± 3.00 (15.00–23.00)	16.60 ± 6.50 (7.00–29.00)	28.00 ± 5.50 (20.00–39.00)	26.80 ± 5.80 (13.00–47.00)	51.70 ± 11.40 (19.00–92.00)
Years between primary and motor onset mean ± SD (range)	1.00 ± 2.10 (0.00–6.00)	2.90 ± 3.60 (0.00–12.00)	0.90 ± 1.30 (0.00–4.00)	3.20 ± 4.00 (0.00–15.00)	1.20 ± 2.50 (0.00–15.00)
Years between primary onset and enrollment mean ± SD (range)	11.30 ± 3.00 (7.00–17.00)	10.10 ± 5.50 (1.00–17.00)	11.40 ± 5.30 (4.00–23.00)	10.00 ± 5.40 (0.00–19.00)	7.00 ± 5.60 (–7.00—47.00)
Follow-up time since enrollment in y mean ± SD (range)	n/a	1.50 ± 1.70 (0.00–6.20)	n/a	1.80 ± 1.70 (0–7.10)	1.90 ± 1.70 (0–7.60)
Sex					
M/F %	56.00/44.00	54.00/46.00	61.00/39.00	51.00/49.00	49.00/51.00
CAG-repeat mean ± SD (range)	77.00 ± 9.00 (66.00–92.00)	75.00 ± 17.00 (48.00–110.00)	58.00 ± 6.00 (49.00–68.00)	57.00 ± 8.00 (41.00–81.00)	44.00 ± 3.00 (40.00–65.00)
Inheritance paternal/maternal %	89.00/11.00	80.00/20.00	76.00/24.00	67.00/32.00	48.00/52.00

Abbreviations: CAG-repeat = cytosine-adenine-guanine repeats in the Huntingtin gene; F = female; M = male; n/a = not applicable; OC = outlier criterion; SC = selection criterium.

AO-HD sample characteristics related to age at primary symptom onset (SC), age at enrollment in the dataset (for HD-JUNIOR, this corresponds to age at death if medical records were obtained posthumously), years between primary symptom and first motor symptom onset (SC), years between primary symptom onset and enrollment in the dataset (OC for Enroll-HD), and follow-up time since enrollment (not applicable for HD-JUNIOR, because data are collected retrospectively). Additional characteristics include sex, CAG-repeat length (SC for patients with adult-onset HD in Enroll-HD), and inheritance.

2.0 Disease characteristics at onset

The prevalence of disease characteristics at onset were analyzed and compared between AO-HD subtypes in pooled data from the 2 datasets (Figure 2). Stratified counts and proportions per dataset are listed in Supplementary Table 2.

In the cJHD subtype, 47.5% of patients (n=46, 95% CI 31.8-63.7) presented with a neurocognitive phenotype, significantly more often than 24.9% of patients with aJHD (n=209, 95% CI 19.3-31.4, p=<.01) and 15% of patients with AHD (n=8 177, 95% CI 14.3-15.8, p=<.001) (Figure 2A). The prevalence of neurocognitive signs at onset in

patients with aJHD was also significantly higher compared to AHD patients (p=<.001). Specified initial disease characteristics were further analyzed in patients with JHD of the HD-JUNIOR dataset (Supplementary Table 3; cJHD n=9, aJHD n=17), because these types of data were not available in the Enroll-HD dataset. Initial neurocognitive changes were most often encountered as learning difficulties (cJHD 3 of 9; aJHD 2 of 18) and attention deficit (cJHD 2 of 9; aJHD 1 of 18). Furthermore, 5 of 9 patients with cJHD presented with developmental regression and 1 of 9 with developmental delay, which were not mentioned in patients with aJHD (Fisher's exact p=.027). Of these 6 of 9 patients with cJHD with changes in development, 4 were related to initial changes in motor development (fine motor skills and walking pattern) and 2 to initial changes in neurocognitive development (repeating class and need for special education).

In the aJHD subtype 47.1% of patients (n=208, 95% CI 40.2-54.1) presented with psychiatric signs and complaints, significantly more often when compared to 31% of patients with AHD (n=8 472, 95% CI 30.1-32.0, p=<.001) (Fig. 2B). Furthermore, the aJHD subtype had in 45.8% of patients (n=212, 95% CI 39.0-52.7) motor signs and symptoms at onset (Fig. 2C), significantly less often when compared with 70% of patients with AHD (n=8 630, 95% CI 69.0-71.0, p=<.001). The most prevalent initial psychiatric change of patients with aJHD within the HD-JUNIOR dataset (Supplementary Table 3) was irritable and aggressive behavior (7 of 16 patients), which was not observed in 9 patients with cJHD at onset (Fisher's exact p=.002).

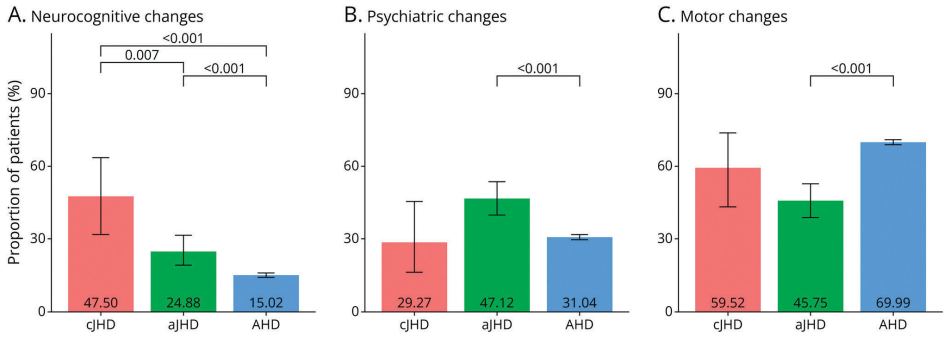


Figure 2. Prevalence and Comparison of HD Disease Characteristics at Onset.

Proportion of patients, 95% CI, and significant p values* per AO-HD subtype (cJHD = red bar; aJHD = green bar; AHD = blue bar) having (A) neurocognitive, (B) psychiatric, and (C) motor changes, whether combined or isolated, at disease onset (pooled datasets). For disease duration and follow-up time, refer to Table 1, and for stratified outcomes per dataset, see Supplementary Table 2. *p Values <0.050 are considered statistically significant and were adjusted for 3 comparisons (cJHD vs aJHD, cJHD vs AHD, and aJHD vs AHD) using the Holm method (inflated p values).

AHD = adult-onset Huntington disease; aJHD = adolescent-onset (juvenile) Huntington disease; AO-HD = AO-defined HD subtype; cJHD = childhood-onset (juvenile) Huntington disease.

3.0 Occurrence of disease characteristics during the disease course

In the next part, the occurrence and severity of changes during the course of the disease will be discussed within the 3 main HD domains, psychiatric, motor, and neurocognitive and in the 'other' domain.

3.1 Psychiatric disease characteristics during the disease course

The occurrence of psychiatric disease characteristics during the disease course was analyzed and compared between AO-HD subtypes in pooled data from the 2 datasets (Figure 3; for disease duration see Table 1). The stratified numbers of patients and proportions per dataset are listed in Supplementary Table 4.

Ever since primary onset, irritability occurred in 91.3% of patients with cJHD (*n*=46, 78.3-97.2), and violence and aggressive behaviour in 73.9% of patients with cJHD (*n*=46, 95% CI 58.6-85.2), significantly more often when compared to 71.4% (*n*=238, 95% CI 65.2-77.0, *p*=<.02) and 55.9% (*n*=238, 95% CI 49.3-62.3, *p*=<.04) of patients with aJHD and 71.0% (*n*=9 501, 95% CI 70.0-71.9, *p*=<.02) and 40.7% (*n*=9 502, 95% CI 39.7-41.7, *p*=<.001) of patients with AHD for irritability and aggressive behaviour respectively (Figure 3A-B). In contrast, depressive complaints occurred in 45.7%of patients with cJHD (*n*=46, 95% CI 31.2-60.8), significantly less often compared to 74.4% of patients with aJHD (*n*=238, 95% CI 68.2-79.7, *p*=<.001) and 74.1% of those with AHD (*n*=9 503, 95% CI 73.2-74.9%, *p*=<.001) (Fig. 3C).

Also, in patients with aJHD violence and aggressive behaviour occurred more often than in patients with AHD (*p*=<.001). Furthermore, apathy occurred in 74.8% of patients with aJHD (*n*=238, 95% CI 68.7-80.1), significantly more often when compared to 52.2% (*n*=46, 95% CI 37.1-66.9, *p*=.01) of patients with cJHD and 65.4% (*n*=9 502, 95% CI 64.4-66.3, *p*=.01) of patients with AHD (Fig. 3D). In addition, psychosis occurred in 23.5% of patients with aJHD (*n*=238, 95% CI 18.4-29.5), which was significantly more often compared to 12.8% (*n*=9 502, 95% CI 12.1-13.5, *p*=<.001) of patients with AHD (Fig. 3E). Perseverative and obsessive behavior occurred in 65.1% of patients with aJHD (*n*=238, 95% CI 58.7-71.2), 69.6% of patients with cJHD (*n*=46, 95% CI 54.3-82.3) and 57.0% of patients with AHD (*n*=9 502, 95% CI 56.0-58.0). No statistically significant differences (p>.05) were observed between patients with cJHD, aJHD and AHD in the occurrence of perseverative and obsessive behavior.

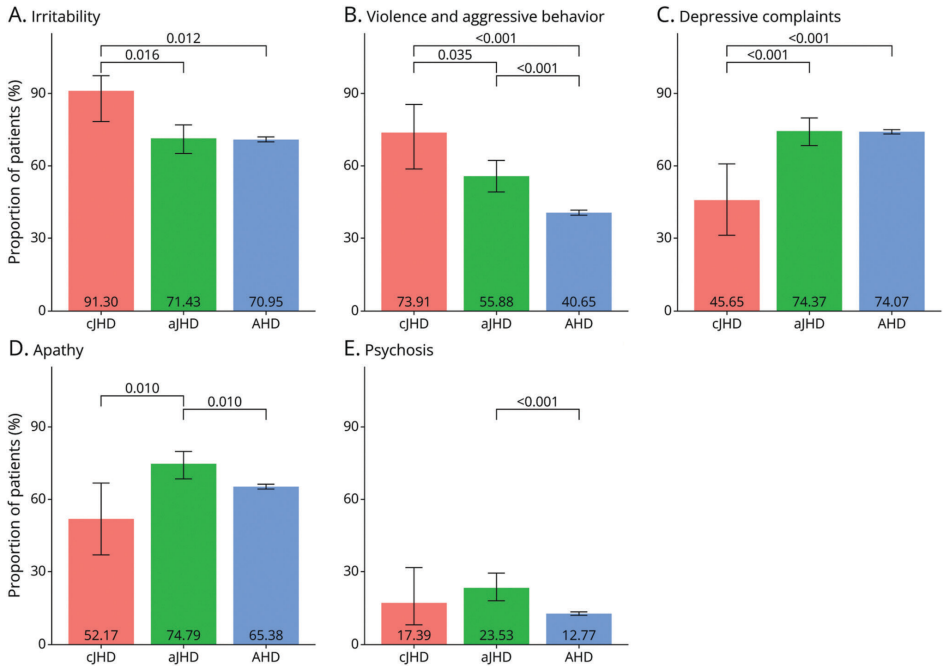


Figure 3. Occurrence and Comparison of Psychiatric HD Disease Characteristics During the Disease Course.

Proportion of patients, 95% CI, and significant p values* per AO-HD subtype (cJHD = red bar; aJHD = green bar; AHD = blue bar) experiencing (A) irritability, (B) violent and aggressive behavior, (C) depressive complaints, (D) apathy, and (E) psychosis as psychiatric disease characteristics (pooled datasets) during the course of the disease. For disease duration and follow-up time, refer to Table 1, and for stratified outcomes per dataset, see Supplementary Table 4. *p Values <0.05 are considered statistically significant and were adjusted for 3 comparisons (cJHD vs aJHD, cJHD vs AHD, and aJHD vs AHD) using the Holm method (inflated p values).

AHD = adult-onset Huntington disease; aJHD = adolescent-onset (juvenile) Huntington disease; AO-HD = AO-defined HD subtype; cJHD = childhood-onset (juvenile) Huntington disease.

3.2 Motor disease characteristics during the disease course

The occurrence of motor disease characteristics during the disease course were analysed and compared between JHD subtypes of the HD-JUNIOR dataset (Table 2; for disease duration see Table 1).

Ever since primary onset, a gait disorder occurred in all cJHD (n=9) and 13 of 18 patients with aJHD. In comparison with aJHD, patients with cJHD more often suffered parkinsonian gait disorder (6/9 vs. 2/18, p=.008) and spastic gait disorder (3/9 vs. 0/17, p=.032). In the motor subdomain ‘speech and swallowing’ dysarthria

occurred in all patients with cJHD (n=9) and 14 of 17 those with aJHD. Patients with cJHD more often suffered from sialorrhea in comparison with patients with aJHD (3/9 vs. 0/18, Fisher's exact p =.029). Also parkinsonism (other than parkinsonian gait disorder) and dystonia occurred often in both JHD subtypes of the HD-JUNIOR dataset. Within this subdomain, patients with cJHD more often experienced loss of fine motor skills (8/9 vs. 8/18, p=.042) and oral dyskinesias (4/9 vs. 1/17, p=.034) in comparison with patients with aJHD. Furthermore, oculomotor disturbances were highly present in both JHD subtypes of the HD-JUNIOR dataset. Between JHD group differences were not observed within this subdomain. In contrast with the higher occurrence of above-mentioned motor symptoms, chorea occurred in 5 of 9 patients with cJHD =, significantly less often when compared to 16 of 17 patients with aJHD (p=.034).

Table 2. The occurrence of motor HD disease characteristics during the disease course and comparison between JHD subtypes of the HD-JUNIOR dataset

			cJHD	aJHD	Fisher's exact p-value
Speech and Swallowing	Anamnesis	Difficulty speech	7/9 (78%)	17/18 (94%)	.250
		Difficulty swallowing	8/9 (89%)	16/18 (89%)	1.000
		Sialorrhea	3/9 (33%)	0/18 (0%)	**.029**
	NE	Dysarthria	9/9 (100%)	14/17 (82.4%)	.529
		Aphasia	1/9 (11.1%)	1/17 (5.9%)	1.000
Walking and Balance	Anamnesis	Difficulty walking	8/9 (89%)	14/18 (78%)	.636
		Difficulty keeping balance	6/9 (67%)	11/18 (61%)	1.000
	NE	Gait disorder;	9/9 (100%)	13/18 (72.2%)	.136
		Parkinsonian gait disorder	6/9 (66.7%)	2/17 (11.8%)	**.008**
		Dystonic gait disorder	2/9 (22.2%)	3/17 (17.6%)	1.000
		Ataxic gait disorder	3/9 (33.3%)	1/17 (5.9%)	.104
		Spastic gait disorder	3/9 (33.3%)	0/17 (0%)	**.032**
		Balance disorder NOS	8/9 (88.9%)	13/17 (76.5%)	.628
Parkinsonism	Anamnesis	Loss of fine motor skills	8/9 (89%)	8/18 (44%)	**.042**
		Stiffness	1/9 (11%)	1/18 (6%)	1.000
	NE	Rigidity	8/9 (88.9%)	15/17 (88.2%)	1.000
		Hypokinesia	6/9 (66.7%)	6/17 (35.3%)	.218
		Bradykinesia	8/9 (88.9%)	14/17 (82.4%)	1.000
		Micrography	2/9 (22.2%)	0/17 (0%)	.111
		Mask face	5/9 (55.6%)	6/17 (35.3%)	.419

Table 2. Continued

			cJHD	aJHD	Fisher's exact p-value
Excessive movement	Anamnesis	Excessive movements extremities	5/9 (56%)	16/18 (89%)	.136
		Excessive movements face	3/9 (33%)	3/18 (17%)	.367
		Tics (vocal or motor)	5/9 (56%)	5/18 (28%)	.219
	NE	Chorea	5/9 (55.6%)	16/17 (94.1%)	**.034**
		Dystonia	6/9 (66.7%)	10/17 (58.8%)	1.000
		Oral dyskinesia	4/9 (44.4%)	1/17 (5.9%)	**.034**
		Motor impersistence	3/9 (33.3%)	9/17 (52.9%)	.429
		Tics vocal	1/9 (11.1%)	1/17 (5.9%)	1.000
		Tics motor	3/9 (33.3%)	2/17 (11.8%)	.302
		Tremor rest	1/9 (11.1%)	0/17 (0%)	.346
		Tremor action	2/9 (22.2%)	2/17 (11.8%)	.591
		Tremor intention	0/9 (0%)	2/17 (11.8%)	.529
		Tremor postural	0/7 (0%)	1/14 (7.1%)	1.000
		Myoclonus	3/9 (33.3%)	2/17 (11.8%)	.302
Oculomotor	NE	Ocular gaze abnormalities	5/9 (55.6%)	9/17 (52.9%)	1.000
		Ocular saccade abnormalities	7/9 (77.8%)	13/17 (76.5%)	1.000
Other	NE	Dysdiadochokinesia	6/9 (66.7%)	7/17 (41.2%)	.411
		Dysmetria	2/9 (22.2%)	1/17 (5.9%)	.268
		Coordination disorder NOS	2/9 (22.2%)	6/17 (35.3%)	.667
		Hyperreflexia	7/9 (77.8%)	4/17 (23.5%)	**.014**
		Scoliosis	2/9 (22.2%)	0/17 (0%)	.111
		Apraxia	1/9 (11.1%)	2/17 (11.8%)	1.000

Results are categorized by neuroanatomical and physiological origin, as well as by source (complaints or signs reported by patients or caregivers during anamnesis, or symptoms identified during neurological examination). The number of participants with each specified sign, symptom, or complaint is presented as a proportion of the total number of participants. Fisher's exact test (two-tailed) was employed to assess the association between JHD subtype and the occurrence of specific motor characteristics. P-values < 0.05 are considered statistically significant and are indicated in bold.

Abbreviations: cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease; NE = Neurological Examination; NOS = Not Otherwise Specified

3.3 Neurocognitive disease characteristics during the disease course

The severity of neurocognitive disease characteristics during the disease course were analysed in JHD subtypes of the HD-JUNIOR dataset (Supplementary Table 5).

Patients with JHD often had a lower-than-average IQ as determined by the primary assessor (cJHD 4 of 7 vs. aJHD 4 of 9). The mean total IQ score in the cJHD group was 80.8±19.4 (years after onset: 3.7±3.1) vs. 75.8±5 (years after onset: 5.4±4.0) in the aJHD group. In general, performance IQ was lower than verbal IQ. This difference was largest in the aJHD group. An executive function disorder was specified in 1 patient with cJHD and 3 patients with aJHD, and an encoding deficit was specified in 4 patients with aJHD.

3.4 Other disease characteristics during the disease course

The occurrence of epileptic seizures during the disease course was analyzed and compared between AO-HD subtypes in pooled data from the 2 datasets. Recurrent epileptic seizures occurred in 23.91% of patients with cJHD (*n*=46, 95% CI 13.10-39.10), significantly more often when compared to 6.33% of patients with aJHD (*n*=237, 95% CI 3.70-10.40, *p*=<.001) and 0.90% of those with AHD (*n*=9472, 95% CI 0.70-1.10, *p*=<.001).

The occurrence of pain during the disease course was analyzed and compared between AO-HD subtypes for the separate datasets. Based on the HD-JUNIOR dataset, 5 of 9 patients with cJHD reported pain throughout their disease. In patients with aJHD, this was 12 of 18 cases. Based on the Enroll-HD dataset, the occurrence of pain interference at baseline was with 42.60% in patients with aJHD (*n*=129, 95% CI 33.70-51.50) significantly higher when compared to 11.80% of patients with cJHD (*n*=17; 95% CI -2.50-26.10, *p* =.014) and 36.60% of patients with AHD (*n*=4262; 95% CI 36.20-37.00, *p*=.040).

4.0 Fitted longitudinal severity and annual progression of motor and neurocognitive disease characteristics

The predicted severity and annual progression of motor and neurocognitive disease characteristics were analysed using longitudinal data from the UHDRS-TMS, UHDRS-SDMT and UHDRS-SIT of the Enroll-HD dataset. We report fitted values from our multivariable regression models for 3 reference patients (Figure 4, Table 3).

By analyzing 6 UHDRS-TMS subclusters, we found distinct patterns in the predicted severity (mean score) and progression (annual change rate) of specified motor symptoms in the 3 AO-HD subtypes. With regard to subcluster ‘dysarthria’, we

found both an increased predicted severity and annual change in the cJHD subtype, followed by aJHD, and in comparison with the AHD subtype, as demonstrated by significantly higher mean scores and annual change rates 5-years after onset (Figure 4A, Table 3). For the subclusters 'parkinsonism' and 'dystonia', we found an increased predicted severity in the cJHD subtype, followed by aJHD, and in comparison with the AHD subtype, as demonstrated by significantly higher mean scores 5-years after onset (Figure 4B-C, Table 3). No between group differences were observed in the predicted annual progression of parkinsonism and dystonia as measured 5-years after onset (Table 3). Alternatively, in the subclusters 'oculomotor' and 'gait & balance', we found an increased predicted annual progression in both JHD subtypes compared to the AHD subtype, as demonstrated by significantly higher annual change scores 5 years after onset (Table 3), but no between group difference was observed in the severity (mean score) of these outcomes (Figure 4D-E, Table 3) Lastly, For the subcluster 'chorea', we found a reduced predicted severity in the cJHD subtype, followed by aJHD, as compared to the AHD subtype, as demonstrated by significantly lower mean scores 5 years after onset (Figure 4G, Table 3). There were no between-group differences in the annual progression of chorea (Table 3).

Apart from motor subclusters, we analyzed differences in the predicted severity and progression of neurocognitive disease characteristics by applying the same models to the UHDRS-SIT (executive functioning) and UHDRS-SDMT (psychomotor speed). Regarding the UHDRS-SDMT, we found an increased predicted annual deterioration in psychomotor processing speed for both JHD subtypes compared to the AHD subtype, as demonstrated by significantly higher mean annual change rates 5 years after onset (Table 3). No between group differences were observed in the predicted annual deterioration of executive functioning, as measured by the SIT assessment (Table 3). In contrast to annual deterioration, the predicted general performance on neurocognitive assessment 5 years after onset was significantly better in the aJHD subtype compared to the AHD subtype, as demonstrated by higher mean SDMT and SIT scores (Table 3).

