Univerzita Karlova
1. lékařská fakulta

Autoreferát disertační práce

UNIVERZITA KARLOVA
1. lékařská fakulta

Clinical Applicability of Cognitive Testing in Huntington Disease

Využití kognitivích testů u Huntingtonovy nemoci v klinické praxi

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2022

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1.lékařské fakulty.

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Abstrakt

Úvod: Huntingtonova nemoc (HN) je dědičné neurodegenerativní onemocnění projevující se poruchami hybnosti, chování a kognitivním deficitem s fatálními následky.

Cíle: Cílem této studie bylo ověřit psychometrické vlastnosti standardní kognitivní baterie používané u HN a stanovit jazykově specifické normativní hodnoty.

Soubor a metodika: V první studii byl porovnáván kognitivní výkon u 106 pacientů v různých stádiích HN a u 100 zdravých kontrol párovaných podle věku, pohlaví a vzdělání. Neuropsychologická baterie zahrnovala Test modalit čísel a symbolů (SDMT), Stroopův test, Test cesty, kategoriální a fonemickou verbální fluenci. U pacientů byl navíc komplexně hodnocen motorický a funkční stav.

Ve druhé studii byl hodnocen kognitivní výkon 3 267 zdravých osob. Práce byla zaměřena na stratifikaci kognitivního výkonu v souvislosti s věkem, pohlavím, rodným jazykem a vzděláním. Dalším cílem bylo na základě získaných dat vytvořit normativní kalukátor pro hodnocení míry kognitivního deficitu v různých jazycích (angličtina, němčina, španělština, italština, polština, francouzština, nizozemština, dánština).

Výsledky: V první studii analýza rozptylu ukázala, že zdravé kontroly dosahovaly významně lepších výsledků než pacienti. Kognitivní výkon koreloval s motorickým a funkčním postižením ($p < 0,001$) nezávisle na věku a délce trvání onemocnění.

Normativní studie prokázala významný nelineární pokles kognitivního výkonu s postupujícím věkem u zdravých osob. Kognitivní výkon ve všech testech byl významně lepší u lidí, kteří dosáhli vyššího vzdělání. Kromě toho byly ve všech testech zjištěny jazykově podmíněné rozdíly v kognitivní výkonu. Z hlediska pohlaví byl zaznemenán u žen trend k lepšímu výkonu v SDMT.

Závěr: Výsledky obou studií poskytují normativní data pro kognitivní baterii specifickou pro HN, která jsou stratifikovaná podle věku, vzdělání, pohlaví a jazyka. Získané normativní hodnoty byly využity pro vytvoření volně přístupného webového kalkulátoru, který umožňuje využití v klinické i výzkumné praxi.

Klíčová slova: Huntingtonova nemoc, Jednotná škála Huntingtonovy nemoci, neuropsychologická testová baterie, kognitivní výkon, normativní data, normativní kalkulačka.

Abstract

Introduction: Huntington disease (HD) is an autosomal dominant neurodegenerative disorder manifested by motor, behavioural and cognitive deficits with fatal consequences.

Aims: This study aims to validate the psychometric properties of a standard cognitive battery used in HD and establish language-specific normative values.

Methods: In the first study, cognitive performance was compared in 106 patients at different stages of HD and 100 healthy controls matched for age, sex, and education. The neuropsychological battery included the Symbol Digit Modalities Test, Stroop Word Reading Test, Stroop Colour Naming Test, Stroop Interference Test, Trail Making Test-A and B, Category and Letter Verbal Fluency. In addition, patients were comprehensively assessed for motor and functional status.

In the second study, the cognitive performance of 3,267 healthy subjects was assessed. The work focused on the stratification of cognitive performance concerning age, gender, language and level of education. Another aim was to establish the language-specific normative values and implement a web-based normative calculator to assess the degree of cognitive deficit in different languages (English, German, Spanish, Italian, Polish, French, Dutch, Danish).

Results: In the first study, analysis of variance showed that healthy controls performed significantly better than patients on all cognitive tests. Cognitive performance was correlated with motor and functional impairment ($p < 0.001$) independent of age and disease duration.

A normative study showed a significant nonlinear decline in cognitive performance with advancing age in healthy subjects. Cognitive performance in all tests was significantly better in subjects who had attained higher education levels. In addition, language-related differences in cognitive performance were found in all tests. In terms of gender, there was a trend towards better performance on the SDMT for females.

Conclusion: The results of both studies provide normative data for a specific cognitive battery stratified by age, education, gender, and language to be used in the field of HD and other neurodegenerative diseases. The normative values obtained were used to create a freely available web-based calculator that allows for clinical and research practice use.

Keywords: Huntington disease, Unified Huntington's Disease Rating Scale, neuropsychological test battery, cognitive performance, normative data, normative calculator.

I. Introduction

Huntington disease (HD) has a severe and profound impact on affected patients and families. It is a hereditary disease usually in middle age and changes how the affected person moves, speaks, thinks, feels, and behaves. It is a fatal, though slowly progressive, disease. The name Huntington disease (HD) is taken from the American physician George Huntington, who published a paper in 1872 that established the hereditary nature of the disease (Huntington, 1872).

1. Genetics of Huntington Disease

The responsible gene for HD was identified in 1993, making an unstable and dynamic pathologic expansion of the cytosine-adenine-guanine (CAG) repeat within the first exon of the gene huntingtin (HTT, OMIM 613004) on the short arm of chromosome 4p16.3 Huntington's disease Collaborative Research, 1993). The *HTT* gene consists of a chain of numerous CAG repeats. For an overview, see Fig.1.

