Juvenile Huntington Disease: towards better understanding its unique disease characteristics

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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.

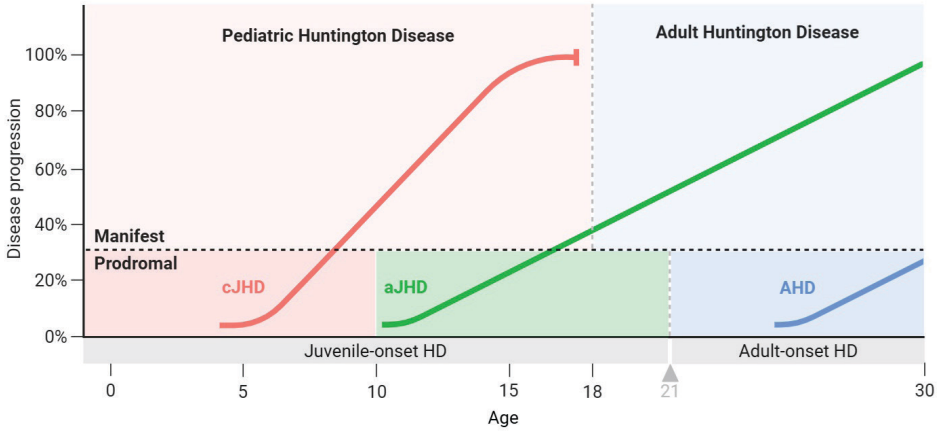

Figure 1. Graphic illustration for Pediatric and Age at Onset-defined HD subtypes.

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.

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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)**.

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