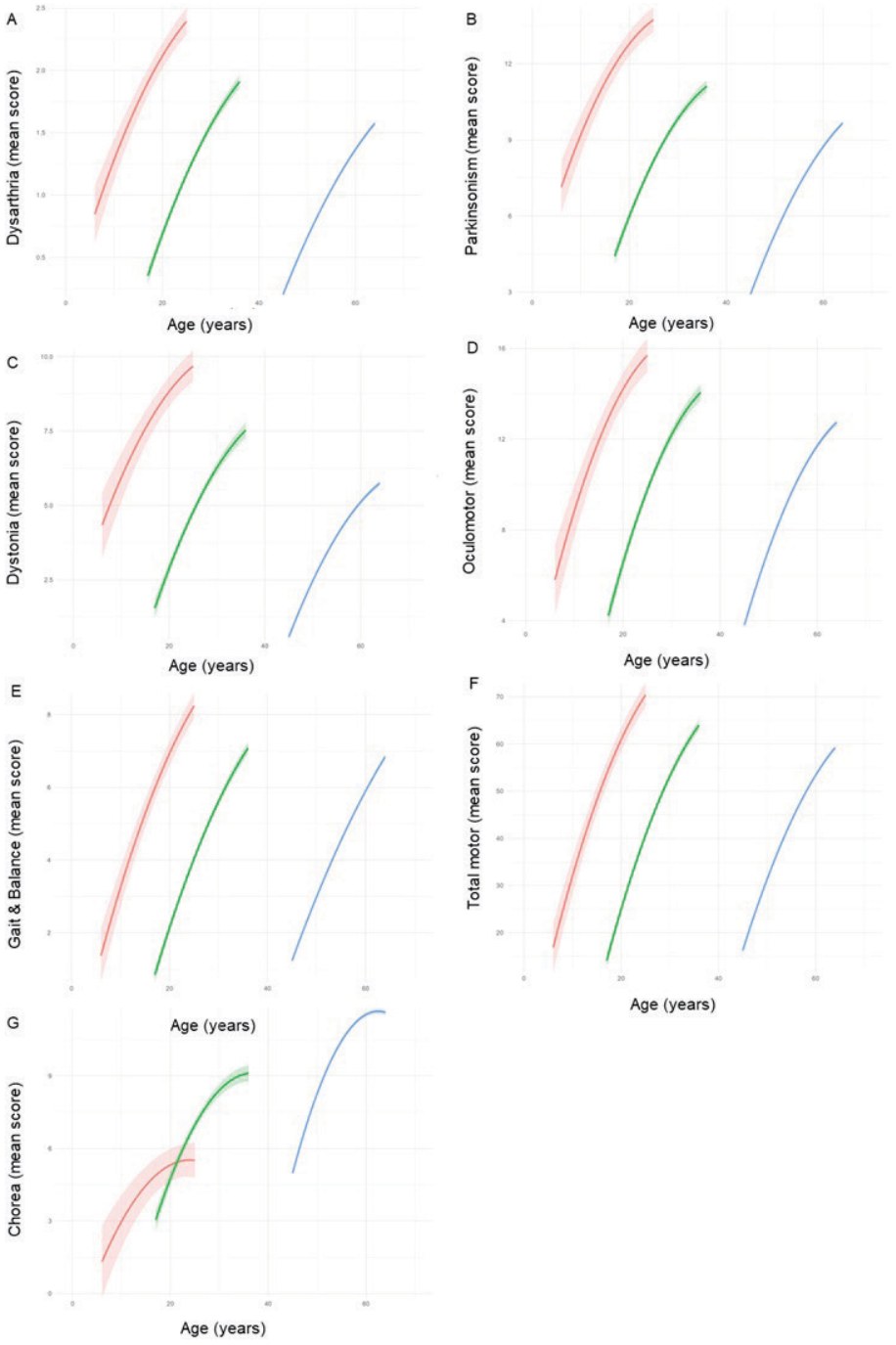


Figure 4. The associated severity of motor subdomain characteristics over time in AO-HD subtypes

Figure 4. Continued

UHDRS-TMS data from the ENROLL-HD dataset were used to assess severity across three AO-HD subtypes: cJHD (red line), aJHD (green line), and AHD (blue line). Scores are graphed as a function of age at measurement for TMS items, with a range of 0 (no abnormalities) to 4 (most severe abnormalities):

(A) 'dysarthria' (max score 4), (B) 'finger taps L + R, rigidity L + R, and body bradykinesia' (max score 20), (C) 'dystonia in: trunk, upper extremities L + R, lower extremities L + R' (max score 20), (D) 'ocular pursuit H + V, saccade initiation H + V, and saccade velocity H + V' (max score 24), (E) 'gait, tandem walking, and retropulsion' (max score 12), (F) UHDRS-TMS total score (max score 124), and (G) 'chorea in: face, buco-oro-laryngeal area, trunk, upper extremities L + R, lower extremities L + R' (max score 28).

Statistics and between-group comparisons of fitted mean scores related to these slopes are detailed in Table 3.

Abbreviations: AO-HD = Age at Onset-defined HD subtype; UHDRS-TMS = Unified Huntington Disease Rating Scale – Total Motor Score; cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease; AHD = Adult-onset Huntington Disease; L = left; R = right; H = horizontal; V = vertical

Table 3. Fitted severity and annual progression of specified motor and neurocognitive clusters in AO-HD subtypes

Outcome measure	AO-HD subtype	Mean score*	SE	Comparison	p-value	Mean annual change*	SE	Comparison	p-value
UHDRS-TMS 'dysarthria'	cJHD	1.38	0.16	aJHD	**<.001**	0.25	0.04	aJHD	**.030**
	aJHD	0.89	0.04	AHD	**<.001**	0.19	0.02	AHD	**.002**
	AHD	0.66	0.01	cJHD	**<.001**	0.10	0.00	cJHD	**.007**
UHDRS-TMS 'parkinsonism'	cJHD	9.85	0.73	aJHD	**<.001**	0.75	0.18	aJHD	.487
	aJHD	7.11	0.18	AHD	**<.001**	0.65	0.09	AHD	.409
	AHD	5.46	0.06	cJHD	**<.001**	0.52	0.03	cJHD	.437
UHDRS-TMS 'dystonia'	cJHD	6.47	0.76	aJHD	**<.001**	0.87	0.22	aJHD	.339
	aJHD	3.87	0.19	AHD	**<.001**	0.72	0.11	AHD	.058
	AHD	2.64	0.06	cJHD	**<.001**	0.44	0.04	cJHD	.142
UHDRS-TMS 'oculomotor'	cJHD	9.86	1.08	aJHD	.204	1.53	0.27	aJHD	.052
	aJHD	8.26	0.27	AHD	**.019**	1.21	0.14	AHD	**.013**
	AHD	7.48	0.09	cJHD	.065	0.77	0.05	cJHD	**.024**
UHDRS-TMS 'gait and balance'	cJHD	4.07	0.51	aJHD	.220	0.79	0.12	aJHD	**.023**
	aJHD	3.33	0.13	AHD	.932	0.63	0.06	AHD	**.005**
	AHD	3.38	0.04	cJHD	.356	0.41	0.02	cJHD	**.009**
UHDRS-TMS 'chorea'	cJHD	3.36	1.02	aJHD	**.020**	0.75	0.30	aJHD	.906
	aJHD	5.76	0.26	AHD	**<.001**	0.81	0.16	AHD	.676
	AHD	8.29	0.09	cJHD	**<.001**	0.67	0.05	cJHD	.966
UHDRS-TMS 'total score'	cJHD	37.03	3.73	aJHD	.448	5.65	0.72	aJHD	.053
	aJHD	33.10	0.94	AHD	.935	4.81	0.37	AHD	**<.001**
	AHD	32.75	0.33	cJHD	.475	3.33	0.13	cJHD	**.007**
UHDRS-SDMT	cJHD	23.13	2.47	aJHD	**.042**	-2,79	0.44	aJHD	.119
	aJHD	28.33	0.63	AHD	**.001**	-2,35	0.23	AHD	**.005**
	AHD	25.29	0.22	cJHD	.651	-1,55	0.08	cJHD	**.024**

Table 3. Continued

Outcome measure	AO-HD subtype	Mean score*	SE	Comparison	p-value	Mean annual change*	SE	Comparison	p-value
UHDRS-SIT	cJHD	27.36	3.03	aJHD	.735	-1,24	0.57	aJHD	.994
	aJHD	29.32	0.66	AHD	**<.001**	-1,21	0.30	AHD	.977
	AHD	25.08	0.20	cJHD	.726	-1,14	0.10	cJHD	.986

* 5-years after onset

Results are categorized by motor and neurocognitive outcome measures, as detailed in the text and Figure 3, according to AO-HD subtype. The left panel displays the predicted mean scores† and standard errors (SE), while the right panel shows the predicted mean annual changes† and SE, along with between-group comparisons for each outcome measure. Row 1 compares cJHD with aJHD, row 2 compares aJHD with AHD, and row 3 compares AHD with cJHD. Statistical significance was adjusted for multiple testing using the Tukey method (inflated p-values). P-values < 0.05, adjusted for the three comparisons (cJHD vs aJHD, cJHD vs AHD, and aJHD vs AHD), are considered statistically significant.

† Scores are based on model coefficients that show the predicted severity and annual change for a female patient 5-years after first symptom onset (cJHD: AO=6, AM=11; aJHD: AO=17, AM=22; AHD: AO=45, AM=50).

Abbreviations: cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease; AHD = Adult-onset Huntington Disease; AO-HD = Age at Onset-defined HD subtype; UHDRS-TMS = Unified Huntington Disease Rating Scale – Total Motor Score; UHDRS-SDMT = Unified Huntington Disease Rating Scale – Symbol-Digit-Modalities-Test; UHDRS-SIT = Unified Huntington Disease Rating Scale – Stroop-Interference-Test; SE = Standard Error; AO = Age at Onset; AM = Age at Measurement

DISCUSSION

This study identifies different disease characteristics at onset, as well as differences in the occurrence, severity and rate of progression of HD clinical characteristics over time in JHD subtypes compared with AHD.

The cJHD population represents the extreme end of the HD spectrum, in which disease progression is known to be accelerated.[7] By comparing a total of 46 patients with cJHD with those with aJHD and AHD, we confirm earlier reported findings such as: (1) a high prevalence of neurocognitive abnormalities at disease onset;[7,13] (2) more often motor changes related to speech, parkinsonism, and oral dyskinesia[6,7] (3) less often chorea;[6,7] (4) neurocognitive changes reminiscent of the AHD phenotype;[20,21] and (5) a higher occurrence of behavioral changes and lower depression complaints.[6,13] Replication of these former findings confirms their association with the cJHD phenotype in comparison with prototypical disease onset in adulthood. We extend this knowledge by showing that the cJHD population more often suffers from (1) secondary developmental regression in motor domains rather than a primary neurodevelopmental delay and (2) a spastic gait disorder. Furthermore, linear mixed regression models suggest faster worsening of dysarthria, gait, balance, oculomotor changes and psychomotor speed over time in association with an earlier onset and in contrast to other motor and neurocognitive subclusters.

The aJHD population is believed to be in closer resemblance with the AHD population compared with cJHD. By comparing 238 patients with aJHD with patients with cJHD and AHD, our study suggests alternative patterns in the aJHD population by (1) a higher prevalence of psychiatric abnormalities and a lower prevalence of motor changes at disease onset; (2) a higher occurrence of psychiatric changes related to psychosis and apathy during the disease course and (3) a higher occurrence of pain interference in daily life. Furthermore, linear mixed regression models suggest that the aJHD subtype has motor changes that are more severe and progress faster when compared to the AHD subtype and better performance on neurocognitive tasks with faster deterioration of psychomotor speed over time. These findings highlight that clinical characteristics in the aJHD population are not directly similar to those in the AHD population or its other counterpart, cJHD.

Multivariable linear mixed regression models were used to describe differences between AO-HD subtypes in the associated severity and progression of specified sub-motor and neurocognitive domains. Because of the unknown relationship of the

independent variables (AO and AM) on the outcome measures in the different AO-HD subtypes and to allow for a relatively flexible description of disease progression, a linear model with quadratic functions of AO and AM on the mean score was used to describe between group differences in associated severity. In addition, linear functions of AO and AM on the mean annual change rate were used to describe between group differences in the associated progression over time. Based on these 2 models, we tentatively conclude that divergent patterns of severity and progression for motor and neurocognitive subclusters are associated with the AO-HD subtypes. Whereas the occurrence and severity of parkinsonism and dystonia are positively associated with the JHD population, their rate of progression over time is comparable to the AHD phenotype. In contrast, faster worsening of symptoms in JHD as compared to AHD is associated with dysarthria, oculomotor and gait and balance changes. For neurocognitive clusters, a pattern of better neurocognitive performance in the aJHD population is paralleled by faster associated worsening of psychomotor speed over time in both JHD subtypes when compared to AHD. These different patterns in severity and progression rate demonstrate that AO and AM influence subclusters in different ways. Of note is the descriptive nature of these models, which should not be interpreted as prediction model or to infer causality. The distribution of datapoints across the age spectrum (X-axis) differs widely between the AO-HD subtypes, which can result in an inaccurate extrapolation over the entire age spectrum in small samples, as is the case in the cJHD subtype. Moreover, the association that we observe is likely to be influenced by covariates that have not been considered in our models.

Our study is the first to report a higher occurrence of apathy and psychosis specifically in the aJHD population. This finding shows that the occurrence of psychosis in HD does not follow a linear relationship with AO or CAG-repeat size. It is of interest that the predilection of this age group is also seen in the onset of idiopathic schizophrenia and might suggest similar risk factors in the onset of this phenotype.

Another interesting finding of our study is the higher occurrence of pain interference in daily life in patients with aJHD, when compared with patients with cJHD and AHD. A higher occurrence of pain has been linked to the JHD population before, but until now a trend toward higher CAGs and therefore earlier onset of disease was observed.[22] Furthermore, no comparison was made with AHD. Although a selection bias could influence our results (SF12 questionnaire in Enroll-HD is an optional

part that is easily left out in case of a to high patient burden during annual visits) assessment of pain by using observational pain scales in addition to more extended self-reported pain scales in both patients with JHD and AHD could help clarifying the prevalence and origin of pain in different AO-HD subtypes.

We cannot exclude the possibility of bias influencing our study results. Information bias might have contributed to the lower estimates for depression in the cJHD population because depressive complaints are easily misrecognized in any childhood population.[23,24] The fact that the HD-JUNIOR dataset uses unspecified medical data, however, does help minimize this risk. A selection bias may influence the cJHD population of Enroll-HD, because more severe cases are less likely to participate in prospective studies. Furthermore, we chose to omit missing cases from analyses. These patients might be different in certain respect from the patients who were included in our analyses, which would lead to some degree of selection bias. Another risk is a recall bias in the retrospectively collected data. Finally, using CAG-repeat length, as used in previous studies, or using age at motor onset for the definition of JHD populations can be more accurate depending on the research question. In this study we choose to define our groups by AO of any HD-related sign, rather than CAG-repeat or age at motor onset. In our opinion this definition relates better to patients presenting in clinical practice, prevents a selection bias of patients with neurocognitive/psychiatric onset yet without a motor phenotype and relates to a certain neurodevelopmental state that potentially influences disease characteristics.

The JHD population is a small heterogeneous group of patients that requires a tailored approach to what is known in HD research, as they represent the extreme end of the HD spectrum. We believe that future studies should include the structural comparison of AO-HD subtypes or different CAG-repeat lengths in all types of (pre)clinical HD research. Furthermore, better identification of clinical characteristics such as developmental changes, gait abnormalities and pain would help to understand their origin and therefore how to treat them. Finally, ongoing (international) collaborations are the only way forward in this very rare form of the disease. Pooling data from several JHD registries worldwide is an important next step in our understanding of JHD. To support these efforts, data from the Dutch HD-JUNIOR registry are available on request.

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.
2. Schultz JL, Moser AD, Nopoulos PC. The Association between CAG-repeat Length and Age of Onset of juvenile-onset Huntington's Disease. *Brain sciences*. Aug 20 2020;10(9)doi:10.3390/brainsci10090575
3. Roos RA. Huntington's disease: a clinical review. *Orphanet J Rare Dis*. Dec 20 2010;5:40. doi:10.1186/1750-1172-5-40
4. 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
5. Achenbach J, Saft C. Data from ENROLL-HD: Is the prevalence of juvenile and pediatric Huntington's disease overestimated? *Parkinsonism & related disorders*. Jul 2021;88:1-2. doi:10.1016/j.parkreldis.2021.05.012
6. Cronin T, Rosser A, Massey T. Clinical Presentation and Features of juvenile-onset Huntington's Disease: A Systematic Review. *Journal of Huntington's disease*. 2019;8(2):171-179. doi:10.3233/jhd-180339
7. Fusilli C, Migliore S, Mazza T, et al. Biological and clinical manifestations of juvenile Huntington's disease: a retrospective analysis. *The Lancet Neurology*. Nov 2018;17(11):986-993. doi:10.1016/S1474-4422(18)30294-1
8. Schultz JL, Nopoulos PC. Autonomic Changes in juvenile-onset Huntington's Disease. *Brain sciences*. Aug 26 2020;10(9)doi:10.3390/brainsci10090589
9. Tereshchenko A, McHugh M, Lee JK, et al. Abnormal Weight and Body Mass Index in Children with Juvenile Huntington's Disease. *Journal of Huntington's disease*. 2015;4(3):231-238. doi:10.3233/jhd-150152
10. Mehanna R, Jankovic J. Systemic Symptoms in Huntington's Disease: A Comprehensive Review. *Mov Disord Clin Pract*. May 2024;11(5):453-464. doi:10.1002/mdc3.14029
11. Aziz NA, Anguelova GV, Marinus J, van Dijk JG, Roos RA. Autonomic symptoms in patients and pre-manifest mutation carriers of Huntington's disease. *Eur J Neurol*. Aug 2010;17(8):1068-74. doi:10.1111/j.1468-1331.2010.02973.x
12. Bakels HS, Roos RAC, van Roon-Mom WMC, de Bot ST. Juvenile-onset Huntington Disease Pathophysiology and Neurodevelopment: A Review. *Movement disorders : official journal of the Movement Disorder Society*. Jan 2022;37(1):16-24. doi:10.1002/mds.28823
13. McAllister B, Gusella JF, Landwehrmeyer GB, et al. Timing and Impact of Psychiatric, Cognitive, and Motor Abnormalities in Huntington Disease. *Neurology*. May 11 2021;96(19):e2395-e2406. doi:10.1212/WNL.0000000000011893
14. Ranganathan M, Kostyk SK, Allain DC, Race JA, Daley AM. Age of onset and behavioral manifestations in Huntington's disease: An Enroll-HD cohort analysis. *Clin Genet*. Jan 2021;99(1):133-142. doi:10.1111/cge.13857
15. Furby H, Moore S, Nordstroem AL, et al. Comorbidities and clinical outcomes in adult- and juvenile-onset Huntington's disease: a study of linked Swedish National Registries (2002-2019). *J Neurol*. Feb 2023;270(2):864-876. doi:10.1007/s00415-022-11418-y
16. Sathe S, Ware J, Levey J, et al. Enroll-HD: An Integrated Clinical Research Platform and Worldwide Observational Study for Huntington's Disease. *Front Neurol*. 2021;12:667420. doi:10.3389/fneur.2021.667420
17. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Medical care*. Mar 1996;34(3):220-33. doi:10.1097/00005650-199603000-00003

18. Team RC. RA language and environment for statistical computing, R Foundation for Statistical. *Computing*. 2020;
19. Wickham H, Averick M, Bryan J, et al. Welcome to the Tidyverse. *Journal of open source software*. 2019;4(43):1686.
20. Langbehn KE, Cochran AM, van der Plas E, et al. Behavioral Deficits in juvenile-onset Huntington's Disease. *Brain sciences*. Aug 11 2020;10(8)doi:10.3390/brainsci10080543
21. Ribai P, Nguyen K, Hahn-Barma V, et al. Psychiatric and cognitive difficulties as indicators of juvenile huntington disease onset in 29 patients. *Archives of neurology*. Jun 2007;64(6):813-9. doi:10.1001/archneur.64.6.813
22. Moser AD, Epping E, Espe-Pfeifer P, et al. A survey-based study identifies common but unrecognized symptoms in a large series of juvenile Huntington's disease. *Neurodegener Dis Manag*. Oct 2017;7(5):307-315. doi:10.2217/nmt-2017-0019
23. Ghandour RM, Sherman LJ, Vladutiu CJ, et al. Prevalence and Treatment of Depression, Anxiety, and Conduct Problems in US Children. *J Pediatr*. Mar 2019;206:256-267. e3. doi:10.1016/j.jpeds.2018.09.021
24. Luby JL, Belden AC, Pautsch J, Si X, Spitznagel E. The clinical significance of preschool depression: impairment in functioning and clinical markers of the disorder. *J Affect Disord*. Jan 2009;112(1-3):111-9. doi:10.1016/j.jad.2008.03.026

SUPPLEMENTARY TABLES

Supplementary Table 1. PDS5 outlier exclusion criteria

Subject ID	Original AO-HD subtype	Reason for exclusion
R439327326	cJHD	Age at enrolment ≥30 years (high risk recall bias), disease duration ≥25 years (>2 SD from mean disease duration with cJHD group)
R707469384	cJHD	Age at enrolment ≥25 years (high risk recall bias), disease duration ≥20 years (>2 SD from mean disease duration with cJHD group)
R35157828X	cJHD	Age at enrolment ≥25 years (high risk recall bias), disease duration ≥20 years within 2 SD from mean, but clinically still very unlikely in case of cJHD phenotype)
R985739546	aJHD	Age at enrolment ≥55 years (high risk recall bias), disease duration ≥35 years (>2 SD from mean disease duration with aJHD group)
R976576146	aJHD	Age at enrolment ≥45 years (high risk recall bias), disease duration ≥25 years (>2 SD from mean disease duration with aJHD group)
R044544548	aJHD	Age at enrolment ≥35 years (high risk recall bias), disease duration ≥25 years (>2 SD from mean disease duration with aJHD group)
R036972168	aJHD	Age at enrolment ≥35 years (high risk recall bias), disease duration ≥20 years (>2 SD from mean disease duration with aJHD group)
R870934436	aJHD	Age at enrolment ≥40 years (high risk recall bias), disease duration ≥20 years (>2 SD from mean disease duration with aJHD group)
R176379066	aJHD	Age at enrolment ≥40 years (high risk recall bias), disease duration ≥20 years (>2 SD from mean disease duration with aJHD group)
R478714630	aJHD	Age at enrolment ≥35 years (high risk recall bias), disease duration ≥20 years (>2 SD from mean disease duration with aJHD group)