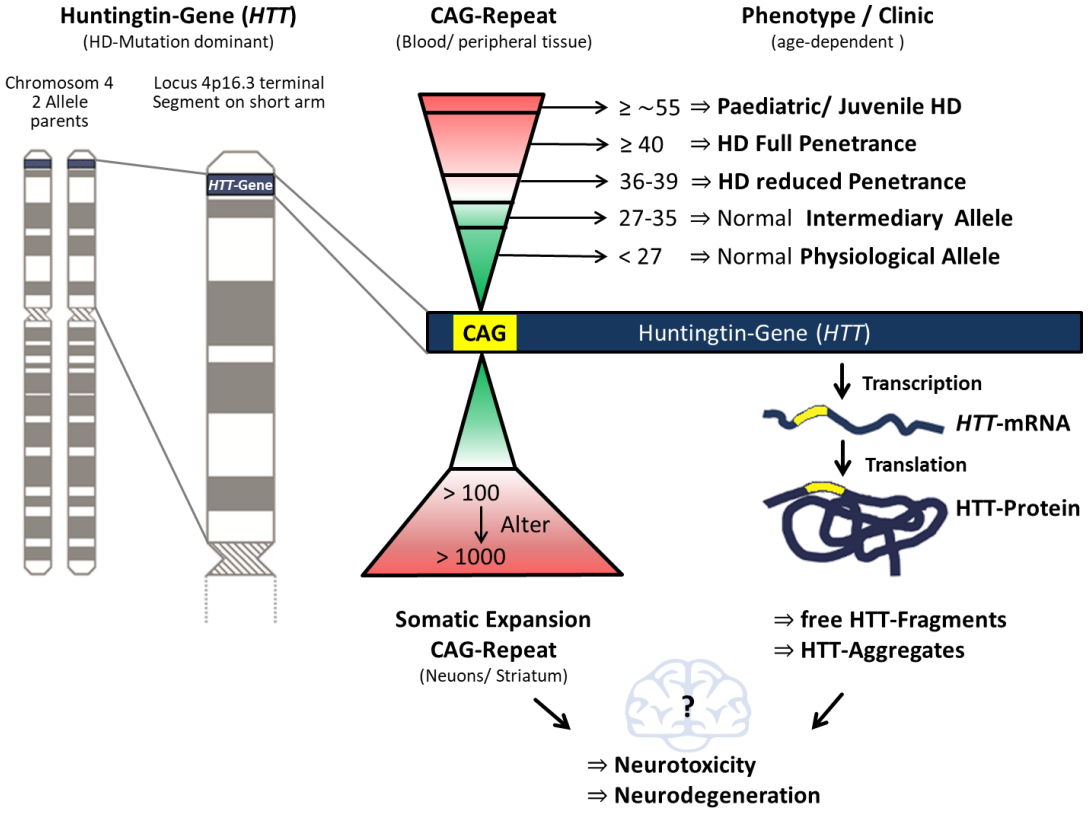

Figure 1: Huntingtin gene structure and genetic mechanism

The number of repeats in *HTT* is inversely associated with disease onset, such that the greater the number, the earlier the onset (Snell et al., 1993, Kieburtz et al., 1994). The onset of the disease is defined as the manifestation of significant motor or neurologic symptoms and occurs on average around 40 (Roos, 2010). The CAG length is also associated with the clinical progression. Individuals with shorter CAG expansions appear to have a better prognosis (Rosenblatt et al., 2006). The number of CAG repeats within the normal allele does not influence the age of onset in Huntington disease (Klempíř et al., 2011).

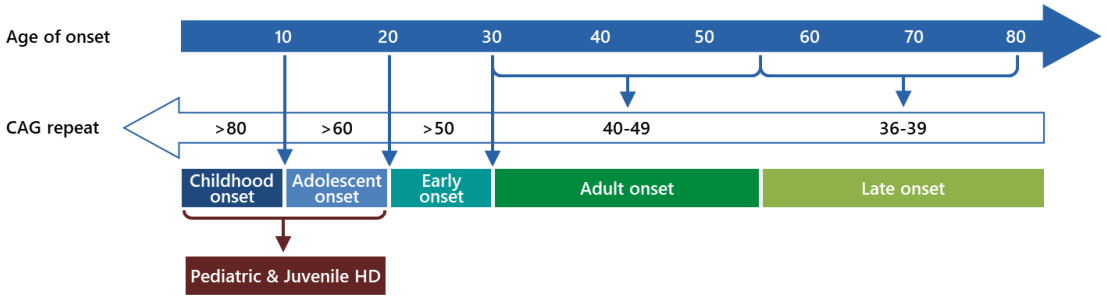

Figure 2: The relationship between CAG length and age of onset.

2. Huntingtin Protein

The *HTT* gene is responsible for coding a protein called huntingtin (HTT), a large (348 kDa), ubiquitously expressed protein (Gusella and MacDonald, 1995). The Huntingtin protein (HTT) is essential for embryonic development: its complete inactivation in huntingtin-knockout mice causes embryonic death before the nervous system formation (Nasir et al., 1995). The HTT becomes vital for neurogenesis and participates in the formation of the central nervous system (CNS) for neural tube formation and neuroblast migration, thereby significantly reduced huntingtin levels are insufficient to support normal development (White et al., 1997). The HTT is also linked to brain-derived neurotrophic factor (BDNF), which is particularly important for the survival of striatal neurons and corticostriatal synapses, as it also controls glutamate release and transmission (Jovanovic et al., 2000). The HTT is a crucial protein in diverse cellular activities. It is involved in multiple cellular pathways: cellular dynamics (cytoskeleton, endocytosis, trafficking, and adhesion), metabolism, protein turnover, gene expression (transcription and RNA processing), and signal transduction (Cattaneo et al., 2005). There is strong evidence that the expression of the mutant HTT (mHTT) and its fragmentation and aggregate formation is the underlying pathological mechanism of HD, leading to neurodegeneration (DiFiglia et al., 1997)

3. Neuropathology - Basal Ganglia and Huntington Disease

The basal ganglia are most affected by the pathological process in HD, especially the striatum. (Vonsattel et al., 1985). BG assures a flow of information that originates in the entire cerebral cortex and projects back to the sole frontal cortex by passing this information through the circuit loops and adjusting in the striatum, the globus pallidus and the subthalamic nucleus. (Albin et al., 1989). Information sent back to the frontal cortex is not a replication of cortical commands but a piece of completely new information processed in specific networks of striatal, pallidal and subthalamic neurons (Yelnik, 2008). These neuronal networks are regulated and consolidated by dopaminergic and cholinergic reinforcement learning, becoming available for automatic execution on cortical request (Prensa et al., 2000).

4. Clinical Manifestation

The main pathognomonic areas underlying the clinical presentation of HD relate to deficits in motor, cognitive, behavioural, and metabolic domains (Bates et al., 2015). The mechanism explaining which area might be affected during the disease is not fully understood and varies widely among individuals. At the onset of the disease, not all of the symptoms are necessarily clinically apparent. However, as the disease progresses, a typical clinical picture develops, but the nature and intensity of individual symptoms may vary. Clinical onset, presentation and

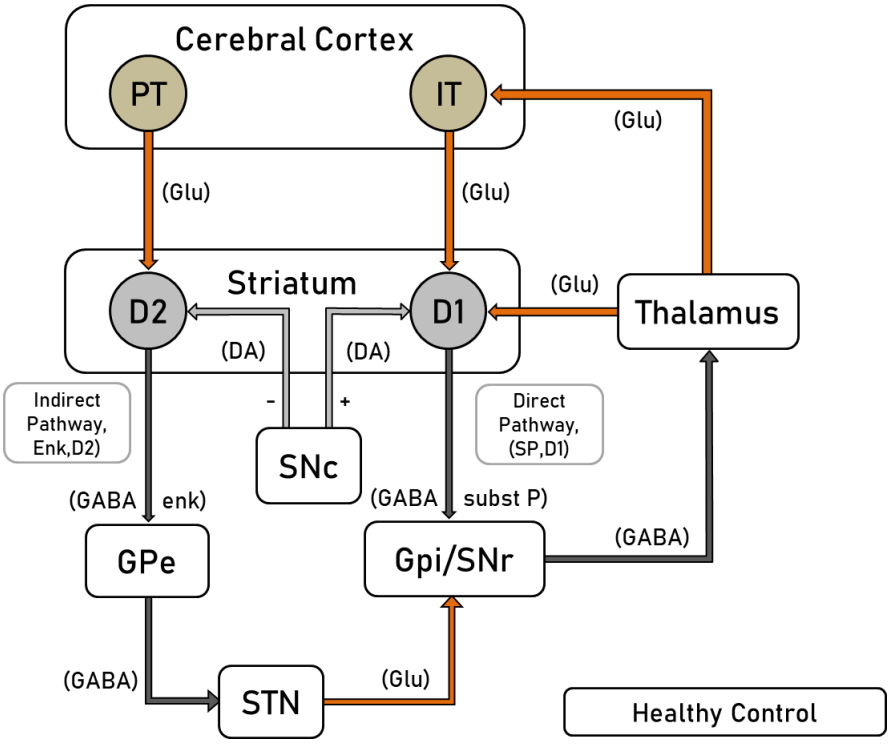

Figure 3: Simplified schematic representation of basal ganglia circuitry
The figure highlights cortical and striatal outputs as originally proposed by Albin et al. 1989.: Red arrows represent excitation; black arrows represent inhibition. Corticopyramidal Neurons (CPNs) of intratelencephalically projection (IT) project to the striatonigral D1-enriched Medium Spiny Neurons (MSNs) of the direct pathway. Pyramidal tract (PT)-type CPNs of the pyramidal tract (PT)-projection to the striatopallidal D2-enriched MSNs of the indirect pathway. Neurotransmitters are indicated in parenthesis. DA, dopamine; enk, enkephalin GABA, gamma-Aminobutyric acid;Glu, glutamate; subst P, substance P; Abbreviations: Gpi, Globus pallidus internus; Gpe, Globus pallidus externus, STN, Subthalamic Nucleus, substantia nigra pars compacta (SNc); substantia nigra pars reticulata (SNr).

progression of HD is determined primarily by the number of CAG triplets (Andrew et al., 1993, Duyao et al., 1993, Snell et al., 1993). HD displays a high level of clinical heterogeneity, even within families, concerning the balance of motor, cognitive, psychiatric, and metabolic features. The adult form of HD is the most common (up to 90%), with a clinical onset between 35-50 years of age (Read, 1993). Typically, the first manifestations usually occur with non-specific psychiatric and cognitive changes. However, these symptoms may facilitate the diagnosis of HD only in case of positive family history. Otherwise, the diagnosis is confirmed in most cases by the appearance of motor symptoms in terms of involuntary choreatic (jerky) movements on the distal parts of the body. In the more advanced stages, dystonia and sometimes myoclonus may occur. As the disease progresses, the hyperkinesia gradually diminishes, and an akinetic rigid syndrome develops the so-called Westphal form. Even in the early stages, bradykinesia and initiation impairments are typical (van Vugt et al., 1996). Other early neurological signs include disturbances in eye pursuit and saccadic movements. Dysarthria and latent dysphagia are common. Patients in the moderate and advanced stages develop gait disturbances with a tendency to fall (Andrich et al., 2007). HD is also associated with weight loss, which can occur at any stage of the disease. (Costa de Miranda et al., 2019). The cause of weight loss is not fully understood. It occurs despite increased caloric intake and is not proportional to dysphagia or the intensity of involuntary movements (Mochel et al., 2007). Advanced and terminal stages include incontinence, severe dysarthria to anarthria with dysphagia, cachexia, akinetic syndrome and severe dementia. The patients are dependent on the care of others. Most often, patients die from complications of infectious diseases. The average survival time is 15-20 years.

5. Cognition in Huntington Disease

Cognitive impairment is also one of the main features of HD and is present in different severity from the premanifest stage across the disease spectrum. However, the severity, frequency, and characterization of cognitive difficulties have not been well-described. Since cognitive deterioration often begins at an early stage, prior to the onset of distinct motor signs and progresses over time, representing a severe burden for a patient, the family, and the environment. (Paulsen, 2011). The neuropsychological profile in premanifest and manifest HD was already described in several studies, including impairment in psychomotor speed, negative emotion recognition and executive functioning (Dumas et al., 2013). Premanifest individuals typically pronounce deficiencies in psychomotor speed, emotion recognition and to some extend in executing functioning and minor deficiencies in memory, language, and global cognitive functioning domains (Dumas et al., 2013, Paulsen and Long, 2014). In manifest HD, adequate functioning remains intact for the longest periods for language and global functional domains. However, with the disease progression, impairments in memory (especially visuospatial), psychomotor speed, negative emotion recognition, and executive functioning maybe expected, resulting in dementia during the end-stage (Dumas et al., 2013).

6. Management of Huntington Disease

The management of Huntington Disease requires a comprehensive and multidisciplinary approach, adjusted to the patients' individual needs and depending on the stage of the disease. Currently, there is no curative treatment for the disease; only symptomatic approaches are available with the aim of symptom relief or symptom control (Tabrizi et al., 2020). There are no recommendations as to when symptomatic pharmacological treatment should be started, which depends on the severity of the condition. Chorea should only be treated with medication when it becomes relevant and causes restrictions in everyday life. Atypical antipsychotics, tetrabenazine and tiapride, can be used to treat chorea (Schultz et al., 2019). However, in patients with depression, tetrabenazine must be used with caution not to worsen the condition (Schultz et al., 2018). Non-Pharmacological approaches the non-pharmacological approaches are of the same importance as the pharmacological intervention. Several approaches are implemented and evaluated; particularly, physiotherapy is useful to optimize gait and balance for as long as possible, reduce falls and maintain the physical condition.