Abbreviations: cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease; SD = standard deviations

Supplementary Table 2. Frequencies and proportions of HD disease characteristics at onset in AO-HD subtypes and stratified by dataset

	childhood-onset JHD		adolescent-onset JHD		adult-onset HD
	ENROLL-HD	HD-JUNIOR	ENROLL-HD	HD-JUNIOR	ENROLL-HD
Motor onset	19/33 (57.6%)	6/9 (66.7%)	92/201 (45.8%)	5/11 (45.5%)	6040/8630 (70.0%)
Neurocognitive onset	14/31 (45.2%)	5/9 (55.6%)	47/198 (23.7%)	5/11 (45.5%)	1228/8177 (15.0%)
Psychiatric onset	9/32 (28.1%)	3/9 (33.3%)	92/197 46.7%)	6/11 (54.5%)	2630/8472 (31.0%)
Mixed onset	11/37 (29.7%)	4/9 (44.4%)	44/225 (19.6%)	3/11 (27.3%)	1815/9490 (19.1%)
Other onset	2/37 (5.4%)	0/9 (0%)	3/225 (1.3%)	0/11 (0%)	31/9490 (0.3%)

Data represent number of patients reporting symptom/number of patients within group (within group percentage)

Supplementary Table 3. Frequencies and proportions of specified disease characteristics at onset in JHD subtypes of the HD-JUNIOR dataset

		cJHD	aJHD	Fisher's exact p-value
Motor	Walking abnormalities	3/9 (33%)	3/16 (19%)	.630
	Balance complaints	0/9 (0%)	2/16 (13%)	.520
	Excessive movements	0/9 (0%)	1/16 (6%)	1.000
	Fine motor skill loss	3/8 (38%)	2/17 (12%)	.283
	Speech problems	0/9 (0%)	2/16 (13%)	.520
	Tics (vocal or motor)	1/9 (11%)	1/16 (6%)	1.000
	Learning difficulties	3/9 (33%)	2/16 (13%)	.312
Neurocognitive	Memory complaints	0/9 (0%)	1/17 (6%)	1.000
	Attention deficit	2/9 (22%)	1/16 (6%)	.530
	Irritable aggressive behavior	0/9 (0%)	7/16 (39%)	**<.027**
	Depressive complaints	0/9 (0%)	3/16 (19%)	.280
	Social withdrawal	1/9 (11%)	0/16 (0%)	.360
Psychiatry	Obsessive compulsive behavior	1/9 (11%)	0/16 (0%)	.360
	Substance abuse	0/9 (0%)	1/17 (6%)	1.000
	Anxiety complaints	1/9 (11%)	0/16 (0%)	.360
	Suicidal behavior	0/9 (0%)	1/17 (6%)	1.000
	Apathy	1/9 (11%)	0/17 (0%)	.346
	Fatigue	1/9 (11%)	0/17 (0%)	.346
	Crying or screaming	1/9 (11%)	0/17 (0%)	.346
'Other'	Pain	1/9 (11%)	2/16 (13%)	1.000
	Developmental delay	1/9 (11%)	0/16 (0%)	.360
	Develepmental regression	5/9 (56%)	0/16 (0%)	**<.002**

Results are catagorized by symptom or sign domain. Shown are the number of participants having specified sign, symptom or complaint / total number of participants (within group proportion having symptom). Fisher's exact test (two-tailed) was used to determine if there was a significant association between JHD subtype and the occurence of a specified disease characteric at onset. p-values <.05 were considered statistically significant and are presented in bold.

Abbreviations: cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease

Supplementary Table 4. Frequencies and proportions of psychiatric disease characteristics during the disease course in AO-HD subtypes and stratified by dataset

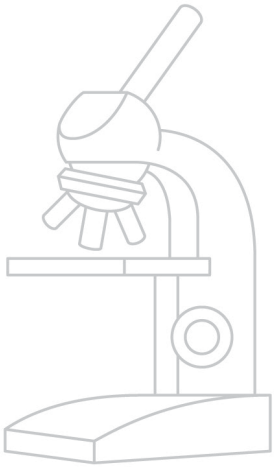
	childhood-onset JHD		adolescent-onset JHD		adult-onset HD
	ENROLL-HD	**HD-JUNIOR**	**ENROLL-HD**	**HD-JUNIOR**	**ENROLL-HD**
Depression	18/37 (48.6%)	3/9 (33.3%)	171/225 (76%)	6/13 (46.2%)	7039/9503 (74.1%)
Irritability	34/37 (91.9%)	8/9 (88.9%)	157/225 (69.8%)	13/13 (100%)	6741/9501 (71%)
Aggression and Violence	26/37 (70.3%)	8/9 (88.9%)	120/225 (53.3%)	13/13 (100%)	3863/9502 (40.7%)
Apathy	22/37 (59.5%)	2/9 (22.2%)	169/225 (75.1%)	9/13 (69.2%)	6212/9502 (65.4%)
Psychosis	5/37 (13.5%)	3/9 (33.3%)	51/225 (22.7%)	5/13 (38.5%)	1213/9502 (12.8%)
Perseveration and Obsession	25/37 (67.6%)	7/9 (77.8%)	147/225 (65.3%)	8/13 (61.5%)	5419/9502 (57%)

Data represent number of patients reporting symptom/number of patients within group (within group percentage)

Supplementary Table 5. Cognitive measures in JHD subtypes of the HD-JUNIOR dataset

	cJHD (n=7)	**aJHD (n=9)**
Years after onset	3.7±3.1 (0-10)	5.4±4.0 (1-14)
Full IQ	80.8±19.4 (60-113)	75.8±5.0 (72-84)
Verbal IQ (VIQ)	80.0±12.4 (62-92)	88.5±8.5 (80-100)
Performance IQ (PIQ)	71.0±11.1 (63-87)	70.0±6.7 (66-80)
VIQ/PIQ Δ	4.8±14.3 (-5-26)	15.2±12.2 (2-34)

Abbreviations: cJHD = childhood-onset (Juvenile) Huntington Disease; aJHD = adolescent-onset (Juvenile) Huntington Disease; n = number of participant's; IQ = Intelligence Quotient; SD = Standard Deviation



CHAPTER 5

Post-mortem 7T MR imaging and neuropathology in middle stage juvenile-onset Huntington Disease: a case report

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INTRODUCTION

Huntington disease (HD) is an autosomal dominant inherited brain disorder, caused by an elongated CAG-repeat in the HTT gene. It typically manifests during adulthood, but in approximately 5% of cases, the disease occurs in minors, referred to as juvenile-onset HD (JHD). [1] JHD patients have a distinct clinical presentation often with early cognitive changes, Parkinsonism and epilepsy in patients with childhood-onset and early cognitive and psychiatric disturbances in adolescent-onset disease. [2] , [3] HD neuropathology is characterised by atrophy, as revealed by a reduction in brain volume, neuronal cell loss and reactive changes in astro and oligodendroglia. These changes are most prominent in the neostriatum (e.g. caudate nucleus and putamen), following a caudorostral gradient with disease progression but extend to other brain regions (e.g. globus pallidus, [hypo]thalamus, cortex, brain stem and cerebellum) as well. Neostriatal findings are formulated in the fivescale Vonsattel grading system, [4] a measure of neuropathological severity. Endstage neuropathology in JHD cases is generally more severe than in adult-onset cases. [3] , [5] Reductions in brain volume and in the volume of specified regions are also apparent in in vivo imaging studies in HD patients, [6] yet a comparison of postmortem imaging and neuropathological findings at the same time point in the same patient is lacking. Furthermore, the majority of postmortem studies are performed on endstage disease. Therefore, an exploration of the relationship between early clinical characteristics and neuropathological grading in HD brain donors who died after a short disease duration has not been undertaken. Here, we report a case study of a JHD brain donor with a moderate clinical disease burden and short clinical disease duration. Ex vivo, in situ ultrahigh field 7T MR imaging revealed bilateral atrophy of the neostriatum, most significantly of the putamen. Neuropathological assessment revealed sparse neuronal loss and limited gliosis of the same regions, in keeping with Vonsattel grade 1. This case report highlights the risk of underestimating neuropathological severity by Vonsattel grading, due to undervaluation of neuropathological changes outside the head of the caudate nucleus (HCN). Atrophy of the putamen was pronounced in this case; therefore, the entirety of the neuropathological findings should always be taken into account in HD brain donors that did not reach endstage disease. Studies like these increase our understanding of how early clinical disease characteristics and imaging are related to neuropathological changes and grading and vice versa.

Clinical characteristics

The patient was a man who died at 21 years of age by legally approved euthanasia. He was clinically diagnosed with HD at 19 years of age, therefore referred to as juvenile (adolescent) onset HD. [2] , [3] Molecular analysis of the *HTT* gene revealed a pathologically expanded CAG-repeat of 57. The earliest symptom of his disease was learning difficulty and this developed 2 years before diagnosis. The patient had a moderate clinical disease burden shortly before death. Clinical characteristics included moderate/common generalised chorea and truncal dystonia, ataxia, balance disorder, mild dysarthria, dysphagia, mild dysexecutive disorder and frequent irritative and aggressive outbursts. Clinical Global Impression of Severity was scored from 4 to 5 on a 7point scale (e.g. 1; not at all ill to 7; extremely ill). [7] Functional disabilities were mild to moderate, including the inability to work, and needing assistance in domestic chores. Patient independence was scored as stage 2 using the Shoulson–Fahn ranking system and a total functional capacity of 6. [8] The short disease duration of 4 years was paralleled by a moderate CAGAge Product score of 490, a measure of disease progression. [9]

METHODS

The patient and his relatives gave informed consent for brain autopsy, postmortem MRI and the pseudonymized use of clinical characteristics and brain tissue for research purposes and publication. The study followed the tenants of the Declaration of Helsinki. Ex vivo, in situ ultrahigh field 7T MR brain imaging was performed within 3 h postmortem delay (PMD) and brain autopsy and dissection within 11 h PMD. Brain dissection was performed following a standard protocol. [10]

RESULTS

By radiological assessment, the donor had severe bilateral atrophy of the putamen and slight to moderate atrophy of the HCN, which was best appreciated at the dorsal side of the caudate head (Figure 1A,C). We could not reliably determine if there was atrophy in the body and tail of the caudate nucleus due to their small size, and therefore, we could not confidently determine if there was a gradient in the caudate nucleus degeneration. There were no signs of atrophy outside the neostriatum.

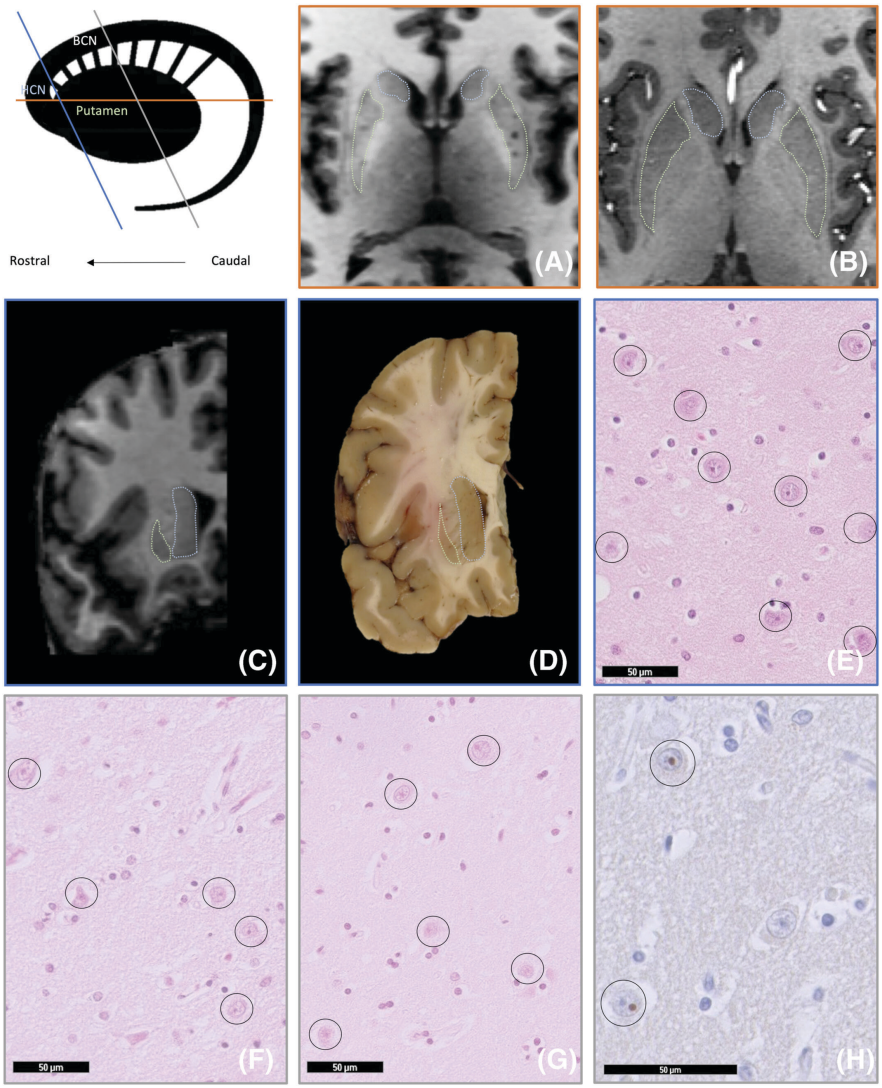


Figure 1. Postmortem 7T MRI imaging and neuropathology of a juvenile-onset Huntington disease (HD) brain donor.

T1weighted image in a transverse plane of the HD brain donor (A) demonstrates severe bilateral atrophy of the putamen (dashed green line) and slight to moderate atrophy of the HCN (dashed blue line) as compared to an age and sexmatched control (B). T1weighted image in the coronal plane of the HD brain donor (C) parallels neuropathology macroscopy findings of the right hemisphere of the same HD donor (D), demonstrating a normal convex contour of the HCN into the lateral ventricle (right side of the dashed blue line) and moderate to severe atrophy of the putamen (dashed green line). H&E staining of the HCN (E) and rostral putamen (not shown) reveals normal neuronal cellularity (circles) and no signs of gliosis. Microscopy of the BCN (F) and caudal putamen (G) reveals mild neuronal loss (fewer circles) and gliosis. Mutant–Huntingtin staining (H) reveals scattered nuclear immunoreactivity (circles) of neurons in the BCN, similar to neurons in the frontal lobe and putamen (not shown). Legend: BCN, body of caudate nucleus; HCN, head of caudate nucleus; H&E, haematoxylin and eosin

By brain autopsy, gross brain weight was 1480 g (normal for this age and sex). The neuropathological assessment revealed no macroscopic evidence of atrophy with a normal contour of the HCN into the lateral ventricles (Figure 1D). Microscopically, the HCN and rostral putamen revealed a normal density of neuronal cell bodies and no signs of astrogliosis (Figure 1E). In the body of the caudate nucleus (BCN; at height of the anterior thalamus), there was a minor loss of neurones and gliosis (Figure 1F). We were not able to microscopically assess the tail of the caudate nucleus in the histopathological sections that were available. Neuronal loss was most prominent in the caudal putamen, including a mild degree of gliosis (Figure 1G). These findings are consistent with a Vonsattel grade 1. Cell distribution and morphology in other striatal and cortical regions appeared normal. Immunohistochemistry for mutantHuntingtin revealed scattered nuclear aggregates in neurons of the frontal lobe, caudate nucleus and putamen (Figure 1H).

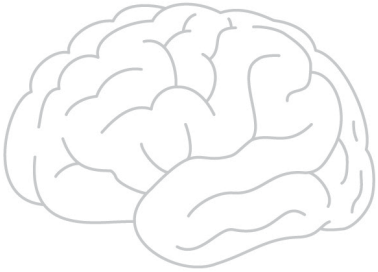
DISCUSSION

Several conclusions can be drawn from this illustrative case report. This patient had a characteristic adolescent-onset HD presentation with cognitive onset of disease, severe psychiatric disturbances and a motor phenotype including ataxia, dystonia and chorea. This moderate disease burden, combined with mild to moderate functional disability and short disease duration, is paralleled by mild neuronal cell loss and gliosis of the neostriatum, which was most prominent in the caudal putamen, without evident cell loss and gliosis in other brain regions. More prominent atrophy of the putamen, as compared with the caudate nucleus, in early HD stages, has been mentioned in the literature before. [11] The discrepancy between moderate disease burden and mild neuropathology most likely relates to the notion that disease burden is primarily caused by neuronal dysfunction and only secondary by neuronal loss. [12] Furthermore, the presence of neuronal mHTT aggregates in our donor with a relatively high CAG-repeat length, and short disease duration is in line with former studies revealing mHTT aggregates even in presymptomatic HD brain donors and correlating with CAG-repeat length. [13] , [14] Severe macroscopic atrophy of the putamen was best appreciated via imaging and confirmed by the microscopic finding that the most prominent neuronal cell loss and reactive gliosis was in this region. Neuropathology in the putamen followed a caudalrostral gradient with the most severe neuronal loss in the caudal putamen, at the level of the thalamus and less neuronal change in the rostral putamen at the level of the nucleus accumbens. This case highlights possible undervaluation of neuropathological severity since

Vonsattel grading is mostly defined by macroscopic volume loss of the HCN (rostral neostriatum) in the lateral ventricle, a change that is usually appreciated only in later stages of the disease. Consideration of macroscopic atrophy of the putamen on coronal sectioning, particularly in HD donors that have not progressed to endstage disease, is therefore warranted. Further multimodal (i.e., clinical, functional, imaging and neuropathology) studies are needed in HD brain donors with relatively short disease duration, in order to improve understanding of how various HD measures relate to one and another and to improve the use of diagnostic, grading and staging criteria in HD.

REFERENCES

1. Quarrell O, O'Donovan KL, Bandmann O, Strong M. The prevalence of juvenile Huntington's disease: a review of the literature and metaanalysis. PLoS Curr. 2012;4:e4f8606b742ef3. doi: 10.1371/4f8606b742ef3
2. 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):986993. doi: 10.1016/s1474-4422(18)30294-1
3. Bakels HS, Roos RAC, van Roon-Mom WMC, de Bot ST. Juvenile-onset Huntington disease pathophysiology and neurodevelopment: a review. Mov Disord. 2022;37(1):1624. doi: 10.1002/mds.28823
4. 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):559577. doi: 10.1097/00005072-198511000-00003
5. Vonsattel JP, Keller C, Cortes Ramirez EP. Huntington's disease neuropathology. Handb Clin Neurol. 2011;100:83100. doi: 10.1016/b978-0-444-52014-2.00004-5
6. Kinnunen KM, Schwarz AJ, Turner EC, et al. Volumetric MRIbased biomarkers in Huntington's disease: an evidentiary review. Front Neurol. 2021;12:712555. doi: 10.3389/fneur.2021.712555
7. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (Edgmont). 2007;4(7):2837.
8. Shoulson I, Fahn S. Huntington disease: clinical care and evaluation. Neurology. 1979;29(1):13. doi: 10.1212/wnl.29.1.1
9. Zhang Y, Long JD, Mills JA, Warner JH, Lu W, Paulsen JS. Indexing disease progression at study entry with individuals atrisk for Huntington disease. Am J Med Genet. 2011;156b(7):751763. doi: 10.1002/ajmg.b.31232
10. Waldvogel HJ, Bullock JY, Synek BJ, Curtis MA, van RoonMom WM, Faull RL. The collection and processing of human brain tissue for research. Cell Tissue Bank. 2008;9(3):169179. doi: 10.1007/s10561-008-9068-1
11. Harris GJ, Pearlson GD, Peyser CE, et al. Putamen volume reduction on magnetic resonance imaging exceeds caudate changes in mild Huntington's disease. Ann Neurol. Jan 1992;31(1):6975. doi: 10.1002/ana.410310113
12. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol. Jan 2011;10(1):8398. doi: 10.1016/s1474-4422(10)70245-3
13. GomezTortosa E, MacDonald ME, Friend JC, et al. Quantitative neuropathological changes in presymptomatic Huntington's disease. Ann Neurol. 2001;49(1):2934. doi:
14. 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. 2022;10(1):55. doi: 10.1186/s40478-022-01364-1



CHAPTER 6

GLUT-1 changes in pediatric Huntington Disease brain cortex and fibroblasts: an observational case-control study

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ABSTRACT

Background: Paediatric Huntington disease with highly expanded mutations (HE-PHD; >80 CAG-repeats) presents atypically, compared to adult-onset Huntington disease (AOHD), with neurodevelopmental delay, epilepsy, abnormal brain glucose metabolism, early striatal damage, and reduced lifespan. Since genetic GLUT-1 deficiency syndrome shows a symptom spectrum similar to HE-PHD, we investigated the potential role of the two main glucose transporters, GLUT-1 and GLUT-3, in HE-PHD.

Methods: We compared GLUT-1 and GLUT-3 protein expression in HE-PHD, juvenile-onset (JOHD), and AOHD brains (n = 2; n = 3; n = 6) and periphery (n = 3; n = 2; n = 2) versus healthy adult controls (n = 6; n = 6). We also investigated mitochondrial complexes and hexokinase-II protein expression.

Findings: GLUT-1 and GLUT-3 expression were significantly lower in HE-PHD frontal cortex ($p = 0.009$, 95% [CI 13.4, 14.7]; $p = 0.017$, 95% [CI 14.2, 14.5]) versus controls. In fibroblasts, GLUT-1 and GLUT-3 expression were lower compared to controls ($p < 0.0001$, 95% [CI 0.91, 1.09]; $p = 0.046$, 95% [CI 0.93, 1.07]). In the frontal cortex, this occurred without evidence of extensive neuronal degeneration. Patients with HE-PHD had deregulated mitochondrial complex expression, particularly complexes II-III, levels of which were lower in frontal cortex versus controls ($p = 0.027$, 95% [CI 17.1, 17.6]; $p = 0.002$, 95% CI [16.6, 16.9]) and patients with AOHD ($p = 0.052$, 95% [CI 17.0, 17.6]; $p = 0.002$, 95% [CI 16.6, 16.7]). Hexokinase-II expression was also lower in HE-PHD frontal cortex and striatum versus controls ($p = 0.010$, 95% [CI 17.8, 18.2]; $p = 0.045$, 95% [CI 18.6, 18.7]) and in frontal cortex versus patients with AOHD ($p = 0.013$, 95% [CI 17.7, 18.1]). Expression JOHD levels were consistently different to those of HE-PHD but similar to those of AOHD.