II. Assessing Cognition in HD: the Relationship Between Cognitive Performance, Functional Decline, and Disease Burden (study I)

1. Study Aims

Cognitive decline is a major feature of Huntington disease. The main objective of the present study was to examine the diagnostic accuracy of the Enroll-HD cognitive battery consisting of the Symbol Digit Modalities Test, Stroop Color Naming Test,Stroop Word Reading Test, Stroop Interference Test, Letter and Categorical Fluency Test, Trail Making Test A and B and MMSE. Further, this study aimed to investigate if the cognitive battery is sufficiently specific and sensitive to discriminate normal cognitive functioning in healthy controls from cognitive deficits in HD patients and differentiate HD patients with more from patients with less pronounced cognitive deficits. In addition, the study aimed to explore the effects of disease burden on the cognitive performance of HD patients and examine the relationship between cognitive, motor, and functional status.

2. Study Methods

Prospective observational study, a total of 256 participants were recruited between 2011 and 2017.

Study Assessments	
Enrolment Procedure	Inclusion and Exclusion Criteria Review
Sociodemographic Information:	Age Genetic test (CAG), Date of test Disease Burden Score (DBS) Date: HD clinical diagnosis Ethnicity Gender Marital status Living & caring situation Years of education, employment status
Medical History:	Comorbidities
Current HD-related Therapies:	Pharmacotherapy Nutritional Supplements Non-Pharmacologic Therapies
Functional Assessments:	UHDRS Total Functional Capacity UHDRS Functional Independence Scale
Motor Assessments:	UHDRS Total Motor Score UHDRS Diagnostic Confidence Index
Cognitive Assessment:	Symbol Digit Modalities Test Stroop Colour Naming Test Stroop Word Reading Test Stroop Interference Test Categorical Verbal Fluency Letter Verbal Fluency Trail Making Test A and B Mini-Mental Status Examination

Table 1: Study Assessments

3. Statistical Analysis & Results

Descriptive statistical methods were applied to describe the study population of HD patients and healthy controls and their demographic characteristics. The demographic and clinical characteristics of HD patients and healthy controls (NC) are displayed in Table 2. For the diagnosis of HD, the genetic test result with the number of CAG for both alleles was recorded, giving the range between 39 to 57 CAG repeats length on the pathological allele and 12 to 28 on the physiological allele.

Mean demographic characteristics of study cohorts Patient group with HD (N = 106) and the healthy control NC (N = 100) group					
	HD (N = 106)		NC (N = 100)		
	M ± SD	Range	M ± SD	Range	p
Age (Y)	48.80 ± 13.39	20-79	48.47 ± 13.53	21-80	.970*
Education (Y)[1]	12.92 ± 2.30	8-20	13.41 ± 2.09	9-19	.054*
Gender (% male)	52.8		39.0		.064†
MMSE[2]	25.46 ± 4.39	9-30	29.58 ± 0.76	27-30	< .001*
CAG repeats[3]	17.86 ± 2.77	12-28 (normal allele)			
	44.22 ± 3.78	39-57 (mutant allele)			

Table 2: Mean demographic characteristics of study cohorts

Sensitivity and Specificity to Detect the Cognitive Impairment

The sensitivity and specificity of the cognitive battery were evaluated and showed significant differences in all test scores for HD and NC (all *p*'s < .001; two-tailed) as displayed in Table 3.

Between-group differences in the cognitive battery between HD (N = 106) and NC (N = 100)					
	HD (*N* = 106)	NC (*N* = 100)			
	M ± SD	*M ± SD*	*t*(df)	*p*	*d*
Letter Fluency (three letters) [1]	21.58 ± 13.50	39.39 ± 12.71	-9.15 (180)	< .001†	1.359
Stroop-Color Naming Test	44.05 ± 20.00	74.11 ± 12.71	-12.948 (179.3)	< .001†	1.794
Stroop-Word Reading Test	62.40 ± 28.76	102.70 ± 13.36	-13.014 (150.2)	< .001†	1.797
Stroop-Interference Test[2]	27.30 ± 13.30	42.83 ± 9.44	-9.326 (166.8)	< .001†	1.347
Symbol Digit Modalities Test	24.37 ± 16.07	47.35 ± 11.51	-11.851 (190.5)	< .001†	1.644
Categorical Fluency	13.79 ± 7.05	23.81 ± 5.43	-11.461 (196.2)	< .001†	1.592
Trail Making Test-A[3]	69.36 ± 53.84	25.44 ± 8.51	8.058 (103.9)	< .001†	-1.140
Trail Making Test-B[4]	144.54 ± 79.50	59.88 ± 29.46	9.762 (118.2)	< .001†	-1.412

Table 3: Sensitivity and specificity to distinct cognitive impairment between manifest patients and healthy controls

Subsequently, the sensitivity and specificity of the cognitive battery to assess cognitive impairment in HD patients compared to normal cognitive functioning in healthy controls were assess by applying the Receiver Operating Characteristic (ROC) analysis to examine the discriminative potential of each neuropsychological test included in the cognitive test battery to assess cognitive impairment in HD patients compared to the NC group.

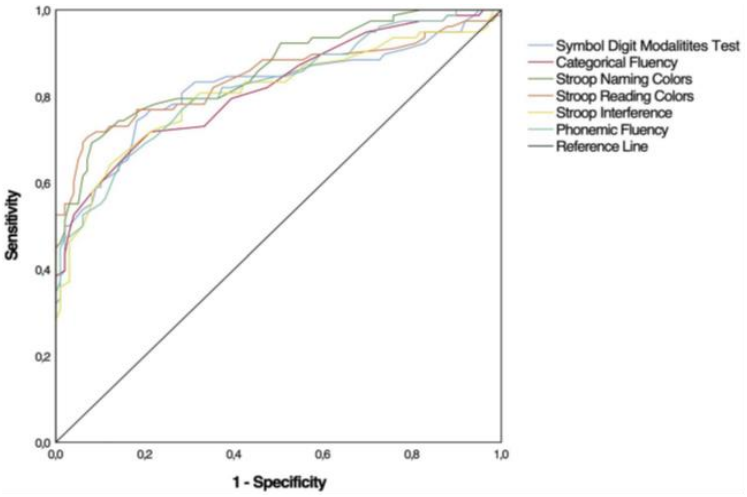

Figure 5: Diagnostic accuracy of the SDMT, Stroop tests and Fluency tests
Receiver-operating characteristic (ROC) discloses sensitivity and specificity of this cognitive battery to assess cognitive impairment and discriminate between HD patients (N = 106) and healthy controls (N = 100).

Sensitivity and Specificity to Distinct Cognitive Impairment Between Patients with More and Less Cognitive Decline

Furthermore, the manifest patients were divided into two subgroups, HD patients with a more pronounced cognitive deficit (HD-CD) and HD patients with a less pronounced cognitive deficit (HD-NC), using the MMSE score as an instrument to assess the general cognitive function. The subgroup HD-NC showed MMSE ≥ 24, and the subgroup HD-CD showed MMSE < 24. Further in the study, the diagnostic accuracy of all tests included in the cognitive battery to discriminate between the cognitive deficit of HD patients with less and more pronounced cognitive impairment and healthy controls was evaluated by one-way between-groups analysis of variance (ANOVA). The statistically significant differences (all p < .001) were recorded in all testscores between all three cohort groups.

Subsequently, ROC analysis was performed to investigate the sensitivity and specificity of each neuropsychological test to discriminate between two patients' subgroups, HD-NC and HD-CD patients. As displayed in Table 4, all tests showed high discriminative potential with all AUC > 0.80 and all p < .001. based on AUC analyses, the highest discriminative potential was detected for the SCNT (AUC = .899) to discriminate between the patients with less and more cognitive impairments, followed by SIT (AUC = .894), SDMT (AUC = .892), SWRT (AUC = .887), CFT (AUC = .863), LFT (AUC = .848), TMT-B (AUC = .835) and TMT-A (AUC = .833) with the lowest discriminative potential.

Sensitivity and specificity of the cognitive battery to discriminate (HD-CD, N = 30) and (HD-NC, N = 70)			
	AUC	95% CI	p
Letter Fluency (3 letters)	.848	.749-.947	< .001
Stroop Colour Naming Test	.899*	.810-.988	< .001
Stroop Word Reading Test	.887	.803-.970	< .001
Stroop Interference Test	.894	.791-.997	< .001
Symbol Digit Modalities Test	.892	.808-.976	< .001
Categorical Fluency	.863	.781-.945	< .001
Trail Making Test-A	.833	.748-.917	< .001
Trail Making Test-B	.835	.754-.915	< .001

Table 4: Sensitivity and specificity of the cognitive battery to discriminate between HD patients with less and more cognitive impairments

Effect of Individual Factors on Cognitive Performance and Relationship of Cognitive, Motor, and Functional Status

In this study, the individual factors were examined using partial correlation analysis to closely examine the effects of the individual factors on the neuropsychological tests and cognitive performance and the relationship between cognitive performance, motor impairment, and functional decline on the other hand.

4. Discussion & Conclusion

The main objective of the first study was to examine the diagnostic accuracy of the Enroll-HD cognitive battery consisting of the Symbol Digit Modalities Test, Stroop Colour Naming Test, Stroop Word Reading Test, Stroop Interference Test, Letter and Categorical Fluency Test, Trail Making Test A and B and MMSE, to assess cognitive impairment and to distinguish between HD patients and healthy controls and between HD patients with more and less pronounced cognitive impairment. Moreover, it aimed to explore the relationship between cognitive, motor, and functional status and the effect of disease burden on cognition.

In summary, the present results provide further evidence that although HD is classically considered to be a motor disorder, cognitive decline already occurs in the early stages of the disease (Paulsen, 2011; Stout et al., 2011) and are highly associated with the deterioration of the patients' functional abilities and interference with everyday life (Beglinger et al., 2012; Paulsen et al., 2014; Ready et al., 2008; Snowden, 2017).

This study confirmed that all cognitive tests included in the examined cognitive battery are sufficiently sensitive and specific to capture cognitive deficits in HD patients compared to healthy controls, as displayed in Table 3. The following tests placed in descending order were found to have the highest discriminatory properties to distinguish between healthy controls and HD patients: Stroop Color Naming Test, Stroop Word Reading Test, TMT-A, Letter Fluency Test, SDMT, Categorical Fluency Test, TMT-B, and Stroop Interference Test. In contrast, it is important to emphasize the non-specificity of the MMSE versus the specificity of the tested cognitive battery for assessing characteristic cognitive deficits in HD patients. However, MMSE will still have its place as a screening tool in clinical practice.

Overall, this cognitive battery's simpler and quick administration seems to be sufficient and enough sensitive in assessing cognitive deficits in HD.

III. Establishing Multilingual Normative Data for the Evaluation of Cognitive Performance in Huntington disease with Normative Calculator (Study II)

1. Study Aims

The study aimed to evaluate the cognitive decline and dissect the disease-associated decline in cognitive performance of HD by minimizing the impact of confounding factors, consisting of age, language, gender, and educational background at a single subject level. For this purpose, this study aimed to develop a normative calculator to convert raw test scores of the cognitive battery consisting of the SDMT, SWRT, SCNT, SIT, TMT-A and TMT B, CFT and LFT to the regression-based *z*-scores based on healthy control samples. The second step aimed to apply a normative calculator to assess individuals affected by HD and identify and evaluate cognitive performance.

2. Study Methods

Study Population

For this study, only the data of the healthy controls, under exclusion of neurological, psychiatric, internal, and other acute and chronicconditions were selected. The process of forming the normative data with multiple steps according to the predefined inclusion and exclusion criteria is described in Figure 6.