Interpretation: Our data suggest a dysfunctional hypometabolic state occurring specifically in paediatric Huntington disease brains.

INTRODUCTION

Huntington Disease (HD) is one of nine autosomal dominant, rare, neurodegenerative, polyglutamine (polyQ) disorders characterised by pathological expansions of a trinucleotide CAG-repeat region encoding polyQ. In HD, the CAG region is located in the HTT gene, which encodes the polyQ region in the huntingtin protein.[1] HD typically manifests between 30 and 40 years of age with a movement disorder (typically chorea), cognitive dysfunction (e.g., abnormal executive functions) and behavioural changes, which are associated with an early progressive neurodegeneration of the cortex and striatum.[2]

Normal CAG-repeat lengths are usually stably inherited, whereas mutant expansions (>36 repeats) can show instability when transmitted to subsequent generations. A mutant CAG-repeat further increases the risk of somatic CAG expansion and can lead to earlier disease onset, at a juvenile age (i.e., <20 years). Highly expanded (HE) mutations (i.e., >80 CAG-repeats) cause paediatric onset of disease (i.e., during the first decade of life).[3]

Patients with HE paediatric HD (HE-PHD) often exhibit neurodevelopmental delay or regression and a particularly severe phenotype, resulting in a shorter lifespan when compared to juvenile-onset HD (JOHD), which has relatively shorter CAG-repeat lengths (i.e., <73),[3] or adult-onset Huntington disease (AOHD).[3] HE-PHD is also associated with a high rate of infantile epilepsy,[3,4] liver steatosis,[5] striatal volume loss with preserved brain cortex,[3,6] and severe striatal glucose hypometabolism.[7]

Abnormal glucose metabolism is a hallmark of several neurodegenerative diseases and is due, at least in part, to altered expression of glucose transporters (GLUTs),[8] which control glucose uptake into the brain. GLUT-1 and 3 are the major transporters of glucose across the blood–brain barrier and into neurons.[9]

GLUT-1 deficiency syndrome is a rare genetic neurometabolic disorder caused by mutations in the SCL2A1 gene, which results in impaired glucose transport into the brain.[10] Clinically, GLUT-1 deficiency syndrome manifests with neurodevelopmental delay, microcephaly, a high rate of infantile epilepsy, cognitive impairment and varying degrees of spasticity, ataxia, and dystonia[11] – a spectrum of symptoms that shares several similarities with HE-PHD.[3,12,13]

We hypothesised that expression of GLUT-1 and GLUT-3 may underlie HE-PHD pathology and differ from AOHD. We therefore compared GLUT-1 and GLUT-3 expression in the brain and peripheral tissues of patients with HE-PHD, JOHD and AOHD, and control subjects. Furthermore, we postulated that defects in glucose uptake are associated with impaired brain energy metabolism in patients with HD. Thus, we evaluated expression of mitochondrial complexes and hexokinase-II (HK-II) in the brains of the same four populations.

METHODS

Study outcomes

Our primary outcome was to compare protein expression of the main glucose transporters (e.g., GLUT-1, GLUT-3) and associated cargo protein (Rab11-A) in the brain and peripheral tissues of patients with HE-PHD, JOHD and AOHD, and control subjects. Our secondary outcome was to compare protein expression of mitochondrial complexes and HK-II, in the same four cohorts. Additional outcomes included brain and peripheral gene expression of SLC2A1 and SLC2A3, which encode GLUT-1 and GLUT-3 (all four cohorts); GLUT-1 localization in the brain (HE-PHD, AOHD and control cohorts only); and cell counts/neurodegeneration in the brain (HE-PHD cohort only).

Study population and tissue acquisition

HE-PHD was defined as HD manifesting with paediatric onset and a mutant HTT expansion length >80 CAG-repeats,[3] a threshold associated with childhood-onset of disease.[3] JOHD was defined as HD manifesting with early age of onset, retrospectively indicated in the approximate range of 18–25 years and HTT expansion length >55 CAG-repeats. AOHD was defined as HD manifesting in adulthood at age > 30 years and a mutant HTT expansion length of ≤55 CAG-repeats.[14] (Table 1). The CAG-Age Product (CAP) score, considered a predictor of HD progression and a commonly used measure of cumulative exposure to the effects of mutant (CAG expanded) Huntingtin, was calculated for all patients.[15]

Table 1. Characteristics of HE-PHD, JHD, AOHD patients and controls involved in fibroblast and brain tissue analyses.

Cohort	Code	Centre	Sex	Tissue	Expanded CAG-repeatExpn	Age of onset (years)	Age/Age of death (years)	CAP at death	Vonsattel grade	HD phase	First main symptoms
	HD86[b]	LUMC	F	FrCx, Striatum	86	4	19	994	3	Final	Bradykinesia, Dystonia
	HD252	LUMC	F	FrCx, Striatum	83	6	17	839	3	Final	Rigidity
HE-PHD	HD707-02[a]	LIRH	M	Fibroblasts	114	3.5	5	NA	NA	Initial	Ataxia, Dystonia
	HD130-05[a], [b]	LIRH	F	Fibroblasts	95	2	6	NA	NA	Moderate	Dystonia
	HD379-05[a], [b]	LIRH	M	Fibroblasts	87	5	7	NA	NA	Moderate	Dystonia
	HD123	LUMC	M	FrCx, Striatum	59	19–22	38	963	2/3	Final	Rigidity, chorea
	HD208	LUMC	M	FrCx, Striatum	68	21–23	37	1270	3	Final	Dystonia, chorea
JOHD	HD247	LUMC	F	FrCx	56	22–25	44	983	2/3	Final	Ataxia, chorea
	HD83-08	LIRH	F	Fibroblasts	56	20–24	35	NA	NA	Advanced	Dystonia, chorea
	HD701-01	LIRH	F	Fibroblasts	62	18	30	NA	NA	Advanced	Dystonia, chorea
	HD255	LUMC	M	FrCx, Striatum	55	30–33	45	960	3	Final	Chorea
	HD246	LUMC	M	FrCx, Striatum	44	35	47	486	3	Advanced	Cognitive changes
	HD234	LUMC	M	FrCx	NK	31	46	NK	3	Final	Behavioural changes, chorea
AOHD	T4161	LIRH	M	FrCx	46	>35	56	691	3	Final	Chorea
	T1991	LIRH	M	FrCx	48	>35	53	688	3	Final	Chorea
	HD249[c]	LUMC	F	FrCx, Striatum	46	35	60	740	3	Final	Chorea
	HD364-06	LIRH	F	Fibroblasts	41	48	50	NA	NA	Initial	Chorea
	HD598-01	LIRH	M	Fibroblasts	40	54	62	NA	NA	Initial	Chorea
	E15-02[c]	LUMC	M	Striatum	NK	NA	54	NA	NA	NA	NA
	E16-07[c]	LUMC	F	FrCx	18/19	NA	61	NA	NA	NA	NA
	E14-138	LUMC	M	FrCx, Striatum	17/17	NA	48	NA	NA	NA	NA
	T4452	LIRH	M	FrCx	15/20	NA	67	NA	NA	NA	NA
	T4233	LIRH	M	FrCx	16/17	NA	74	NA	NA	NA	NA

Table 1. Continued

Cohort	Code	Centre	Sex	Tissue	Expanded CAG-repeatExpn	Age of onset (years)	Age/Age of death (years)	CAP at death	Vonsattel grade	HD phase	First main symptoms
Controls	T4434	LIRH	F	FrCx	18/27	NA	79	NA	NA	NA	NA
	CTR1	OPBG	F	Fibroblasts	NK	NA	NA	NA	NA	NA	NA
	CTR2	OPBG	F	Fibroblasts	NK	NA	NA	NA	NA	NA	NA
	CTR3	LIRH	F	Fibroblasts	NK	NA	NA	NA	NA	NA	NA
	CTR4	LIRH	M	Fibroblasts	NK	NA	NA	NA	NA	NA	NA
	CTR5	LIRH	F	Fibroblasts	NK	NA	NA	NA	NA	NA	NA
	CTR6	LIRH	M	Fibroblasts	NK	NA	65	NA	NA	NA	NA

AOHD, adult-onset Huntington disease; CAP, CAG age product; F, female; FrCx, frontal cortex; HD, Huntington disease; HE-PHD, highly expanded paediatric Huntington disease; LIRH, Lega Italiana Ricerca Huntington; LUMC, Leiden University Medical Center; JHD, juvenile Huntington disease; M, male; NA, not applicable; NK, not known; OPBG, Ospedale Pediatrico Bambino Gesù.

[a]Detailed clinical and genetic description in Graziola et al.[1]

[b]Individual with documented epilepsy.

[c]Sample used for IHC experiments.

Post-mortem brain tissue samples of the frontal cortex and striatum were taken from donor brains of deceased patients with either HE-PHD (n = 2), JOHD (n = 3) or AOHD (n = 6), and healthy adult controls (n = 6) (age-matched to AOHD only, owing to the lack of available donor brains from healthy children) and collected at the Department of Pathology, Leiden University Medical Center (LUMC), Leiden, the Netherlands (Table 1).

Human fibroblast cell lines were generated at the IRCCS Bambino Gesú Children's Hospital (OPBG), Rome, Italy following skin punch biopsies taken from three patients with HE-PHD, two patients with JOHD, two patients with AOHD (all recruited from the Lega Italiana Ricerca Huntington [LIRH] Foundation outpatient clinic) and six age-matched controls (four recruited from the LIRH Foundation and two recruited from the OPBG; Table 1). These patients had also been entered into the ENROLL-HD study, the world's largest observational study for patients with HD and their families, at the LIRH Foundation site.[3]

Sample size determination

Owing to the rarity of HD, especially HE-PHD, no formal sample size calculations were performed. Indeed, HE-PHD is so rare that its prevalence has yet to be determined. Furthermore, obtaining HE-PHD brain specimens is particularly challenging since it relies on brain donations from deceased children, which is generally considered an exceptional event. Our available sample size of 30 subjects, including patients and healthy controls (brain donors, n = 17; fibroblast donors, n = 13), was therefore based on the number of samples obtainable within a reasonable timeframe for analysis.

Analysis of protein and transcripts

Deep frozen human brain samples and fibroblasts were prepared in radioimmunoprecipitation (RIPA) buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 0.1% SDS, pH 7.4) and phosphatase and protease inhibitors (Sigma–Aldrich). Detailed laboratory procedures for protein expression analysis have been previously described.[16] Briefly, 20 µg of protein preparations from patients and controls were separated via SDS-PAGE using Criterion™ TGX Stain-Free™ precast gels (Bio-Rad Laboratories or ThermoFisher) and transferred to a nitrocellulose or polyvinylidene difluoride membranes by Trans-Blot Turbo Transfer System (Bio-Rad Laboratories). Membranes were probed with the following primary antibodies: GLUT-1 (Ab15309, Abcam, 1:1000, RRID:

AB_301844), GLUT-3 (Ab15311, Abcam, 1:1000, RRID: AB_301846), Rab11-A (sc-166912, Santa Cruz Biotec., 1:1000, RRID: AB_10611645), HK-II (sc-130358, Santa Cruz, 1:1000, RRID:AB_2295219), OXPHOS (Ab110411, Abcam, 1:5000, RRID:AB_2756818), NDUFUB8 (NBP2-75586, NOVUS, 1:5000), and VDAC (PA1-954A, Invitrogen, 1:1000, RRID:AB_2304154). GAPDH (MA5-15738, Invitrogen, 1:1000, RRID: AB_10977387) and anti-Vinculin (V9264, Sigma–Aldrich, 1:1000, RRID: AB_10603627) were the housekeeping proteins used for normalization in brain tissue and fibroblasts, respectively. Immunodetection was performed with horseradish peroxidase (HRP)-conjugated secondary antibodies anti-rabbit (1:10000; L005661, Bio-Rad Laboratories) or anti-mouse (1:10000; L005662, Bio-Rad Laboratories or 1:30000, Jackson Immuno-Research). Blots were then imaged by the ChemiDoc MP imaging system using Chemiluminescence settings. Western blot results were quantified and visualised as percentage of variation relative to controls using Image Lab 6.1 software (Bio-Rad Laboratories). All experiments were performed in triplicate. Total RNA was isolated from patients' and controls' fibroblasts using the Total RNA Purification Plus Kit (Norgen Biotek Corp, Canada) and, for each sample, 2 μg of total RNA was reverse transcribed according to the manufacturer's protocol for M-MLV reverse transcriptase (Promega Italia, Italy). cDNAs were amplified (TaqMan assays) in triplicate with primers for SLC2A1 (Hs00892681_m1), SLC2A3 (Hs00359840_m1), Rab11-A (Hs00366449_g1), Rab11-B (Hs00188448_m1), and GAPDH (Hs99999905_m1) conjugated with fluorochrome FAM (Applied Biosystems Italia). The level of expression was measured by real-time quantitative reverse transcriptase PCR (qRT-PCR) using cycle threshold (Ct). The Ct was obtained by subtracting the Ct value of the gene of interest from the Ct value of the housekeeping gene (GAPDH). Data were analysed using the $2^{-\Delta\Delta Ct}$ method and reported as fold difference relative to controls. The analysis was performed using the QuantStudio™ 12K FlexSoftware v2.2 (Applied Biosystems). All PCR reactions were performed using a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems).

Immunohistochemistry and cell counts

Formalin-Fixed-Paraffin-Embedded 5 μm brain sections were cut and stained using haematoxylin and eosin (H&E). Immunostaining was performed for GLUT-1 (Ab15309, Abcam, 1:500, RRID: AB_301844) and NeuN (AB104225, Abcam, 1:500, RRID: AB_10711153). Sections were deparaffinised and rehydrated, and subsequent antigen retrieval was performed in citrate buffer (pH 6.0) using a pressure cooker

(15 min). Afterwards, endogenous enzyme block was performed by a 10-min incubation in 3% H_2O_2 in demineralised water, and non-specific antibody block was performed by a 1-h incubation in 1% BSA in PBS-T. Primary antibody incubation was performed overnight (GLUT-1 at room temperature, NeuN at 4 °C), followed by incubation with an anti-rabbit HRP secondary antibody (sc-2030, Santa-Cruz, 1:200) for 1 h at room temperature. Visualization of immunostaining was performed with chromogen 3,30'-diaminobenzidine (DAB). Finally, sections were counterstained with haematoxylin, dehydrated and cover slipped. The slides were digitised using an automatic bright field microscope (Philips Ultra Fast Scanner, Philips, Netherlands) for microscopic evaluation and taking pictures. For easy visualization purposes of GLUT-1 immunopositivity, a binary mask of the DAB signal was established by use of free Image-J software (colour deconvolution, grey scale 8-bit, threshold grey value 0–188, binary mask).

The total number of neurons in HE-PHD donors was estimated by stereological analyses of NeuN-stained slides by use of free QuPath software (positive cell detection, optical density sum, cell intensity threshold: DAB OD max) (Supplementary Fig. S1). Per slide, the mean number of neurons per mm^2 was calculated in three separate areas by analyzing cortical layers I to VI. Cell counts were performed by a single individual (HSB).

Fibroblast cell lines

Human cultured fibroblasts were obtained from skin biopsy of patients and aged matched controls. Human fibroblasts were cultured in Dulbecco's Modified Eagle Medium high glucose (4.5 g/l) supplemented with 10% foetal bovine serum, 50 µg/ml uridine and 110 mg/l sodium pyruvate. To analyse cell cycle status, 5×10^5 fibroblasts from patients and controls were plated and harvested at about 80% confluence. 2×10^6 cells were washed with PBS and centrifuged for 5 min at 1500 rpm at 4 °C. The obtained pellet was fixed with a cold solution of methanol/acetone 2:1 (v/v) by gently vortexing, and incubated over night at 4 °C. The next day, cells were centrifuged at 1100 rpm for 5 min at 4 °C and the pellet was re-suspended in a solution containing propidium iodide 500 µg/ml and RNAse 1 mg/ml and incubated for 30 min at room temperature. After incubation, cells were analysed by flow cytometry (BD LSRFortessa X-20, BD Biosciences).

Statistics

Patient data were expressed as mean ± standard error of the mean (SEM). For brain tissue, densitometry data were first log-transformed and then analysed using Analysis of Variance (ANOVA) to assess the overall differences among groups, i.e., controls, HE-PHD, JOHD, and AOHD. Subsequently, post-hoc comparisons were conducted using the Tukey multiple comparisons of means test or the Wilcoxon rank sum exact test, in case of deviation from normality. Normality was assessed using the Shapiro–Wilk normality test. Multiplicity was not considered in this study. All statistical tests were two-tailed, and the level of significance was set at $p < 0.05$. Statistical analyses were performed using the R software (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria).

Ethics

Samples were provided to the LIRH Foundation for research purposes. This study was performed in accordance with the ethical principles outlined in the World Medical Association Declaration of Helsinki. Pseudo-anonymization of all donors was preserved by using a coded system for the tissue samples. Clinical study protocols and informed consent forms for patients and healthy controls were approved by the Institutional Review Board of the LIRH Foundation on 28th October 2022 (n. 10.281022).

Role of the funding source

The funding source had no role in study design, data collection and interpretation, analysis, writing of this report, or in the decision to submit the paper for publication.

RESULTS

Study population and tissue acquisition

In total, 17 deceased donors were identified for the brain tissue analyses (HE-PHD, n = 2; JOHD, n = 3; AOHD, n = 6; healthy adult controls, n = 6) and 13 individuals participated in the fibroblast analyses (HE-PHD, n = 3; JOHD, n = 2; AOHD, n = 2; healthy adult controls, n = 6) (Fig. 1; Table 1).

All patients in the HE-PHD cohort (n = 5) showed childhood onset of disease at <10 years of age (Table 1). The HTT mutation was inherited maternally in one patient and transmitted paternally in all other patients.

Post-mortem delay (<12 h) was comparable between controls, patients with HE-PHD, JOHD and AOHD. All striatal samples from patients with HD were categorised as Vonsattel neuropathological grade 3 (Table 1). Further clinical and neuropathological characteristics of the HE-PHD brain donors are also included in Table 1.

Patients of any sex were eligible for inclusion in this study. Sex was self-reported by participants or, for deceased donors, documented per the patient's medical records.

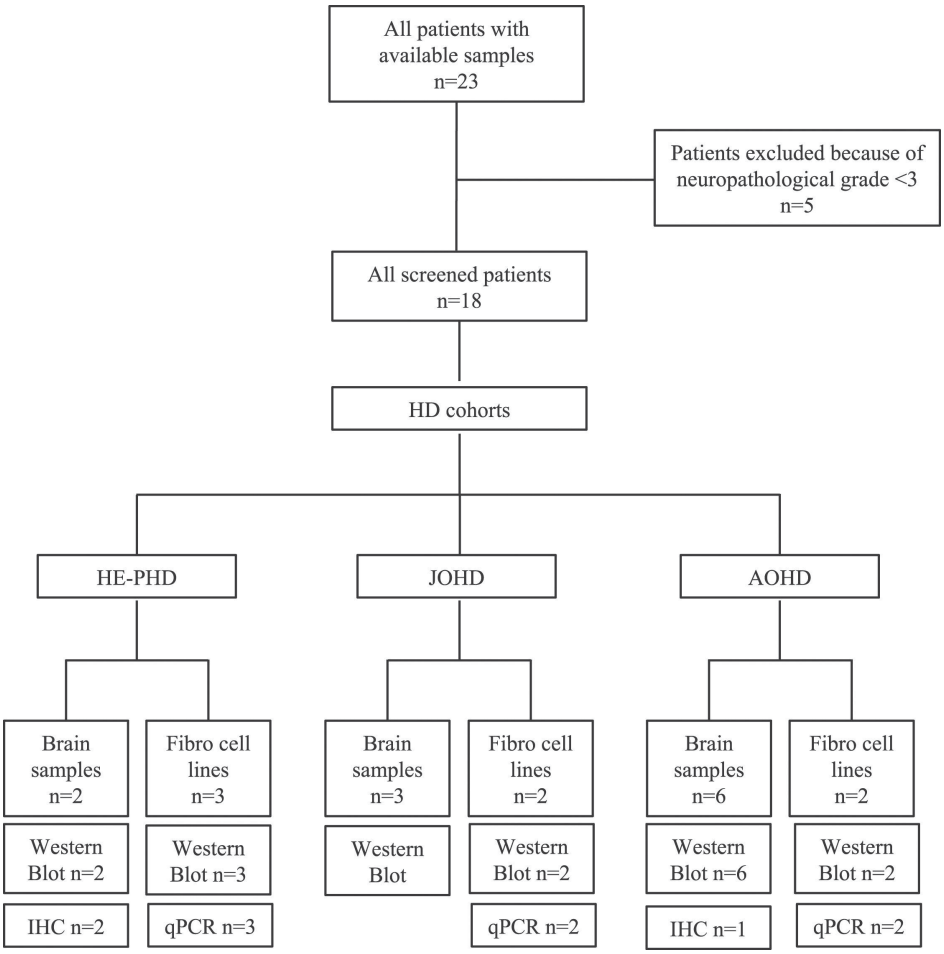


Figure 1. Study design, participants, and procedures. IHC = immunohistochemistry.

Low GLUT-1 expression in HE-PHD tissues

The ANOVA test for GLUT-1 protein expression revealed a statistically significant difference among groups in the frontal cortex ($F(3, 10) = 5.93$, $p = 0.014$), but not in the striatum ($F(3, 4) = 3.054$, $p = 0.15$). In the frontal cortex of donors with HE-PHD, the GLUT-1 protein expression level was ~4 or 5 times lower than in the JOHD, AOHD, or control samples (Fig. 2a and b; Table 2). The protein expression level of GLUT-1 was also lower in the striatum of donors with HE-PHD than in all other groups, although this difference was not statistically significant (Fig. 2c and d; Table 2). In contrast, neither JOHD nor AOHD samples showed any significant difference in GLUT-1 expression in the frontal cortex or in the striatum when compared to the control samples (Tukey test, frontal cortex: JOHD $p = 0.884$, 95% CI [−1.25, 0.77], AOHD $p = 0.704$, 95% CI [1.21, −0.57]; striatum: JOHD $p = 0.407$, 95% CI [−5.81, 2.35], AOHD $p = 0.698$, 95% CI [−2.99, 5.17]) (Fig. 2a–d). The expression levels of GLUT-1 protein and SLC2A1 mRNA were 2 to ~7 times statistically significantly lower in peripheral fibroblasts of donors with HE-PHD than in all other groups (Fig. 2e–i; Table 3). On the other hand, GLUT-1 protein and SLC2A1 mRNA levels were statistically significantly greater in peripheral fibroblasts of donors with JOHD (Wilcoxon rank sum exact test, protein $W = 132$, $p = 0.0002$, 95% CI [0.65, 2.35]; mRNA $W = 126$, $p = 0.0003$, 95% CI [1.23, 1.77]) and AOHD (Wilcoxon rank sum exact test, protein $W = 123$, $p = 0.0016$, 95% CI [0.17, 0.62]; mRNA $W = 100$, $p = 0.0332$, 95% CI [0.05, 2.49]) than in controls (Fig. 2e–i).