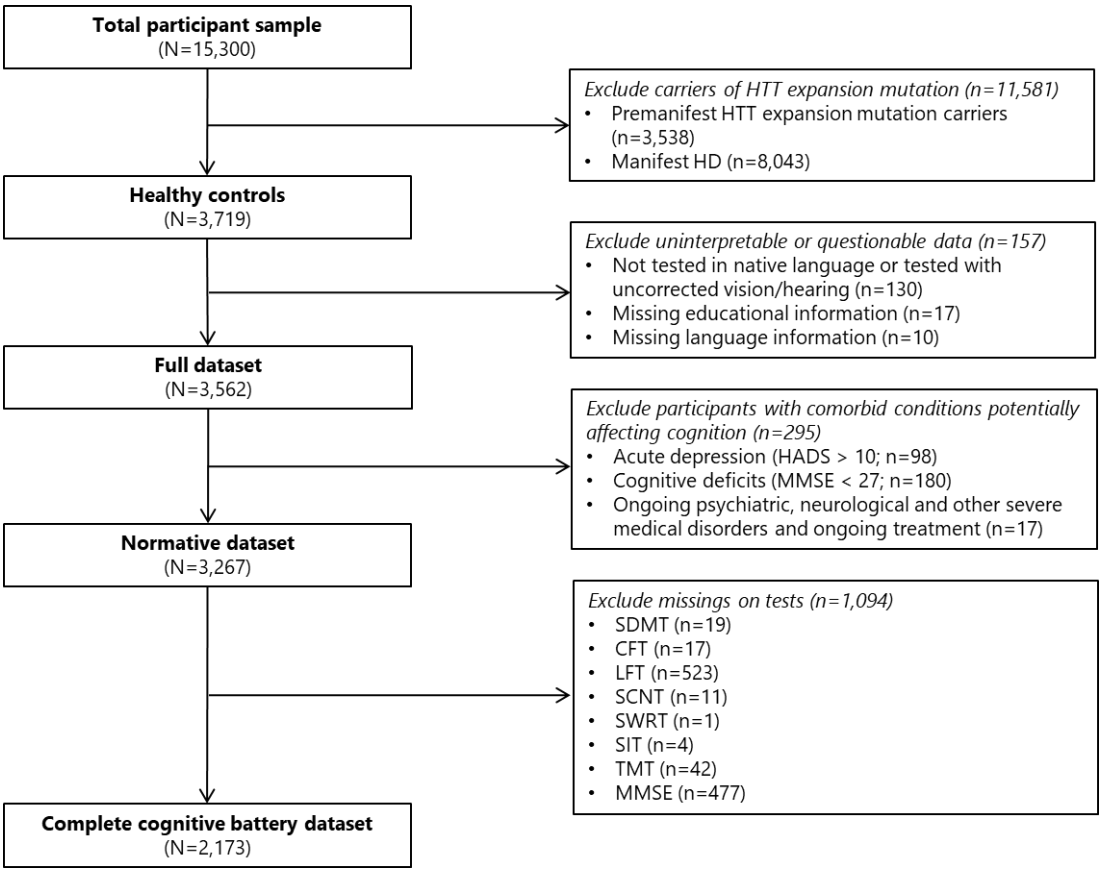

Figure 6: Flow chart describing the selection process defining a 'Normative Dataset'
The flow chart depicting the selection process defines a 'Normative Dataset' and a 'Complete Cognitive Battery Dataset'. The results reported in the main text are based on the Normative dataset (N=3,267). Results from the Complete cognitive battery dataset (N=1,173) and Full dataset (N=3,562) were used to check the robustness of the findings.

3. Statistical Analysis

Descriptive statistical methods were applied to describe the normative data set consisting of participants and their demographic characteristics. Continuous variables were characterized by means (*M*), standard deviations (*SD*), medians, and ranges. Categorical variables were expressed as percentages to the whole sample. Table norms consisting of *M* and *SD* stratified by age, an education level (≤12 years and >12 years of education), gender and language were constructed when feasible.

Development of Normative Values

General Approach and Model Selection

Choosing a proper model for this study was based upon the rationale that the developed normative values need to consider the impact of age, gender, language, and education level on the scores obtained from the examined neuropsychological test (SDMT, SWRT, SCNT, SIT, TMT-A and B, CFT, VFT). The possible missing values on the psychological test scores were also accounted for in the final decision. Furthermore, Generalized Additive Models (GAMs), including all predictors and using tensor splines to model possibly nonlinear additive effects of age and years of education, may also be used to establish the normative values (Wood et al., 2012). To evaluate the predictive performance of each demographic variable, models were fitted using either all four predictors or dropping one predictor at a time. Models were compared via leave-one-out information criterion (LOO-IC) as an approximate measure of expected predictive accuracy. In the further steps of this study, multiple nonlinear Bayesian regression analysis approaches were run between the studied cofounding parameters, such as age, gender, education level and language and scores of the neuropsychological test of the studied cognitive battery to determine the model of best fit.

Bayesian Model

This study uses the Bayesian approach for statistical analysis. Bayesian modelling provides information about the probability of the model parameters made as a combination of the predictions about the parameters and what is learned about the parameters from the data (Baldwin and Larson, 2017). The prediction about the probability of the parameters is known as the "prior" as it represents the parameter predictions prior to processing the data. In contrast, combining the prior and the data (Equation) produces the posterior distribution because it is created after seeing the data. (Baldwin and Larson, 2017).

$$P\ (parameter|data) = \frac{P\ (data\ |\ parameter)\ \ P(parameter)}{P(data)}$$

Equation 1: Bayesian Theorem
The Conditional probability – Bayesian Theory where p (parameters) is the prior and p (parameters /data) is the posterior (read as the probability of the parameters given the data)

Based on the Bayesian modelling, estimation of a normative distribution of the cognitive test scores at different levels of relevant sociodemographic variables (e.g., age, gender, language, and education level) will be done. The normative distribution is used to predict an outcome of a single individual based on their above-mentioned sociodemographic characteristics.

The *z* score on test *j* was calculated as shown in Equation 2 for each individual.

$$z_{ij} = \frac{y_{ij} - \hat{y}_{ij}}{\sqrt{\sigma_{ij}^2 + \sigma_{nj}^2}}$$

Equation 2: *Construction of z score: The z score on test j is calculated for each individual i. The individual's actual score is y_{ij}, and further $\hat{y}_{ij}$ is the individual's mean predicted score, σ_{ij}^2 is the variance of predictive distribution for the individual i and σ_{nj}^2 is the residual variance of the model for cognitive measure j.*

4. Results

Language-related Differences in Cognitive Performance

The analysis detected the language-dependent differences in cognitive performance for all cognitive tests under an adjustment for education, gender, and language. The largest data set was obtained from English native speakers was used as a reference for the comparisons between the performance in the neuropsychological tests for other language groups. In this study, the following language groups were examined: English, German, Spanish, Italian, Polish, French Danish, Dutch, and Spanish Latin-America. All data collected were obtained from native speakers in of respective language. The mean expected performance on all cognitive tests for each language group and their 95% PPIs are depicted in Figure 7.