GLUT-1 localization was predominantly found in endothelial cells lining parenchymal capillaries of HE-PHD, AOHD and control brain tissues. In the frontal cortex of HE-PHD donors, a patchy lack of GLUT-1 immunopositivity and reduced expression was observed, as compared to the adjacent subcortical white matter within the same donor (Fig. 2j). Also, in the frontal cortex, a smaller length and less branching complexity of capillaries was visualized in donors with HE-PHD, as compared to donors with AOHD and control (Fig. 2j). GLUT-1 visualization in the caudal neostriatum of HE-PHD donors revealed areas with low or no immunopositivity, as compared to the adjacent capsular white matter showing a regular signal (Fig. 2j). In addition, the length and branching complexity of GLUT-1 positive capillaries in the striatum appeared lower as compared to donors with AOHD and control (Fig. 2j).

Limited neurodegeneration in the frontal cortex of patients with HE-PHD

Microscopic analysis of the frontal cortex revealed limited signs of degeneration (i.e., eccentric nucleus and eosinophilic cytoplasm) and neuronal loss in one donor

with HE-PHD (HD86; mean neuronal cell count, 349 per mm^2), and normal structure and architecture of the neocortex in the other (HD252; mean neuronal cell count 493 per mm^2) (Supplementary Fig. S2a and b). Conversely, in both patients there was remarkable neuronal loss in the striatum, in line with Vonsattel grade 3.

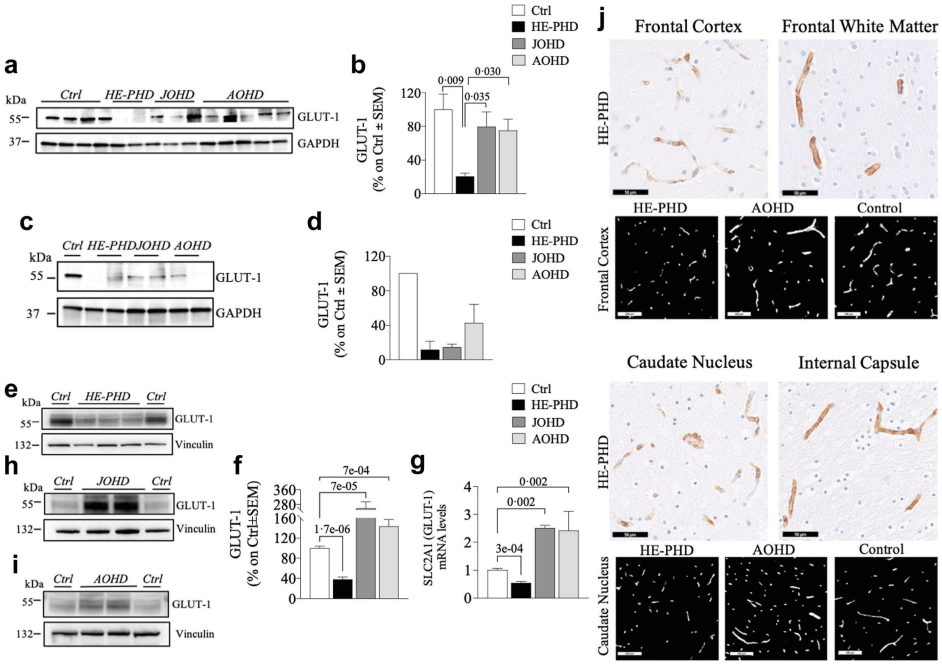


Figure 2. GLUT-1 in brain tissues and fibroblasts of patients with HE-PHD, JOHD and AOHD and controls.

Representative Western blot images and densitometric evaluation of GLUT-1 protein levels normalised to GAPDH or Vinculin protein levels in the FrCx (a, b) striatum (c, d) and fibroblasts (e, f, h, i) of patients with HE-PHD, JOHD and AOHD and healthy adult controls. All densitometric values are reported as a percentage of controls (set at 100%) and are the mean ± SEM of three independent experiments ($p < 0.05$, one-way ANOVA with Tukey multiple comparisons of means or Wilcoxon rank sum exact post-hoc tests). qRT-PCR analysis of SLC2A1 (GLUT-1) total mRNA in fibroblasts of patients with HE-PHD, JOHD and AOHD and controls (g). Relative mRNA levels were normalised using GAPDH and were calculated as $2^{-\Delta Ct}$. Results are the mean ± SEM of three independent experiments ($p < 0.05$, one-way ANOVA with Tukey multiple comparisons of means post-hoc test). IHC staining of GLUT-1 in the FrCx (white matter) of HE-PHD donor HD252 (j). Smaller length and lower branching complexity of immune-positive capillaries in the FrCx of HE-PHD donor HD252, as compared to AOHD donor HD249 and healthy control donor E16-07. Scale bars: 100 µm. GLUT-1 IHC staining in the striatum (caudate nucleus, internal capsule) of HE-PHD donor (HD86) (j). Binary representation of the DAB signal, illustrating smaller length and lower branching complexity of immune-positive capillaries in the caudate nucleus of HD86, as compared to AOHD donor HD249 and healthy control donor E15-02. Scale bars 50 µm. The use of the same loading control in different figures serves as a representative image. Each individual protein has been normalized against its respective loading control.

Table 2.

Protein	Cohort	Frontal cortex	Striatum
GLUT1	HE-PHD	12.5 ± 0.19 (10.1, 14.9)	9.83 ± 1.30 (–6.67, 26.3)
	Ctrl	14.0 ± 0.19 (13.4, 14.7) [p = 0.009]	12.8 ± 0.12 (11.2, 14.4) [p = 0.129]
	JOHD	13.8 ± 0.27 (12.7, 15.0) [p = 0.035]	11.0 ± 0.07 (10.1, 12.0) [p = 0.65]
	AOHD	13.7 ± 0.21 (13.1, 14.3) [p = 0.030]	11.7 ± 0.56 (4.57, 18.8) [p = 0.376]
GLUT3	HE-PHD	13.1 ± 0.44 (7.50, 18.7)	13.3 ± 0.20 (10.7, 15.9)
	Ctrl	14.3 ± 0.05 (14.2, 14.5) [p = 0.017]	14.5 ± 0.02 (14.3, 14.7) [p = 0.022]
	JOHD	13.0 ± 0.70 (10.0, 16.1) [p = 0.987]	13.3 ± 0.21 (10.7, 16.0) [p = 0.999]
	AOHD	13.7 ± 0.21 (13.2, 14.3) [p = 0.440]	13.8 ± 0.15 (11.9, 15.7) [p = 0.309]
Rab11-A	HE-PHD	16.3 ± 0.02 (16.1, 16.5)	16.5 ± 0.49 (10.3, 22.7)
	Ctrl	17.4 ± 0.08 (17.1, 17.6) [p = 0.011]	17.6 ± 0.01 (17.6, 17.6) [p = 0.170]
	JOHD	16.9 ± 0.34 (15.5, 18.4) [p = 0.175]	17.3 ± 0.31 (13.3, 21.3) [p = 0.377]
	AOHD	17.4 ± 0.09 (17.1, 17.6) [p = 0.010]	17.3 ± 0.12 (15.8, 18.7) [p = 0.404]
HK-II	HE-PHD	16.7 ± 0.21 (14.0, 19.4)	16.7 ± 0.59 (9,2, 24.2)
	Ctrl	18.0 ± 0.12 (17.8, 18.2) [p = 0.010]	18.6 ± 0.01 (18.6, 18.7) [p = 0.045]
	JOHD	17.5 ± 0.44 (15.6, 19.4) [p = 0.131]	17.7 ± 0.22 (14.8, 20.5) [p = 0.283]
	AOHD	17.9 ± 0.06 (17.7, 18.1) [p = 0.013]	17.9 ± 0.14 (16.0, 19.7) [p = 0.193]
Complex I	HE-PHD	16.9 ± 0.62 (9.0, 24.8)	18.1 ± 0.11 (17.4, 20.1)
	Ctrl	17.6 ± 0.19 (17.0, 18.3) [p = 0.155]	18.7 ± 0.01 (18.7, 18.8) [p = 0.230]
	JOHD	17.9 ± 0.21 (17.4, 18.5) [p = 0.049]	18.4 ± 0.03 (18.0, 18.9) [p = 0.633]
	AOHD	17.8 ± 0.06 (17.6, 18.0) [p = 0.064]	18.8 ± 0.11 (17.4, 20.1) [p = 0.173]
Complex II	HE-PHD	16.7 ± 0.15 (14.8, 18.6)	15.8 ± 0.19 (13.3, 18.2)
	Ctrl	17.4 ± 0.09 (17.1, 17.6) [p = 0.027]	17.3 ± 0.01 (17.3, 17.4) [p = 0.024]
	JOHD	17.2 ± 0.13 (16.6, 17.7) [p = 0.150]	17.0 ± 0.33 (12.8, 21.2) [p = 0.048]
	AOHD	17.3 ± 0.11 (17.0, 17.6) [p = 0.052]	16.7 ± 0.19 (14.3, 19.2) [p = 0.099]
Complex III	HE-PHD	15.6 ± 0.41 (10.3, 20.8)	17.6 ± 0.43 (12.1, 23.1)
	Ctrl	16.7 ± 0.05 (16.6, 16.9) [p = 0.002]	19.1 ± 0.01 (19.1, 19.2) [p = 0.029]
	JOHD	16.1 ± 0.21 (15.2, 17.0) [p = 0.061]	18.4 ± 0.03 (17.9, 18.8) [p = 0.215]
	AOHD	16.6 ± 0.01 (16.6, 16.7) [p = 0.002]	18.3 ± 0.03 (18.0, 18.7) [p = 0.246]
Complex IV	HE-PHD	16.0 ± 0.75 (6.48, 25.6)	16.2 ± 0.51 (9.76, 22.6)
	Ctrl	17.6 ± 0.11 (17.2, 17.9) [p = 0.217]	18.9 ± 0.01 (18.9, 19.1) [p = 0.010]
	JOHD	17.1 ± 0.94 (13.1, 21.2) [p = 0.501]	17.5 ± 0.15 (15.6, 19.3) [p = 0.120]
	AOHD	17.8 ± 0.13 (17.4, 18.1) [p = 0.123]	17.8 ± 0.28 (14.3, 21.4) [p = 0.058]
Complex V	HE-PHD	15.7 ± 0.14 (13.9, 17.5)	16.8 ± 0.31 (12.8, 20.8)
	Ctrl	16.2 ± 0.13 (15.8, 16.6) [p = 0.161]	18.2 ± 0.02 (18.2, 28.5) [p = 0.012]
	JOHD	16.2 ± 0.21 (15.3, 17.0) [p = 0.246]	17.4 ± 0.02 (17.2, 17.6) [p = 0.172]
	AOHD	16.2 ± 0.14 (13.9, 17.5) [p = 0.156]	17.3 ± 0.07 (16.3, 18.2) [p = 0.277]

Assessment of highly expanded (HE) Paediatric Huntington disease (PHD) compared with control (Ctrl), juvenile-onset (JOHD), and Adult-Onset HD (AOHD) individuals. Summary of average log-transformed protein expression levels ± SEM, Confidence Intervals (lower, upper), and [p-values] in the frontal cortex and striatum of highly expanded paediatric (HE-PHD) compared to juvenile-onset HD (JOHD), adult-onset HD (AOHD) and control (Ctrl) individuals.

Table 3.

Protein	Cohort	Levels
GLUT1	HE-PHD	0.38 ± 0.17 (0.27, 0.48)
	Ctrl	1.00 ± 0.04 (0.91, 1.09) [p = 1.7e-06]
	JOHD	2.59 ± 0.35 (1.68, 3.49) [p = 7e-05]
	AOHD	1.43 ± 0.14 (1.08, 1.79) [p = 7e-04]
Gene exp.		
SLC2A1	HE-PHD	0.54 ± 0.05 (0.41, 0.67)
	Ctrl	1.02 ± 0.08 (0.83, 1.21) [p = 3e-04]
	JOHD	2.51 ± 0.09 (2.25, 2.76) [p = 0.002]
	AOHD	2.43 ± 0.69 (0.65, 4.20) [p = 0.002]
Protein		
GLUT3	HE-PHD	0.87 ± 0.04 (0.79, 0.97)
	Ctrl	1.05 ± 0.03 (0.93, 1.07) [p = 0.046]
	JOHD	1.09 ± 0.09 (0.99, 1.20) [p = 0.002]
	AOHD	1.12 ± 0.09 (0.89, 1.34) [p = 0.045]
Gene exp.		
SLC2A3	HE-PHD	0.89 ± 0.12 (0.61, 1.19)
	Ctrl	1.11 ± 0.18 (0.67, 1.55) [p = 0.359]
	JOHD	1.18 ± 0.17 (0.75, 1.62) [p = 0.204]
	AOHD	1.70 ± 0.58 (0.19, 3.20) [p = 0.573]
Protein		
Rab11-A	HE-PHD	1.06 ± 0.06 (0.93, 1.19)
	Ctrl	1.00 ± 0.05 (0.89, 1.11) [p = 0.434]
	JOHD	1.14 ± 0.09 (1.04, 1.23) [p = 0.840]
	AOHD	1.06 ± 0.08 (0.85, 1.26) [p = 1]
Gene exp.		
Rab11-A	HE-PHD	0.93 ± 0.08 (0.73, 1.13)
	Ctrl	1.00 ± 0.32 (0.92, 1.08) [p = 0.443]
	JOHD	0.87 ± 0.06 (0.71, 1.02) [p = 0.569]
	AOHD	1.66 ± 0.52 (0.33, 2.99) [p = 0.175]

Assessment of highly expanded (HE) Paediatric Huntington disease (PHD) compared with control (Ctrl), juvenile-onset (JOHD), and Adult-Onset HD (AOHD) individuals. Summary of average protein and gene expression levels ± SEM, Confidence Intervals (lower, upper), and [p-values] in fibroblast cell lines of highly expanded paediatric (HE-PHD) compared to juvenile-onset HD (JOHD), adult-onset HD (AOHD) and control (Ctrl) individuals.

Low GLUT-3 and Rab11-A expression in HE-PHD tissues

The protein expression levels of GLUT-3 were globally significantly different among groups, both in the frontal cortex (ANOVA test, F (3, 10) = 7.05, p = 0.0079) and in the striatum (ANOVA test, F (3, 4) = 11.6, p = 0.019). Compared to controls, protein

expression levels of GLUT-3 were significantly lower in both the frontal cortex and striatum of donors with HE-PHD (Fig. 3a–d; Table 2). GLUT-3 was also lower in donors with AOHD compared to controls in the frontal cortex (Tukey test, p = 0.066, 95% CI [−1.28, 0.14]) (Fig. 3a–d), and lower in donors with JOHD compared to controls in both the frontal cortex (Tukey test, p = 0.014, 95% CI [−6.26, −5.32) and striatum (Tukey test, p = 0.025, 95% CI [−2.09, −0.21]). In peripheral fibroblasts, *SLC2A3* gene expression was not statistically significantly different between any group (Table 3), whereas its protein expression was statistically significantly lower in donors with HE-PHD when compared to donors with JOHD, AOHD and controls (Supplementary Fig. S1a–e; Table 3).

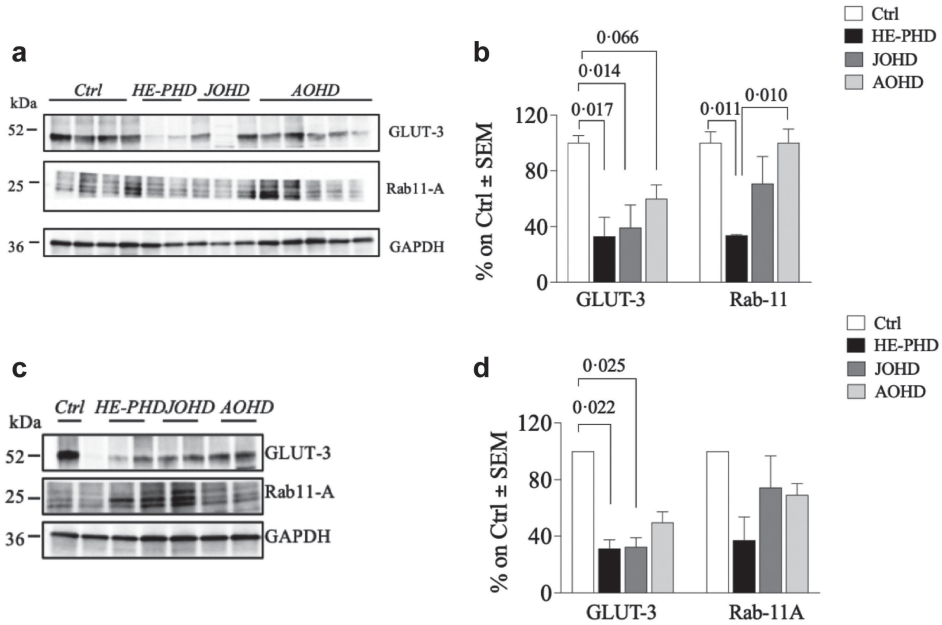


Figure 3. Evaluation of GLUT-3 and Rab11-A levels in brain tissues of patients with HE-PHD, JOHD, AOHD and controls.

Representative Western blot images and densitometric evaluation of GLUT-3 and Rab11-A protein levels normalised to GAPDH protein levels in the FrCx (a, b) and striatum (c, d) of patients with HE-PHD, JOHD and AOHD and healthy adult controls. All densitometric values are reported as a percentage of controls (set at 100%) and are the mean ± SEM of three experiments ($p < 0.05$, one-way ANOVA with Tukey multiple comparisons of means post-hoc test). The use of the same loading control in different figures serves as a representative image. Each individual protein has been normalized against its respective loading control.

Expression levels of the cargo protein Rab11-A were significantly lower in the frontal cortex of donors with HE-PHD compared to both controls (Tukey test, p = 0.011,

95% CI [−1.91, −0.25]) and AOHD (Tukey test, p = 0.010, 95% CI [−1.87, −0.27]) (Fig. 3a and b). Data collected in the striatum did not show any differences in Rab11-A protein levels between the HE-PHD cohort and any other cohort (Fig. 3c and d). Rab11-A protein levels in fibroblasts also did not differ between the HE-PHD cohort and any other cohort (Supplementary Fig. S1a, d–g; Table 3).

Impairment in glucose uptake is associated with defects of mitochondrial machinery in patients with HE-PHD

Mitochondrial complex expression was dysregulated in the brains of patients with HD. In particular, levels of complex II and complex III subunits were significantly lower in the frontal cortex of donors with HE-PHD, compared to controls (Tukey test, p = 0.027, 95% CI [−1.22, −0.074] and p = 0.002, 95% CI [−1.82, −0.48], respectively) and compared to patients with AOHD (Tukey test, p = 0.052, 95% CI [−1.09, 0.005], and p = 0.002, 95% CI [−1.73, −0.43], respectively) (Fig. 4a, c; Table 2). Furthermore, the expression level of the complex I subunit in HE-PHD was statistically significantly lower compared to donors with JOHD (Tukey test, p = 0.049, 95% CI [−2.10, −0.006]), and numerically lower compared to donors with AOHD, following a trend towards statistical significance (Tukey test, p = 0.064, 95% CI [−1.87, 0.048]) (Fig. 4a, c; Table 2). We also evaluated levels of complex IV and V (ATP synthase) and voltage-dependent anion-selective channel, a protein that plays a key role in maintaining high rates of oxidative phosphorylation.[17] No statistically significant differences between groups were observed for either protein in the frontal cortex.

Deregulation of mitochondrial complexes expression was also observed in the striatum, with statistically significantly lower levels of all complexes, except complex I, seen in patients with HE-PHD compared to controls (Fig. 4d, f; Table 2).

The HE-PHD cohort showed statistically significantly lower levels of HK-II protein expression in the frontal cortex and striatum, compared to controls (Tukey test, p = 0.010, 95% CI [−2.30, −0.33], and p = 0.045, 95% CI [−3.78, −0.06], respectively) and in the frontal cortex compared to AOHD (Tukey test, p = 0.013, 95% CI [−2.18, −0.27]) (Fig. 4a, b, d, e; Table 2). In contrast, no difference in HK-II protein expression in the frontal cortex was observed between the AOHD and control cohorts (Tukey test, p = 0.982, 95% CI [0.86, −0.67]; striatum, p = 0.443, 95% CI [2.62, −1.10]). In peripheral fibroblasts, HK-II protein expression differed only between the JOHD and control cohorts (Tukey test, p = 0.014, 95% CI [0.07, 0.77]) (Supplementary Fig. S1h–k).

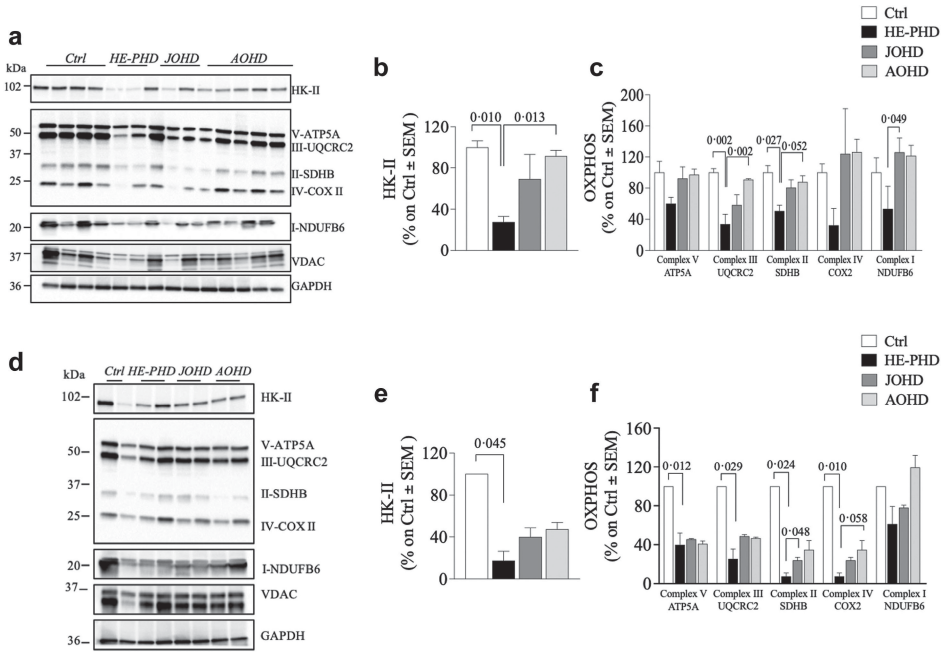


Figure 4. Evaluation of HK-II and mitochondrial machinery complexes (OXPHOS) levels in brain tissues of patients with HE-PHD, JOHD, AOHD and controls.

Representative Western blot images and densitometric evaluation of HK-II and OXPHOS protein levels normalised to GAPDH protein levels in the FrCx (a–c) and striatum (d–f) of patients with HE-PHD, JOHD and AOHD and healthy adult controls. All densitometric values are reported as a percentage of controls (set at 100%) and are presented as the mean ± SEM of three independent experiments ($p < 0.05$, one-way ANOVA with Tukey multiple comparisons of means post-hoc test). The use of the same loading control in different figures serves as a representative image. Each individual protein has been normalized against its respective loading control.

DISCUSSION

Neurodegenerative conditions, such as Alzheimer, Huntington, and Parkinson diseases, are associated with brain glucose hypometabolism, which is contributed to by impaired GLUT-1 and GLUT-3 glucose transporters.[8] Interestingly, dysregulation in GLUT transporters has also been observed in aberrant brain neurodevelopment,[18] micromalformations[19] and epilepsy, all conditions that have been associated with HD.[3,4,20,21]

Patients with HE-PHD manifest signs and symptoms that are uncommon to AOHD, including high frequency of epileptic seizures, early onset hypokinetic-rigid syndrome/dystonia and developmental delay.[3,4] Interestingly, certain HE-PHD clinical features,

such as epilepsy, are also observed in patients with GLUT-1 deficiency syndrome, where genetic defects in this transporter result in a chronic shortage of glucose in the brain.[22] This suggests that alterations in glucose transport or metabolism may also occur in HE-PHD and could explain some of its unique symptoms, compared to AOHD.

In the context of HD, this study demonstrates reduced protein expression of GLUT-1 and GLUT-3 in the frontal cortex and striatum of brains from patients with HE-PHD, compared to brains from patients affected since adulthood, including JOHD, and from controls. Our results are partly in line with others, who have reported significantly lower GLUT-1 and GLUT-3 protein expression in the striatum of late-stage AOHD (classified as neuropathological Vonsattel grade 3), compared to earlier-stage AOHD (neuropathological Vonsattel grades 1 and 2).[20] However, they found no evidence of reduced GLUT-1 and GLUT-3 expression in AOHD cortex,[23] whereas in our frontal cortex samples we found statistically significantly lower GLUT-1 expression in patients with HE-PHD versus controls, and statistically significantly lower GLUT-3 expression in all cohorts (HE-PHD, JOHD and AOHD) versus controls.

Furthermore, we identified reduced protein levels of Rab11-A in HE-PHD frontal cortex. Rab11-A is a small GTPase that has a critical role in regulating the trafficking of GLUT-3 to the neuronal cell surface,[24] experiments in HD cell models have shown reduced neuronal GLUT-3[25] and Rab11-A expression levels and highlights the relevance of altered glucose transportation in models with HE mutations.

In HE-PHD brain samples, low levels of GLUT-1 and GLUT-3 were observed alongside low levels of mitochondrial complexes involved in energy metabolism. In the frontal cortex, significant differences versus controls and versus AOHD were seen for complexes II and III and versus JOHD for complex I. Results from in vitro studies have revealed calcium abnormalities in mitochondria from patients and transgenic mice with HD, and that these defects are a direct effect of HE polyQ.[26] Our findings in brain tissues affected by HE HTT mutations, therefore, corroborate these in vitro observations.

Furthermore, we also observed significantly lower levels of HK-II in HE-PHD but not AOHD brains compared to control. Hexokinases catalyse the first committed step of glucose metabolism, by phosphorylating glucose to glucose-6-phosphate.[27] HK-II also protects cells from death during hypoxia and functions as a sensor of glucose availability, inducing apoptosis in response to glucose depletion.[28]

Our finding, that expression levels of GLUT-1, GLUT-3, mitochondrial complexes and HK-II were significantly and selectively lower in HE-PHD frontal cortex and striatum, shows that a disease-relevant biological dysfunction (i.e., hypometabolic state) occurs without any substantial neuronal loss in the frontal cortex of these patients. Relatively preserved brain cortex has been observed previously in HE-PHD by magnetic resonance imaging (MRI), magnetic resonance spectroscopy and neuropathological studies,[3] and is in line with findings from ongoing studies using volumetric MRI in paediatric-onset patients (Sabatini, personal communication). Nevertheless, the disease pathophysiology of patients with HE HTT mutations, concurs with a more severe disease and worse prognosis.

To validate the findings from our study on brain tissues of patients with HE-PHD, we conducted an analysis of metabolic profiles in fibroblasts obtained from patients with HE-PHD. Our results revealed significantly reduced levels of GLUT-1 in both gene and protein expression, and a somewhat less pronounced decrease in GLUT-3 protein expression. However, no significant differences were observed in gene expression when compared to the healthy control. This discrepancy between findings in the brain tissues versus fibroblasts is likely explained by the fact that GLUT-3 is not the main transporter of glucose in peripheral cells such as fibroblasts.[29] While our data emphasize that the primary pathology in HE-PHD is found in the brain, they also highlight the probable existence of a peripheral component to the disease, as also suggested by our recent study which identified liver steatosis in patients with HE-PHD.[5] The evidence of peripheral biological abnormalities may represent an important observation considering currently ongoing experimental therapies aimed at lowering mutated HTT in both the brain and periphery.[30]

Since a direct relationship between GLUT function, neurodegeneration and neurodevelopment is still poorly understood, additional studies in HD and other neurodegenerative diseases are therefore needed due to its potential role in HD pathogenesis[20] and therapy perspectives.[31] In fact, growing evidence suggests an important modulatory role for glucose transport in the HD mitochondrial deregulation, emphasizing that abnormal energy metabolism could interfere with brain neurodevelopment and may represent a critical therapeutic target for HD, as also suggested for adulthood disease.[32] For instance, the GLUT-1 role in HE-PHD pathology suggests that a ketogenic diet could be beneficial for patients with HE-PHD when initiated in the early stages of the disease.[11] The high-fat ketogenic diet may result in permanent ketosis and provide the brain with an alternative energy

source. The beneficial effects of the ketogenic, normocaloric, with low carbohydrate intake diet, currently used in epilepsy and movement disorders, are well documented in children with GLUT-1 deficiency and may offer new therapeutic approaches to HE-PHD. This is especially important given that there is no validated experimental protocol so far to at least address any disease-modifying treatment against HE-PHD.

We are aware that our study has a number of limitations, the main one being that our HE-PHD brain donor cohort is limited to only two patients. This is due to the extremely rare occurrence of this variant. HD itself is a rare disease and only a small proportion of about 6% patients have juvenile-onset disease,[14] and an even smaller proportion have HE-PHD. Thus, the opportunity to acquire a donor brain from a patient with HE-PHD is exceptionally rare. Likewise, owing to a general lack of donor brains from healthy controls, especially child donors, it was not possible to age-match our healthy control donor brain cohort to our HE-PHD cohort. It is therefore possible that some of the differences we observed between patients with HE-PHD and controls may have been due to differences in age rather than pathophysiology. Another limitation is due to the analysis of frontal cortex only, while an extensive study of several parts of brain and cortical regions might have offered additional insights. Unfortunately, we cannot provide a fine stratification of brain areas in our current context. This argues again that more cases and more extensive examination of the cerebral cortex are required to draw any firm conclusions.

An additional limitation is due to the clinical stratification of our adult cohort, specifically concerning the retrospective reconstruction of the approximate age of onset of JOHD. To limit such a bias, we included in the JOHD cohort only those patients with mutation size >55 CAG-repeats, a mutation size which is widely believed to be associated with JOHD.[14] Notwithstanding these limitations, our findings shed light on the pathology of this devastating disease variant and highlight differences in potential biological mechanisms underlying HE-PHD and AOHD. Such differentiation may be important not only from a therapeutic perspective but also to ensure appropriate inclusion of paediatric patients into Huntington disease clinical trials.[14]

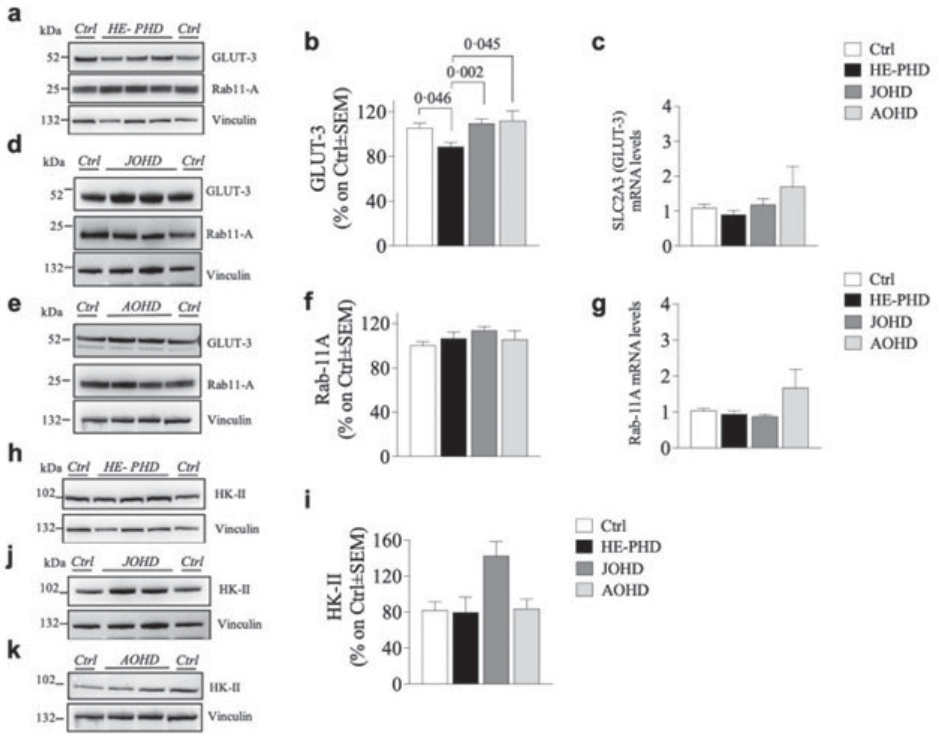
Finally, our study highlighted a difference in GLUT-1 expression between HE-PHD and JOHD. Such a biological difference, in addition to the CAG mutation length, suggests there may be a need to reconsider the old classification of HD variants and abandon the terminology “juvenile-onset”, which currently includes some adult-onset patients and instead replace this with the term “paediatric-onset” HD, to represent the category of patients that appears clinically and biologically different from adulthood HD.

REFERENCES

1. MacDonald M.E., Ambrose C.M., Duyao M.P., et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971–983. doi: 10.1016/0092-8674(93)90585-e.
2. Tabrizi S.J., Langbehn D.R., Leavitt B.R., et al. Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet Neurol. 2009;8(9):791–801. doi: 10.1016/S1474-4422(09)70170-X.
3. Fusilli C., Migliore S., Mazza T., et al. Biological and clinical manifestations of juvenile Huntington's disease: a retrospective analysis. Lancet Neurol. 2018;17(11):986–993. doi: 10.1016/S1474-4422(18)30294-1.
4. Cloud L.J., Rosenblatt A., Margolis R.L., et al. Seizures in juvenile Huntington's disease: frequency and characterization in a multicenter cohort. Mov Disord. 2012;27(14):1797–1800. doi: 10.1002/mds.25237.
5. Squitieri F., Monti L., Graziola F., Colafati G.S., Sabatini U. Early liver steatosis in children with pediatric Huntington disease and highly expanded CAG mutations. Park Relat Disord. 2022;103:102–104. doi: 10.1016/j.parkreldis.2022.08.027.
6. Tereshchenko A., Magnotta V., Epping E., et al. Brain structure in juvenile-onset Huntington disease. Neurology. 2019;92(17):e1939–e1947. doi: 10.1212/WNL.0000000000007355.
7. Zhou X.Y., Lu J.Y., Zuo C.T., Wang J., Sun Y.M. juvenile-onset hypokinetic-rigid Huntington's disease: a case with dual-tracer positron emission tomography. Quant Imaging Med Surg. 2021;11(1):479–482. doi: 10.21037/qims-19-992.
8. Głuchowska K., Pliszka M., Szablewski L. Expression of glucose transporters in human neurodegenerative diseases. Biochem Biophys Res Commun. 2021;540:8–15. doi: 10.1016/j.bbrc.2020.12.067.
9. Benarroch E.E. Brain glucose transporters: implications for neurologic disease. Neurology. 2014;82(15):1374–1379. doi: 10.1212/WNL.0000000000000328.
10. Seidner G., Alvarez M.G., Yeh J.I., et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nat Genet. 1998;18(2):188–191. doi: 10.1038/ng0298-188.
11. Leen W.G., Klepper J., Verbeek M.M., et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. Brain. 2010;133(Pt 3):655–670. doi: 10.1093/brain/awp336.
12. Ribaï P., Nguyen K., Hahn-Barma V., et al. Psychiatric and cognitive difficulties as indicators of juvenile huntington disease onset in 29 patients. Arch Neurol. 2007;64(6):813–819. doi: 10.1001/archneur.64.6.813.
13. Nopoulos P.C., Aylward E.H., Ross C.A., et al. Smaller intracranial volume in prodromal Huntington's disease: evidence for abnormal neurodevelopment. Brain. 2011;134(1):137–142. doi: 10.1093/brain/awq280.
14. Quarrell O.W.J., Nance M.A., Nopoulos P., et al. Defining pediatric huntington disease: time to abandon the term Juvenile Huntington disease? Mov Disord. 2019;34(4):584–585. doi: 10.1002/mds.27640.
15. Zhang Y., Long J.D., Mills J.A., et al. Indexing disease progression at study entry with individuals at-risk for Huntington disease. Am J Med Genet B Neuropsychiatr Genet. 2011;156(7):751–763. doi: 10.1002/ajmg.b.31232.
16. Di Domenico F., Tramutola A., Barone E., et al. Restoration of aberrant mTOR signaling by intranasal rapamycin reduces oxidative damage: focus on HNE-modified proteins in a mouse model of down syndrome. Redox Biol. 2019;23 doi: 10.1016/j.redox.2019.101162.

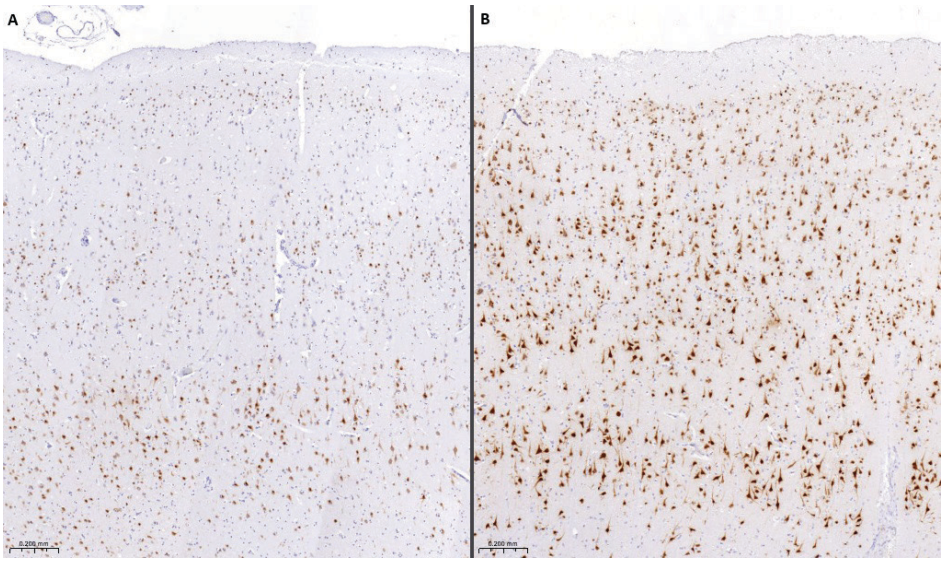
17. Klepinin A., Ounpuu L., Mado K., et al. The complexity of mitochondrial outer membrane permeability and VDAC regulation by associated proteins. J Bioenerg Biomembr. 2018;50(5):339–354. doi: 10.1007/s10863-018-9765-9.
18. Tramutola A., Lanzillotta C., Di Domenico F., et al. Brain insulin resistance triggers early onset Alzheimer disease in down syndrome. Neurobiol Dis. 2020;137 doi: 10.1016/j.nbd.2020.104772.
19. Ghosh C., Myers R., O'Connor C., et al. Cortical dysplasia in rats provokes neurovascular alterations, GLUT1 dysfunction, and metabolic disturbances that are sustained post-seizure induction. Mol Neurobiol. 2022;59(4):2389–2406. doi: 10.1007/s12035-021-02624-2.
20. Barnat M., Capizzi M., Aparicio E., et al. Huntington's disease alters human neurodevelopment. Science. 2020;369(6505):787–793. doi: 10.1126/science.aax3338.
21. Hickman R.A., Faust P.L., Rosenblum M.K., Marder K., Mehler M.F., Vonsattel J.P. Developmental malformations in Huntington disease: neuropathologic evidence of focal neuronal migration defects in a subset of adult brains. Acta Neuropathol. 2021;141(3):399–413. doi: 10.1007/s00401-021-02269-4.
22. De Vivo D.C., Trifiletti R.R., Jacobson R.I., Ronen G.M., Behmand R.A., Harik S.I. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N Engl J Med. 1991;325(10):703–709. doi: 10.1056/NEJM199109053251006.
23. Gamberino W.C., Brennan W.A. Glucose transporter isoform expression in Huntington's disease brain. J Neurochem. 1994;63(4):1392–1397. doi: 10.1046/j.1471-4159.1994.63041392.x.
24. McClory H., Williams D., Sapp E., et al. Glucose transporter 3 is a rab11-dependent trafficking cargo and its transport to the cell surface is reduced in neurons of CAG140 Huntington's disease mice. Acta Neuropathol Commun. 2014;2:179. doi: 10.1186/s40478-014-0178-7. BioMed Central Ltd.
25. Solìs-Maldonado M., Mirò M.P., Acuña A.I., et al. Altered lactate metabolism in Huntington's disease is dependent on GLUT3 expression. CNS Neurosci Ther. 2018;24(4):343–352. doi: 10.1111/cns.12837.
26. Panov A.V., Gutekunst C.A., Leavitt B.R., et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci. 2002;5(8):731–736. doi: 10.1038/nn884.
27. Han R., Liang J., Zhou B. Glucose metabolic dysfunction in neurodegenerative diseases—new mechanistic insights and the potential of hypoxia as a prospective therapy targeting metabolic reprogramming. Int J Mol Sci. 2021;22(11):5887. doi: 10.3390/ijms22115887.
28. Mergenthaler P., Kahl A., Kamitz A., et al. Mitochondrial hexokinase II (HKII) and phosphoprotein enriched in astrocytes (PEA15) form a molecular switch governing cellular fate depending on the metabolic state. Proc Natl Acad Sci U S A. 2012;109(5):1518–1523. doi: 10.1073/pnas.1108225109.
29. Simpson I.A., Dwyer D., Malide D., Moley K.H., Travis A., Vannucci S.J. The facilitative glucose transporter GLUT3: 20 years of distinction. Am J Physiol Metab. 2008;295(2):E242–E253. doi: 10.1152/ajpendo.90388.2008.
30. Bhattacharyya A., Trotta C.R., Narasimhan J., et al. Small molecule splicing modifiers with systemic HTT-lowering activity. Nat Commun. 2021;12(1):1–12. doi: 10.1038/s41467-021-27157-z. 2021 121.
31. Braz B.Y., Wennagel D., RatiÃ© L., et al. Treating early postnatal circuit defect delays Huntington's disease onset and pathology in mice. Science. 2022;377(6613) doi: 10.1126/science.abq5011.
32. Saft C., Zange J., Andrich J., et al. Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. Mov Disord. 2005;20(6):674–679. doi: 10.1002/mds.20373.

SUPPLEMENTARY FIGURES



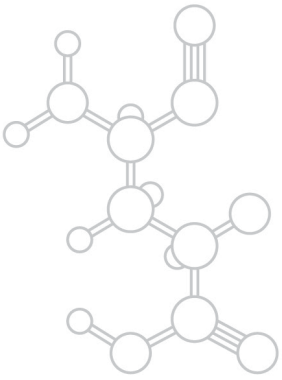
Supplementary Figure S1. Evaluation of GLUT-3, Rab11-A and HK-II levels in fibroblasts of HE-PHD, JOHD, AOHD patients and controls.

Representative western blot images and densitometric evaluation of GLUT-3, Rab11-A (a, b, d, e, f-g) and HK-II (h-k) protein levels normalized to Vinculin protein levels in fibroblasts of HE-PHD, JOHD, AOHD patients and age-matched controls. All densitometric values are reported as a percentage of controls (set at 100%) and are the mean ± SEM of three independent experiments. ($p<0{\cdot}05$, one-way ANOVA with Tukey post-hoc test). qRT-PCR analysis of GLUT-3 and Rab11-A total mRNA in fibroblasts of HE-PHD, JOHD, AOHD patients and age-matched controls (c, g). Relative mRNA expression levels are reported as a percentage of GAPDH expression levels (set at 100%) and are the mean ± SEM of three experiments ($p<0{\cdot}05$, with Tukey post-hoc test). The use of the same loading control in different figures serves as a representative image. Each individual protein has been normalized against its respective loading control.



Supplementary Figure S2. NeuN staining in the frontal cortex of HE-PHD patients

IHC staining of NeuN in the FrCx of HE-PHD HD86 (a) and HD252 (b) donors.