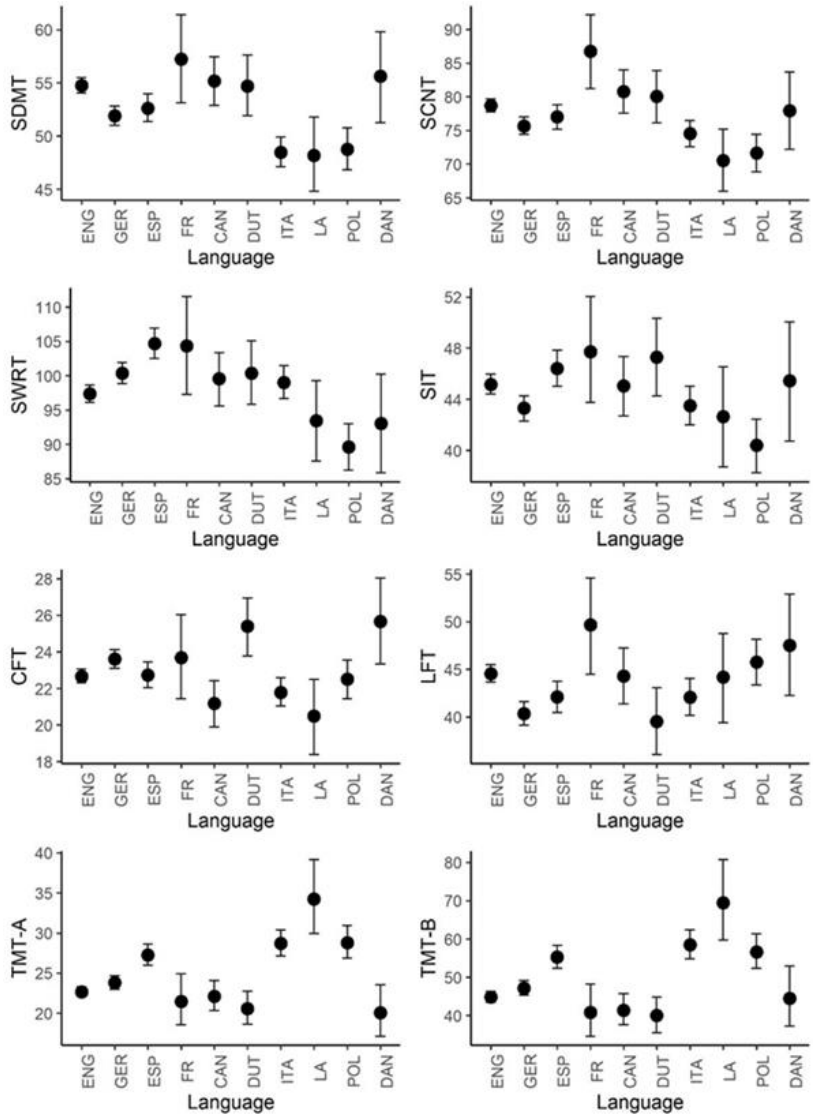

Figure 7: Language differences in tested cognitive measures

The displayed values represent posterior means and their 95% PPI interval; posteriors values of non-English languages were compared to English as a reference category; consequently, negative values indicate lower score, and positive values indicate higher score than the one obtained in English native speakers.

Despite the low numbers of normal controls for Danish, Dutch, French and Spanish Latin-America (< 50), there were significant differences in several neuropsychological tests in these languages, as showed in Table 8. The study also detected the differences in performance even in the tests considered "language-independent", such as SDMT and TMT-A and B. The Spanish, Italian and Polish native speakers, performed worse than French-Canadian and German native speakers, who performed similarly to English native speakers in TMT-A and B. The performance in SDMT in German native speakers was worse than the one observed in English native speakers but better than Polish and Italian native speakers. The differences between native speakers in performance in SDMT, TMT-A and TMT-B remained significant even after adjustment for age and educational levels between the various groups of native speakers.

On language-dependent tasks such as the SWRT, there were clear differences between groups of native speakers: Spanish native speakers performed best on the SWRT. In contrast, Polish native speakers performed worse than all other language groups on all three Stroop subtests. Further, Polish native speakers' performance on CFT and LFT was like that of English native speakers. However, German and Spanish native speakers (and to a lesser extent Italian native speakers) appeared to perform worse on the LFT.

Normative Calculator

An interactive normative calculator was created as a server-based web application to efficiently evaluate the patients' cognitive performance in daily clinical practice and research. The normative calculator uses lookup tables created from the above models containing every possible combination of values: z-scores, derived from the scores of the neuropsychological tests SDMT, SWRT, SCNT, SIT, TMT-A and TMT-B, CFT and VFT, and demographic variables (i.e., age, gender, education level, language). The web application provides a user interface to transform the measured (raw) values from the neuropsychological tests into *z*-scores using these lookup tables.

The application of normative calculators can be explained with a practical example. In contrast to the measured (raw) values, a z-score allows an immediate interpretation of the result from the neuropsychological tests. By shifting the distribution of the test score to a mean (M) of zero and scaling it to a standard deviation (SD) of one, a measured test score can be considered "in the normal range" if the corresponding z-score is no more than one standard deviation away from zero, i.e. between -1 and 1. Without further information, it may be unclear how to classify, e.g. a raw value of 60 symbols measured in the SDMT, corresponding with a z-score of 0.64, and refer to a slightly above average result, though still within the normal range. To emphasize the immediate applicability, a colour-coded interpretation of the resulting percentile is added, e.g. "superior" (green), "average" (yellow) or "severely impaired" (red).