CHAPTER 7

Summary and Discussion

SUMMARY

The aim of this thesis was to address several unresolved questions regarding the juvenile-onset HD (JHD) and Pediatric HD (PHD) populations through a translational approach. While numerous therapeutic trials are currently underway in the adult-onset HD (AHD) population,[1] focused on modifying disease progression, the JHD population, although part of the same HD continuum, presents with unique disease characteristics that necessitate a tailored approach distinct from that of the AHD population. Key issues related to the epidemiology of JHD and PHD, the capacity of these populations to participate in clinical trials, clinical disease characteristics of JHD, underlying pathophysiological mechanisms, and brain maturation in pediatric patients remain inadequately addressed. Resolving these questions is crucial to ensure that these patients are not excluded from future treatment options.

We have shown that the JHD and PHD populations in the Netherlands are even smaller than previously suggested, comprising less than 1% of the entire clinically manifest HD population (**Chapter 2**). Due to significant diagnostic delays in the JHD population, more than half of patients with JHD is not available for clinical trials under 18 years of age (PHD). Additionally, we have demonstrated that functional competence at the time of diagnosis is diminished in JHD patients, and that the CAP^{100} score, a measure of disease progression, is invalid for use in the JHD population. These findings highlight the need for alternative approaches in the design of interventional trials targeting these populations and novel inclusion criteria tailored to the JHD and PHD population.

We summarized clinical and neuropathological disease characteristics of JHD as reported in the literature and provided a pathophysiological perspective to explain differences with the prototypical AHD phenotype (**Chapter 3**). While toxic gain-of-function disease mechanisms in relation to CAG-repeat length explain age at disease onset and progression in HD, CAG-dependent modulation or loss of normal HTT function in neurodevelopment might explain some of the unique clinical disease characteristics that are seen in the JHD population, such as developmental delay, epilepsy, behavioral disorder and psychosis. Additionally, the pediatric age of onset in PHD cases may influence ongoing postnatal brain maturation processes. These potential differences in pathophysiology and brain development have important implications for future therapeutic strategies and underscore the need for a personalized approach to treatment in the JHD population.

We revealed that both the cJHD and aJHD populations exhibit distinct patterns in the prevalence, severity, and progression of clinical characteristics at onset and throughout the disease course, when compared to the prototypical AHD population (**Chapter 4**).

Specifically, the cJHD population demonstrated: (1) the highest prevalence of neurocognitive deficits at onset, and, during the disease course (2) the most severe and rapid progression of specified motor and neurocognitive subclusters, (3) the highest occurrence of irritability, violence, and aggressive behavior, and (4) the highest prevalence of epilepsy. In contrast, the aJHD population exhibited: (I) the highest prevalence of psychiatric disturbances at onset, and, during the disease course, (II) more severe and faster progression of motor and neurocognitive subclusters compared to AHD, (III) the highest prevalence of apathy and psychosis, and (IV) the highest prevalence of pain interference with daily life. These distinct patterns of clinical characteristics underscore the necessity of stratifying JHD subtypes separately when compared to AHD. Moreover, our findings suggest that many clinical features align with CAG-repeat length or age at onset, while others appear to be influenced by the age at which specific clinical characteristics emerge, indicating moderating effects of brain maturation.

We revealed the correlation between clinical, radiological and neuropathological disease characteristics in an aJHD brain donor who died mid-stage disease (**Chapter 5**). Our findings indicate that a moderate clinical and functional disease stage, along with a short disease duration of 4 years, correlates with mild to moderate radiological and neuropathological disease characteristics which were most prominent in the putamen. Additionally, we emphasized the importance of conducting a comprehensive neuropathological evaluation, rather than relying solely on Vonsattel grade, as our analysis revealed that neuropathological changes were more comprehensive than can be appreciated by the Vonsattel grading system.

Lastly, we demonstrated diminished RNA and protein expression of glucose transporters and mitochondrial complexes in high-expansion cJHD brains and fibroblasts, compared to aJHD, AHD patient and healthy control material (**Chapter 6**). These findings suggest that glucose metabolism is impaired in high-expansion cJHD, a pattern that contrasts partially with aJHD and AHD. This indicates that distinct pathophysiological mechanisms may be at play in the high-expansion cJHD subtype, but not in other HD subtypes. Furthermore, patients with mutations in glucose transporter genes (e.g. GLUT1) exhibit disease characteristics like those of the cJHD population, such as developmental delay and epilepsy, which may help explain the atypical clinical features observed in the cJHD phenotype.

In the next part we will discuss these results in a broader overarching perspective and provide recommendations and future perspectives on (1) the definition of the JHD and PHD population, (2) practical implementations and (3) the pathophysiological framework and neurodevelopment.

DISCUSSION

Juvenile-onset and Pediatric Huntington Disease nomenclature and selection criteria

As mentioned in the introduction of this thesis, the definition of "JHD" is rather arbitrary and not bound to any obvious criteria such as unique disease characteristics or onset on pediatric (≤17) or adult (≥18) age. The additional definition "PHD" for cases between 0-17 years with clinically manifest HD was introduced to resolve regulatory issues in clinical trial design relating to manifest HD on pediatric vs adult age.[2] Our finding relating to the extremely low prevalence of PHD (**Chapter 2**) drives the awareness that conventional clinical trial design is not feasible in such a small patient population and urges regulatory authorities to the use of alternative trial designs.

Yet both the term JHD and PHD do not tell us anything concerning unique clinical characteristics in (part of) these populations, which troubles the selection of homogeneous patient populations and getting insight in pathophysiological differences between HD subtypes. There is no straightforward answer to how to optimally define JHD nomenclature and selection criteria. By dividing JHD patients into a childhood-onset (cJHD) and adolescent-onset (aJHD) phenotype, we have shown that both cJHD and aJHD as compared to AHD have distinct patterns in the occurrence, severity and progression of disease characteristics (**Chapter 4**). Age at onset is an useful measure to distinguish JHD subpopulations from AHD, as it relates to developmental stages relevant to disease expression. However, defining JHD based on age of onset is complicated by the different types of onsets (motor, psychiatric, neurocognitive). Relying on motor onset alone, as is often done in clinical trial designs, may exclude JHD cases where isolated non-motor symptoms appear first, which is seen in approximately 50% of JHD cases (**Chapter 4**). The introduction of the HD-ISS,[1] which includes neurocognitive assessments, partially addresses this issue, though up to now it is only validated for AHD cases. Also other selection criteria have been used to refer to sub-JHD populations sharing unique disease patterns. For instance "Highly-Expanded JHD" (HE-JHD, CAG-repeats ≥80), which progresses more rapidly with resulting shorter survival and prevalent epileptic seizures compared to "Low-Expansion" JHD cases (LE-JHD, CAG-repeats <80).[3] Additionally, we have shown that glucose transporters and mitochondrial complexes are selectively diminished in HE-JHD brain material compared to LE-JHD and AHD (**Chapter 6**). CAG-repeat length can explain much of the variability in

motor onset age,[4] but considerable overlap exists between cJHD, aJHD, and AHD, limiting its usability as a sole criterion for unique JHD subpopulations.

These studies reveal the need for accurate and internationally approved JHD subtype selection criteria to ensure valid methodology and reliable study results in the different JHD subtypes. Our study results suggest stratification is needed between JHD cases with (1) high expansions (≥80) or onset in childhood with (2) JHD cases that have lower expansions or onset in adolescence. To establish a both sensitive and specific stratification of JHD subtypes, all types of clinical onset should be considered, as well as the conditional and combined use of clinical severity markers (e.g. age onset, rate of progression), unique disease characteristics (e.g. epileptic seizures) and molecular disease markers (e.g. CAG-repeat length, somatic expansion index). International agreement and implementation of such selection criteria can be harbored via the established international JHD working group of the EHDN.

Practical implementations

Our findings of an extremely small PHD population (**Chapter 2**) and clinically distinct JHD population (**Chapter 2 and 4**), reveals the need for a tailored clinical and research approach, differing from standard HD practices. The practical implementation of this tailored approach influences types of research designs, collaborations, the validation and use of (clinical) assessment tools, and prediction models and, ultimately, the type of clinical care that is offered and implementation of therapeutic strategies. In the paragraph below several directives are offered in light of these practical implementations for future research.

Since the identification of the HTT gene in 1993,[5] substantial progress has been made in our understanding of HD disease characteristics and pathophysiology, with contributions from research organizations and patient advocacy groups driving funding, collaborations, and standardized tools. While JHD has benefited some of these advancements, the fundamental differences between JHD and AHD has been overlooked in key areas, particularly in the applicability of the Unified Huntington Disease Rating Scale (UHDRS). The UHDRS,[6,7] developed in 1996, is widely used to assess motor, neurocognitive, psychiatric, and functional symptoms of HD. Concerns about its applicability to JHD and PHD populations were raised over a decade ago,[8] yet no real advances have been made to modify and validate UHDRS scales to include the juvenile subtype. As our data reveals, the neurocognitive and

functional assessments in the UHDRS lack validity for JHD (**Chapter 2 and 4)**, hindering insights into affected cognitive domains and functional decline. Amongst other strategies, digital neurocognitive assessments and age-independent functional measures hold the promise of bridging this gap. A suitable UHDRS for the entire HD population – including JHD – is crucial to the necessity of including JHD patients in comparative studies alongside AHD, which in turn offers deeper insight into pathophysiological differences that may inform more tailored clinical care and therapeutic strategies.

Another overlooked topic in the JHD population is the use of prediction models for disease stage and progression (e.g., PIN,[9] CAP,[10] HD-ISS[1]). These models rely on a combination of clinical, functional, biomarker and molecular disease characteristics and are designed to properly identify candidates for clinical trials or patient materials for pre-clinical studies. So far, these prediction models are not validated for the JHD population, as is also demonstrated in this thesis by the CAP^{100} outcomes (**Chapter 2**). The lack of valid disease stage and progression markers in the JHD population hampers our insight into possible pathophysiological differences in AO-HD subtypes. Redesigning prediction models to include a somatic expansion index, quadratic CAG terms, interaction terms with smaller allele CAGs, and incorporating revised neurocognitive and functional assessments could improve their relevance and accuracy for the entire HD population.

In contrast to the invalidity of clinical and prediction markers for the JHD population, is the common use of HD disease models resembling a juvenile phenotype. To ensure early and prominent phenotypic disease, many insights in HD molecular mechanisms are based on mouse models carrying CAG-repeats in the extremely high range (CAG-repeats >100). Although useful to the JHD population, it remains to be seen if the same mechanisms are relevant to the entire HD spectrum or only to a small proportion of it, being HE-JHD. To substantiate the relevance of these molecular findings, comparison between CAG-repeat lengths in the mild (40-50), moderate (50-80) and severe (>80) range are needed. In turn this structural comparison between CAG-HD subtype models will benefit our understanding of pathomechanisms both in the classical adult-onset and JHD phenotypes.

Because of its rarity, broad international collaboration is another key aspect in moving the JHD research field forward. In this regard, significant progress has already been made by the sharing of knowledge and resources in JHD working

groups and by the foundation of the HDYO JOIN-HD registry.[11] However, there is still considerable potential to deepen and expand these efforts. For example, by standardizing clinical care assessments and adding these in multinational datasets, by allocating and sharing JHD patient materials and by sharing interim research findings on a larger scale. This way researchers have access to more diverse and larger JHD patient populations, which is crucial for improving the generalizability and robustness of study results. The exchange of interim findings between international teams can help accelerate the making of new research protocols and speed up the translation of new insights into clinical practice or therapeutic strategies.

A last topic worth addressing is clinical trial design and therapeutic strategies. With the extremely small number of PHD and JHD patients (**Chapter 2**), the design of conventional interventional trials is unrealistic. In our opinion, adopting flexible personalized approaches such as N=1 cross over designs and compassionate use programs with therapeutic agents tested in the AHD population should be strongly considered. Lastly, it may be worth reconsidering treatments that were unsuccessful in AHD trials but could potentially offer benefits for some JHD patients due to the different clinical course of the juvenile form. Re-testing these therapies in the JHD population might yield promising results, especially if the mechanisms of the disease in younger patients differ from those seen in adults.

JHD pathophysiology

HD pathophysiological framework

Since the recognition in 1993 that HD pathophysiology in general is triggered by a germline expansion of the CAG-repeat (≥36) in the *HTT* gene,[5] it has become evident in recent years that further somatic expansion of the CAG-repeat in mainly neuronal cells of the HD brain plays an important mediating factor in the disease mechanism.[12] This somatic expansion is influenced by the length of the germline CAG-repeat itself, as well as *cis*-acting loss of mHTT CAA interruption and *trans*-acting SNPs in DNA-repair genes.[13-15] Eventually, this process enters into a cascade of multi -spatial and -cellular degenerative and reactive processes, with the medium spiny neurons (MSNs) of the caudolateral basal ganglia to be the earliest and most severely affected.[16,17] Another emerged extension on this pathophysiological framework are the more recently acquired insights in neurodevelopmental alterations, which have been observed in several HD models, materials and even patient *in vivo* studies.[18-21]

Although not fully elucidated, HTT's role in neurodevelopment suggests that these aberrations might be caused by dominant-negative loss-of-function mechanisms. Hypotheses exist regarding how neurodevelopmental defects may contribute to the clinical picture in HD,[22] however, much remains to be understood in this regard.

Neurodevelopmental context

The recognition that JHD patients exhibit a distinct clinical phenotype compared to prototypical AHD – and how this relates to the pathophysiological framework outlined above – formed the foundation of this thesis. The prevailing hypothesis that HD pathophysiology, driven by the HTT-CAG-repeat expansion, follows one continuum, was challenged in this thesis by an alternative hypothesis: are there specific pathomechanisms that contribute differently or more significantly in the JHD population? An important consideration when analyzing differences between AO-HD subtypes, is the different neurodevelopmental context that patients are in when they experience HD symptoms (**Chapter 3**). Postnatal brain maturation is a physiological process that continues well into early adulthood. Whereas AHD patients generally have a fully matured brain when HD pathomechanisms succumb, in JHD patients' neurodevelopmental changes are still ongoing when pathomechanisms occur and are therefore prone to interaction. This interaction is likely contributing to distinct clinical disease outcomes when compared to AHD. In this context we speculated on contributing pathomechanisms and interacting processes on certain highly prevalent symptoms in JHD, being developmental alterations, epileptic seizures and psychosis/behavioral disorder **(Chapter 3**). In the following subparagraph we will draw hypotheses regarding contributing disease mechanisms and neurodevelopmental interaction based on some of our own study results, and offer future directions for research. Furthermore, we will highlight some opportunities for future studies based on others' work.

Hypotheses and future directions

Based on longitudinal clinical data in the 3 defined AO-HD subtypes, we predicted distinct patterns of severity and progression across sub-motor and neurocognitive domains in the AO-HD subtypes (**Chapter 4**). For the submotor domains parkinsonism and dystonia we visualized a pattern of early occurrence and more severe changes in early-onset phenotypes compared to AHD, but a similar rate of progression over time in the 3 AO-HD subtypes. In contrast, in submotor domains dysarthria, oculomotor and gait and balance specifically a faster rate of progression

was associated with early onset phenotypes. In the neurocognitive domain, the aJHD population was predicted to have a better initial performance, but both JHD subtypes were associated with a faster decline over time in psychomotor speed function compared to AHD. The predicted differences in severity and progression rates suggest that the predictors 'age at onset' and 'age at measurement' have a differential impact on sub-motor and neurocognitive clusters. Based on these predictions, one can hypothesize that an early and more severe clinical phenotype of parkinsonism and dystonia with similar progression rates is more likely to be influenced by early neurodevelopmental defects, whereas a faster progression over time of dysarthria, oculomotor, gait and balance and psychomotor speed is more likely to be caused by neurodegenerative pathomechanisms. Although it is a difficult task of answering such hypotheses in small patient populations, the value of post-mortem HD neuropathology studies may prove insightful in untangling the contribution of neurodegenerative vs neurodevelopmental pathomechanisms on certain predominant clinical features.

Another interesting area for future research is the underlying pathomechanism of psychosis. We showed that this disease characteristic is specifically more common in aJHD patients compared to cJHD and AHD (**Chapter 4**). Notably, the same age-prevalence distribution is seen for the onset of psychosis (DSM-5: Schizophrenia Spectrum and Other Psychotic Disorders) in the general population, with a primary psychotic episode often occurring during adolescence. As is suggested by this age predilection, the pathogenesis of psychotic disorders is thought to relate to a lack of physiological synaptic pruning on adolescent age causing overabundance of synaptic connections in the post pubertal brain.[23] Given this, one could speculate about potential common pathways underlying the onset of psychosis in HD. In particular the suggested interaction between HD pathomechanisms in JHD and ongoing neurodevelopmental processes, such as synaptic pruning, provides an interesting hypothetical framework for future studies. Morphological and quantitative analysis of cell populations in post-mortem brain tissue from different AO-HD subtypes at various ages may offer insights into these mechanisms.

Furthermore, we have shown that the glucose receptor GLUT1 and mitochondrial complexes are specifically downregulated in high-expansion cJHD brains when compared to lower expansion aJHD and AHD brains (**Chapter 6**). These findings suggest that high-expansion cJHD patients may suffer brain glucose hypometabolism, which makes an interesting new investigational target for future

studies. The notion that the clinical phenotype cJHD partially overlaps with GLUT1-deficiency syndrome may imply an effect of glucose hypometabolism on symptoms like epilepsy. Analyzing glucose in blood and CSF and or ^{18}F-FDG PET imaging in cJHD patients can offer insight in the relation between epilepsy and brain glucose metabolism.

A particularly informative study in the context of developmental neural circuitry characteristics formation is the KIDS-HD study, which, among other outcomes, investigates fMRI-based functional circuitries in HD-Expanded Gene Carrier (HDEGC) minors who are decades removed from disease onset.[20,24] Their findings have provided valuable insights in spatial remodeling of functional circuitries that may compensate for early disease mechanisms in the brains of children and adolescent who are destined to develop HD clinical characteristics later in life. Although functional circuitry alterations can also relate to the presence of clinical symptoms, up to now, these results do not teach us anything on the relationship between AO-HD subtypes and manifest clinical characteristics. Yet it holds promise for an alternative study design in which manifest JHD and AHD patients are compared in relation to the occurrence of specific symptoms, such as epileptic seizures. This way it could address questions related to neural circuitry functionality across different AO-HD subtypes and in relation to clinical symptoms.

Finally, another avenue of future research would be the relationship between the HTT interactome and age. In many Mendelian inherited disorders, complex genotype-phenotype relationships are likely to involve abnormal multi-omic interactions between the disease-causing gene and other genes. While several studies have investigated perturbed interactions of (m)HTT in relation to various pathophysiological aspects of HD,[25-27] the relationship between the multi-omic HTT interactome and age has not yet been explored. Investigating this relationship could provide valuable information regarding age-related phenotypes in HD. Open-access resources, such as the Allen Brain Atlas, offer valuable data on the transcriptome of the developing brain, which could help address these questions.

CRITICAL LIMITATIONS

While this thesis provides novel insights into the clinical, molecular, and pathophysiological characteristics of JHD and PHD, several limitations must be acknowledged. The extreme rarity of these populations resulted in small sample

sizes, limiting statistical power and generalizability. The retrospective and cross-sectional study designs introduce potential biases due to incomplete longitudinal data and the limited validity of assessment tools such as the UHDRS in juvenile populations. A further limitation concerns the insufficient consideration of disease progression as a mediating factor in the analysis of AO-HD subtypes, potentially obscuring dynamic interactions between age at onset, CAG-repeat length, and evolving clinical phenotypes. Lastly, methodological variability between institutions and registries may have introduced inconsistencies in data collection and classification. Future studies in JHD and PHD research will continue to face challenges related to population size, data harmonization, and model validity. Overcoming these will require international collaboration, standardized diagnostic and assessment frameworks, longitudinal study designs, and the development of age-appropriate clinical and molecular markers to ensure reproducibility and translational relevance.

CONCLUDING REMARKS

The findings of this thesis emphasize the need for a tailored approach to conduct research in JHD and PHD, which differs from the standard practices for AHD. Several factors support this conclusion. First, the JHD and PHD populations are small and clinically distinct. The small population size makes traditional clinical trials difficult, while the unique clinical characteristics of JHD require a more personalized approach. Second, current assessment tools and prediction models are not validated for JHD and PHD, hampering the accurate assessment of disease progression. Third, the interaction between HD pathophysiology and the ongoing brain development in JHD and PHD requires special attention. The disease affects a developing brain, which likely contributes to the distinct clinical presentation compared to AHD. Future research should focus on (1) re-developing and validating assessment tools and prediction models to include the JHD and PHD populations, thereby enabling structural comparison of AO-HD subtypes, (2) further investigating the different pathophysiological mechanisms in JHD, particularly in the cJHD subgroup, (3) Studying the interaction between HD pathophysiology and brain development in JHD and PHD, and (4) considering flexible, personalized treatment approaches, such as N=1 cross-over designs and compassionate use programs. By following these recommendations, we can improve the care of JHD and PHD patients and hopefully pave the way for more effective treatments.

REFERENCES

1. Tabrizi SJ, Schobel S, Gantman EC, et al. A biological classification of Huntington's disease: the Integrated Staging System. *Lancet Neurol*. Jul 2022;21(7):632-644. doi:10.1016/S1474-4422(22)00120-X
2. 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
3. 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
4. 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
5. 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.
6. Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. *Mov Disord*. Mar 1996;11(2):136-42. doi:10.1002/mds.870110204
7. Callaghan J, Stopford C, Arran N, et al. Reliability and factor structure of the Short Problem Behaviors Assessment for Huntington's disease (PBA-s) in the TRACK-HD and REGISTRY studies. *The Journal of neuropsychiatry and clinical neurosciences*. Winter 2015;27(1):59-64. doi:10.1176/appi.neuropsych.13070169
8. Brewer HM, Barker, Roger A., Quarrell OWJ. Challenges in assessment. In: Quarrell OWJ, Brewer HM, Squitieri F, Barker RA, Nance MA, Landwehrmeyer GB, eds. *Juvenile Huntington's Disease: and other trinucleotide repeat disorders*. Oxford University Press; 2009:0.
9. Langbehn DR, Fine EM, Meier A, Hersch S. Utility of the Huntington's Disease Prognostic Index Score for a Perimanifest Clinical Trial. *Mov Disord*. May 2022;37(5):1040-1046. doi:10.1002/mds.28944
10. Warner JH, Long JD, Mills JA, et al. Standardizing the CAP Score in Huntington's Disease by Predicting Age-at-Onset. *J Huntingtons Dis*. 2022;11(2):153-171. doi:10.3233/JHD-210475
11. Mason R, Byrne L, Papoutsi M, et al. F12 Join-HD: the juvenile-onset initiative for Huntington's disease. BMJ Publishing Group Ltd; 2022.
12. Handsaker RE, Kashin S, Reed NM, et al. Long somatic DNA-repeat expansion drives neurodegeneration in Huntington's disease. *Cell*. 2025;188(3):623-639. e19.
13. Handsaker RE, Kashin S, Reed NM, et al. Long somatic DNA-repeat expansion drives neurodegeneration in Huntington disease. *bioRxiv*. 2024:2024.05.17.592722. doi:10.1101/2024.05.17.592722
14. 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
15. 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
16. 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

17. 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
18. 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
19. 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
20. van der Plas E, Langbehn DR, Conrad AL, et al. Abnormal brain development in child and adolescent carriers of mutant huntingtin. *Neurology*. Sep 3 2019;93(10):e1021-e1030. doi:10.1212/wnl.0000000000008066
21. Scahill RI, Zeun P, Osborne-Crowley K, et al. Biological and clinical characteristics of gene carriers far from predicted onset in the Huntington's disease Young Adult Study (HD-YAS): a cross-sectional analysis. *Lancet Neurol*. Jun 2020;19(6):502-512. doi:10.1016/S1474-4422(20)30143-5
22. 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
23. Sakai J. Core Concept: How synaptic pruning shapes neural wiring during development and, possibly, in disease. *Proc Natl Acad Sci U S A*. Jul 14 2020;117(28):16096-16099. doi:10.1073/pnas.2010281117
24. Tereshchenko AV, Schultz JL, Bruss JE, Magnotta VA, Epping EA, Nopoulos PC. Abnormal development of cerebellar-striatal circuitry in Huntington disease. *Neurology*. May 5 2020;94(18):e1908-e1915. doi:10.1212/wnl.0000000000009364
25. Estevez-Fraga C, Altmann A, Parker CS, et al. Genetic topography and cortical cell loss in Huntington's disease link development and neurodegeneration. *Brain*. Nov 2 2023;146(11):4532-4546. doi:10.1093/brain/awad275
26. Wanker EE, Ast A, Schindler F, Trepte P, Schnoegl S. The pathobiology of perturbed mutant huntingtin protein-protein interactions in Huntington's disease. *J Neurochem*. Nov 2019;151(4):507-519. doi:10.1111/jnc.14853
27. Bonsor M, Ammar O, Schnoegl S, Wanker EE, Silva Ramos E. Polyglutamine disease proteins: Commonalities and differences in interaction profiles and pathological effects. *Proteomics*. Jun 2024;24(12-13):e2300114. doi:10.1002/pmic.202300114

APPENDICES

Nederlandse samenvatting

Dankwoord

List of publications

List of abbreviations

Curriculum Vitae

NEDERLANDSE SAMENVATTING

In dit proefschrift worden de resultaten gepresenteerd van onderzoek naar de kinder- en jeugdvorm van de ziekte van Huntington (Juvenile-onset Huntington Disease, JHD). De ziekte van Huntington (HD) is een zeldzame erfelijke hersenziekte die autosomaal dominant overerft. Dit houdt in dat ieder kind van een ouder met HD 50% kans heeft om de ziekte ook te krijgen. In Nederland hebben ongeveer 1000 mensen ziekteverschijnselen van HD. De genetische oorzaak van HD ligt in het Huntingtine-gen (HTT) op chromosoom 4, waarbij een verlengde herhaling van de DNA-sequentie Cytosine-Adenine-Guanine (CAG) verantwoordelijk is voor het ontstaan van de ziekte. De lengte van deze CAG-herhaling kan over generaties toenemen, wat leidt tot een begin van de ziekte op jongere leeftijd. De meeste mensen met HD ervaren de eerste symptomen tussen de 30 en 50 jaar, maar er zijn ook patiënten bekend die al op peuterleeftijd of pas op oudere leeftijd (boven de 80 jaar) ziekteverschijnselen ontwikkelden. De ziekteverschijnselen kenmerken zich door achteruitgang van de motoriek en cognitie (denkvermogen) en door psychiatrische stoornissen. HD is een progressieve ziekte, wat inhoudt dat ziekteverschijnselen toenemen naarmate de ziekte voortduurt. Hierdoor worden mensen met HD toenemend afhankelijk van anderen. De gemiddelde ziekteduur is 17 tot 20 jaar. Alhoewel sommige ziekteverschijnselen kunnen worden behandeld met medicatie (zoals depressie of overbeweeglijkheid), is er op dit moment nog geen medicatie beschikbaar die de ziekteprogressie kan vertragen of zelfs stoppen.

In de introductie van dit proefschrift (**hoofdstuk 1**) wordt een kader geschetst van bekende genetische en klinische aspecten van HD, de veronderstelde ziektemechanismen en hoe deze zich verhouden tot de kinder- en jeugdvorm van HD. JHD betreft patiënten die de eerste symptomen vóór of op de leeftijd van 20 jaar ontwikkelen en komt voor bij 1 tot 5% van alle HD-patiënten. De genetische oorzaak van JHD is identiek aan die van volwassen HD, namelijk een verlengde CAG-herhaling in het HTT-gen, maar bij JHD-patiënten is deze herhaling doorgaans langer. Daarnaast zijn er tussen JHD en volwassen HD-patiënten verschillen waar te nemen in het (1) type en de ernst van de ziekteverschijnselen (2) de ziekteduur en (3) de ernst van afwijkingen die in de hersenen kunnen worden waargenomen. Omdat er momenteel geneesmiddelenonderzoek wordt gedaan bij volwassen HD-patiënten, is het belangrijk om de unieke kenmerken van JHD beter te begrijpen, zodat gerichte behandelingen ontwikkeld kunnen worden. Dit omvat het identificeren van het aantal beschikbare JHD-patiënten voor klinische studies en het verbeteren van ons begrip van de verschillen in de ziekteprogressie en hersenafwijkingen tussen

JHD en patiënten met eerste klachten op volwassen leeftijd (AHD). Daarnaast moet er rekening worden gehouden met de invloed van hersenontwikkeling, aangezien JHD zich voordoet in een nog ontwikkelend brein.

In **hoofdstuk 2** worden de resultaten weergegeven van onderzoek naar het aantal JHD-patiënten in Nederland dat beschikbaar is voor geneesmiddelenonderzoek. Daarnaast is er gekeken naar de ernst van de ziekte wanneer de diagnose JHD wordt gesteld, omdat dit inzicht geeft in het vermogen van JHD-patiënten om deel te nemen aan geneesmiddelenonderzoek. De HD onderzoeksgroep van het LUMC heeft in het kader van dit onderzoek in 2020 een register geopend waarin genetische -, klinische -, en persoonsgegevens worden verzameld van JHD patiënten in Nederland, met de naam HD-JUNIOR. Daarnaast is er voor dit onderzoek gebruik gemaakt van een internationaal register waarin gegevens worden verzameld van ruim 20.000 HD-patiënten wereldwijd (ENROLL-HD). Uit dit onderzoek bleek dat er begin 2024 in totaal 9 levende JHD-patiënten in Nederland (NL) waren en dat dit 0.84 tot 1.25% bedroeg van de gehele HD patiënten populatie in NL. Daarnaast was er 1 levende JHD-patiënt die op dat moment nog jonger dan 18 jaar was, dit wordt Pediatrische HD (PHD) genoemd, wat 0.09 tot 0.14% bedroeg van de gehele HD-populatie in NL met ziekteverschijnselen. Tussen 2000 en 2020 werden in NL iedere 5 jaar gemiddeld 4 patiënten met JHD gediagnosticeerd. Tussen het moment van eerste ziekteverschijnselen en diagnose zat gemiddeld 4 jaar, wat ervoor zorgde dat in 55% van JHD gevallen de diagnose pas werd gesteld op volwassen leeftijd. In deze gevallen kon alleen achteraf worden vastgesteld dat de eerste ziekteverschijnselen al op kinder- of jongerenleeftijd aanwezig waren. Verder werd aangetoond dat 92% van JHD-patiënten in NL al functionele beperkingen had op het moment dat ze werden gediagnosticeerd. Ook waren jongeren met JHD minder zelfstandig op het moment van diagnose dan volwassen HD-patiënten. Bovendien bleek een veelgebruikte maat voor ziekteprogressie bij volwassen HD-patiënten (CAP^{100}) niet geschikt voor gebruik bij JHD. De conclusies uit dit onderzoek zijn, dat de JHD-populatie in Nederland kleiner is dan eerder werd aangenomen en dat de vertraging in diagnose en functionele beperkingen de beschikbaarheid voor deelname aan geneesmiddelenonderzoek aanzienlijk beïnvloedt.

In **hoofdstuk 3** worden de resultaten van literatuuronderzoek naar verschillen tussen JHD en volwassen HD uitgelicht en wordt er een perspectief geboden om deze verschillen te verklaren. Behalve dat er een relatie wordt gelegd tussen ziekteverschijnselen en de geobserveerde afbraak van hersencellen, wordt er ook een relatie gelegd tussen ziekteverschijnselen en een afwijkende hersenontwikkeling. Deze afwijkende hersenontwikkeling kan tweeledig zijn. Aan de ene kant kan

hersenontwikkeling afwijkend zijn door de onderliggende genetische afwijking in het HTT gen, aan de andere kant doordat afbraak van hersencellen de normale hersenontwikkeling – die nog in volle gang is bij JHD-patiënten – kan verstoren. Het is belangrijk om beiden mogelijkheden voor ogen te houden wanneer de JHD-populatie wordt onderzocht en wordt vergeleken met volwassen HD.

In **hoofdstuk 4** worden de prevalentie, ernst en progressie van ziekteverschijnselen vergeleken tussen JHD-patiënten met eerste verschijnselen op de kinderleeftijd (cJHD, tot en met 10 jaar), adolescentie (aJHD, 11-20 jaar) en volwassen HD (AHD). Voor dit onderzoek is er gebruik gemaakt van gegevens uit het Nederlandse JHD register (HD-JUNIOR) en het internationaal register waarin gegevens worden verzameld van ruim 20.000 HD patiënten wereldwijd (ENROLL-HD). Het onderzoek toonde aan dat, in vergelijking met AHD, cJHD vaker gepaard gaat met cognitieve problemen en aJHD met psychiatrische symptomen als eerste uiting van de ziekte. Gedurende de ziekte werden psychiatrische symptomen, zoals prikkelbaarheid en agressie, vaker geobserveerd bij JHD-patiënten in vergelijking tot AHD. Bij aJHD kwam specifiek initiatiefloosheid (apathie) en psychose vaker voor in vergelijking met AHD. Ook werd een hogere mate van epilepsie en pijn beschreven bij JHD-patiënten in vergelijking met AHD. Met behulp van voorspellingsmodellen werd aangetoond dat motorische symptomen in relatie tot spraak (dysartrie), stijfheid (parkinsonisme) en afwijkende spieraanspanning (dystonie) het ernstigst waren in cJHD, gevolgd door aJHD, in vergelijking met AHD. Daarnaast werd een versnelde progressie van spraak-, loop-, balans- en oogbewegingsstoornissen waargenomen bij JHD in vergelijking met AHD. In contrast, overbeweeglijkheid (chorea) was minder ernstig in cJHD, gevolgd door aJHD, in vergelijking met AHD. Verder werd aangetoond dat de achteruitgang van psychomotorische snelheid sneller verloopt bij cJHD, gevolgd door aJHD, in vergelijking met AHD. De conclusie is dat er duidelijke verschillen zijn tussen cJHD, aJHD en AHD in zowel de presentatie als de progressie van de ziekte, wat belangrijke implicaties heeft voor toekomstig onderzoek en de zorg voor deze patiënten.

In **hoofdstuk 5** wordt een aJHD casus beschreven die na een relatief korte ziekteduur kwam te overlijden, en de hersenen beschikbaar heeft gesteld voor onderzoek. In dit onderzoek werd de ziekte-ernst en -duur in relatie gebracht tot de geobserveerde hersenafwijkingen, via radiologisch en pathologisch onderzoek. Voor dit onderzoek werd er na overlijden beeldvorming verricht van de hersenen in een 7T MRI-scanner, waarna de hersenen uit werden genomen voor pathologisch onderzoek. Uit dit onderzoek kwam naar voren, dat er sprake was van een matige ziekte ernst bij overlijden. Beeldvormend onderzoek toonde volume verlies van de hersenen, voornamelijk van

het putamen. Bij pathologisch onderzoek was er sprake van een voor de leeftijd normaal hersengewicht en waren er op het oog geen afwijkingen te zien. Met behulp van de microscoop werd er matige hersenschade en celverlies geconstateerd, vooral in het putamen, wat slechts een Vonsattel graad 1 opleverde. Deze gradering houdt in dat de pathologische afwijkingen passend bij HD mild waren. Uit deze studie kunnen we concluderen dat de matige ziekte ernst en korte ziekteduur bij deze aJHD patiënt in relatie staat tot matig celverlies, meest uitgesproken in het putamen, en dat die het beste waar te nemen is met behulp van beeldvormend onderzoek. Verder kunnen we concluderen dat het belangrijk is om niet alleen de Vonsattel graad te beoordelen, maar ook het totale beeld, omdat de pathologische afwijkingen uitgebreider waren dan op basis van de Vonsattel graad kon worden vastgesteld.

In **hoofdstuk 6** worden de resultaten van onderzoek naar de expressie en lokalisatie van glucose transporters in HD-materiaal beschreven. Voor dit onderzoek werd er gebruik gemaakt van hersenmateriaal en huidbiopten van een aantal cJHD, aJHD, AHD en gezonde controle donoren. Er werd gekeken naar de expressie en lokalisatie van de twee glucose transporters, GLUT1 en GLUT3, en naar de expressie van Rab11-A, mitochondriële complexen en hexokinase-II. Uit dit onderzoek kwam naar voren dat GLUT1 expressie verlaagd was in de frontaalkwab, maar niet het striatum, van cJHD hersendonoren in vergelijking met aJHD, AHD en gezonde controle hersendonoren. Dit terwijl het celverlies in de frontaalkwab van de cJHD donoren minder uitgesproken was dan afwijkingen in het striatum. Daarnaast werd er in cJHD hersenen verminderde expressie van GLUT3, Rab11-A, mitochondriële complexen en hexokinase-II aangetoond in verschillende regio's en in vergelijking met verschillende fenotypes (aJHD, AHD en gezonde controles). Uit deze studie kunnen we concluderen dat in cJHD de opname van glucose in het brein aangetast lijkt en dat dit geassocieerd is met afwijkingen in mitochondriële complexen (die zorgen voor de energievoorziening van de cel). Dit is deels in contrast met wat in aJHD en AHD-hersenen werd gezien. Het is opvallend dat een deel van de atypische ziekteverschijnselen bij cJHD, zoals epilepsie en een ontwikkelingsachterstand, ook worden gezien bij mensen die een genmutatie hebben in het GLUT1-gen. Dit zou erop kunnen wijzen dat deze ziekteverschijnselen in de cJHD populatie samenhangen met een defect in glucose metabolisme.

In de discussie van dit proefschrift (**Hoofdstuk 7**) wordt een samenvatting gegeven van de onderzoeksbevindingen uit hoofdstuk 2 tot en met 6 en hierna een discussie gevoerd met suggesties voor toekomstig onderzoek aan de hand van 3 onderwerpen: (1) JHD en PHD-naamgeving, (2) praktische implementaties en (3) een pathofysiologisch kader in samenhang met de hersenontwikkeling in de jeugd.

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LIST OF PUBLICATIONS

1. **Bakels HS**, van der Zwaan KF, Van Zwet E, Reijntjes R, Sprenger GP, Knecht TA, et al. Comparison of the Clinical Spectrum of Juvenile- and Adult-Onset Huntington Disease: A National Cohort and Enroll-HD Observational Study. Neurology. 2025;104(10):e213525.

2. **Bakels HS**, Feleus S, Rodriguez-Girondo M, Losekoot M, Bijlsma EK, Roos RAC, et al. Prevalence of juvenile-onset and Pediatric Huntington's Disease and Their Availability and Ability to Participate in Trials: A Dutch Population and Enroll-HD Observational Study. J Huntingtons Dis. 2024;13(3):357–68.

3. Sprenger GP, van Zwet EW, **Bakels HS**, Achterberg WP, Roos RA, de Bot ST. Prevalence and burden of pain across the entire spectrum of Huntington's disease. J Neurol Neurosurg Psychiatry. 2024;95(7):647–55.

4. Tramutola A, **Bakels HS**, Perrone F, Di Nottia M, Mazza T, Abruzzese MP, et al. GLUT-1 changes in paediatric Huntington disease brain cortex and fibroblasts: an observational case-control study. EBioMedicine. 2023;97:104849.

5. **Bakels HS**, Feleus S, van Dis V, de Bot ST. More than a co-incidence? Comment on: Amyotrophic lateral sclerosis is over-represented in two Huntington's disease brain bank cohorts: further evidence to support genetic pleiotropy of pathogenic HTT gene expansion. Acta Neuropathol. 2023;145(2):257–8.

6. **Bakels HS**, van Duinen SG, de Bresser J, van Roon-Mom WMC, van der Weerd L, de Bot ST. Post-mortem 7T MR imaging and neuropathology in middle stage juvenile-onset Huntington disease: A case report. Neuropathol Appl Neurobiol. 2023;49(1):e12858.

7. **Bakels HS**, Roos RAC, van Roon-Mom WMC, de Bot ST. juvenile-onset Huntington Disease Pathophysiology and Neurodevelopment: A Review. Mov Disord. 2022;37(1):16–24.

8. Arkink EB, Schoonman GG, van Vliet JA, **Bakels HS**, Sneeboer MA, Haan J, et al. The cavernous sinus in cluster headache - a quantitative structural magnetic resonance imaging study. Cephalalgia. 2017;37(3):208–13.

9. Bugiani M, Kevelam SH, **Bakels HS**, Waisfisz Q, Ceuterick-de Groote C, Niessen HW, et al. Cathepsin A-related arteriopathy with strokes and leukoencephalopathy (CARASAL). Neurology. 2016;87(17):1777–86.

10. Klok MD, **Bakels HS**, Postma NL, van Spaendonk RM, van der Knaap MS, Bugiani M. Interferon-alpha and the calcifying microangiopathy in Aicardi-Goutieres syndrome. Ann Clin Transl Neurol. 2015;2(7):774–9.

11. Dubbink HJ, **Bakels H**, Post E, Zwarthoff EC, Verdijk RM. TERT promoter mutations and BRAF mutations are rare in sporadic, and TERT promoter mutations are absent in NF1-related malignant peripheral nerve sheath tumors. J Neurooncol. 2014;120(2):267-72.

LIST OF ABBREVIATIONS

AHD	Adult-onset Huntington Disease
AO-HD	Age at Onset-defined Huntington Disease
aJHD	Adolescent-onset Juvenile Huntington Disease
BDNF	Brain-Derived Neurotrophic Factor
CAP100	CAG-Age Product (normalized at 100)
CAG	Cytosine-Adenine-Guanine
CAA	Cytosine-Adenine-Adenine
cJHD	Childhood-onset Juvenile Huntington Disease
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
EMA	European Medicines Agency
ENROLL-HD	International Prospective Longitudinal Registry Study in Huntington's Disease
FAN1	Fanconi Anemia-Associated Nuclease 1
HD	Huntington Disease
HD-JUNIOR	Dutch Registry for Juvenile Huntington Disease
HDEGC	HD Expanded Gene Carriers
HTT	Huntingtin (gene)
IQR	Interquartile Range
JHD	Juvenile Huntington Disease
mHTT	Mutant Huntingtin
MLH1	MutL Homolog 1
MRI	Magnetic Resonance Imaging
MSH3	MutS Homolog 3
PDS5	5th Periodic Dataset of Enroll-HD
PHD	Pediatric Huntington Disease
polyQ	Polyglutamine
PRD	Proline-Rich Domain
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism

STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TFC	Total Functional Capacity
UHDRS	Unified Huntington Disease Rating Scale
UHDRS-FAS	Unified Huntington Disease Rating Scale – Functional Assessment Scale
UHDRS-IS	Unified Huntington Disease Rating Scale – Independence Score
UHDRS-TFC	Unified Huntington Disease Rating Scale – Total Functional Capacity

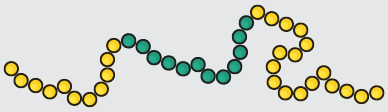
CURRICULUM VITAE

Hannah Stéphanie Bakels was born on October 28, 1988, in Berkel-Enschot, the Netherlands. She completed her pre-university education (Atheneum) on the St. Odulphus Lyceum in Tilburg before earning a Bachelor's degree in Psychology from Leiden University in 2011. Following this, she pursued a Research Master in Neurosciences, specializing in Clinical Neurosciences, at VU University Amsterdam, which she completed in 2013. In the same year, she joined the Zigma Graduate Entry Program at VU Medical Center, where she studied Medicine, graduating with a Medical Degree in 2017. During her studies, Hannah completed various research internships in neurology (trigeminal autonomic cephalalgias), pediatric neurology (white matter diseases), and neuro-oncology (malignant peripheral nerve sheath tumors). She also gained (extra)curricular clinical experience in pediatrics and pathology.

In 2017, Hannah began her professional career as a resident physician in clinical genetics, focusing on onco- and neurogenetics, at Amsterdam University Medical Center. Her interest in genetic brain disorders led her to apply for a research physician/PhD position in the Huntington's Disease (HD) research team at Leiden University Medical Center (LUMC) in 2019.

At LUMC, Hannah worked extensively with HD patients in both clinical and research settings. She was a sub-investigator in several clinical trials, including ENROLL-HD, TRIHEP3, GENERATION-HD1, PROOF-HD, PIVOT-HD, and VICO-SCA. Her PhD work included establishing the national Juvenile Huntington Disease (JHD) registry, HD-JUNIOR, and coordinating collaborative research projects within LUMC and internationally with JHD expert Ferdinando Squitieri. She also contributed to the restructuring of the work flow with HD brain material in the LUMC and actively participated in the European Huntington Disease Network's JHD working group.

In 2019, Hannah married Pieter, and in 2021, they welcomed their son, Ben. In May 2024, she began a clinical residency in Pathology at LUMC, with the goal of specializing in neuropathology and brain bank research, while continuing her work in HD research.



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