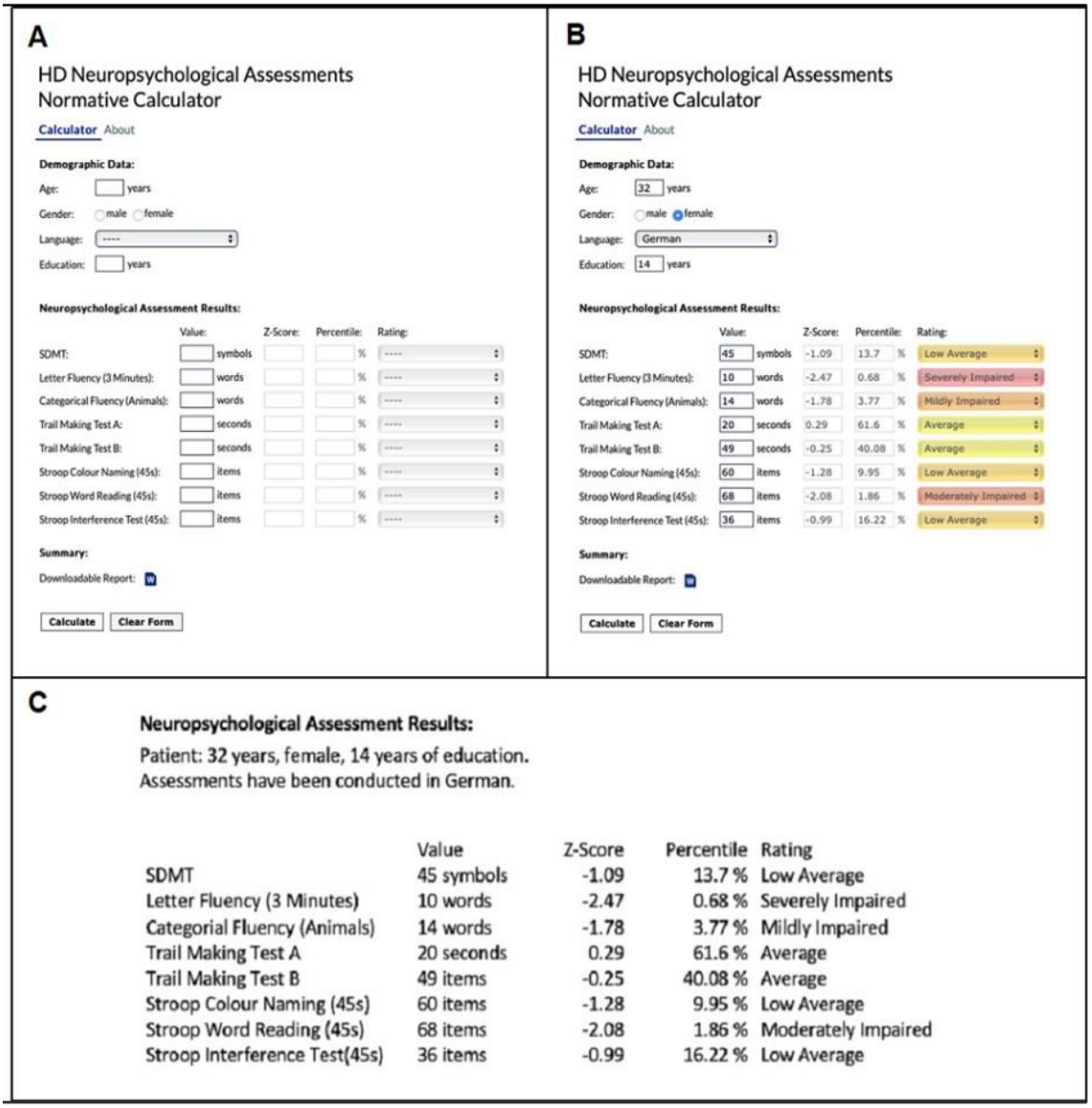

Figure 10: The normative calculator
From upper left to right: A) Entry forms (demographics and raw values obtained for each cognitive test) with automatic export function including a downloadable report in RTF format, B) Example output for a 32-year-old HD patient, C) Downloadable report in RTF-format

Results Interpretation in Clinical Practice: Illustrative Case Study

In the illustrative case study, a practical example of using normative values is presented. During the study, a 32-year-old female patient, a Polish native speaker living in Germany with known HD, molecularly confirmed with a genetic test giving a result of 18/44 CAG repeats, presented at the clinic to evaluate subjective memory impairment and difficulties in concentration. The patient undertook the whole cognitive battery in the Polish language. She obtained in SWRT a raw value of 77. This value was interpreted by the normative calculator within normal range z=-.83 when considering Polish normative values, however in the below-average. When the raw value was evaluated by considering the normative values from other countries (e.g., German) than z=-1.52 detected, this indicates a different interpretation, referring to the mild cognitive deficit. An overview of the z-scores in different languages is displayed in Figure 11.

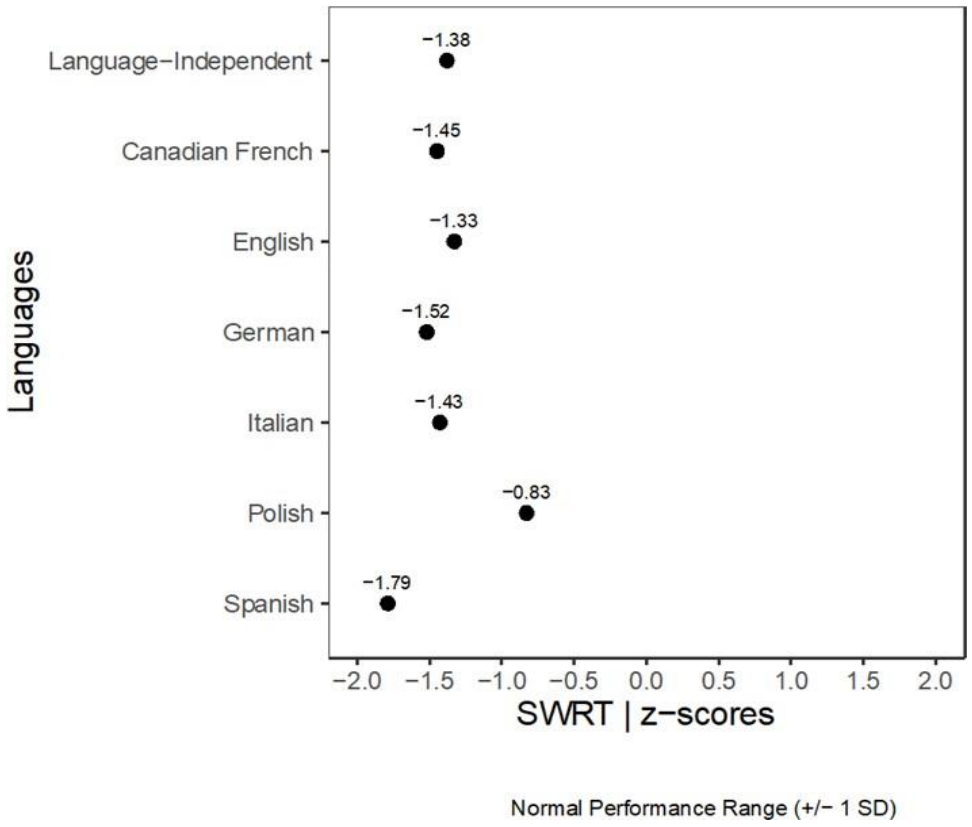

Figure 11: Illustrative case study
The figures show a clinical case of a 32-year-old female manifest HD patient, the raw value of 77 in Stroop Word Reading Test (SWRT) language-dependent differences in evaluating performance with different normative values in different languages.

5. Discussion & Conclusion

This study examined the most essential confounding factors impacting the performance in the cognitive battery consisting of SDMT, SWRT, SCNT, SIT, TMT-A and TMT-B, LCF, VFT. The main findings were as follows:

(1) The cognitive performance of healthy controls are influenced by sociodemographic characteristics, including age, education level, gender and language;
(2) Cognitive performance on all tests was significantly better with higher educational levels.
(3) There were language-dependent performance differences on all neuropsychological tests.
(4) There was a significant decline in cognitive performance with increasing age in most tests (except LFT), and
(5) Regarding gender differences, there was a trend towards better female performance. However, this was significant only for performance in SDMT.

The study provides normative data for a cognitive test battery to assess patients affected by HD or other primary or secondary neurodegenerative disorders (e.g., FTD, MS, PD, PSP), based on a dataset of *N*=3,267 healthy controls. The study looked at the several confounding factors impacting performance in the respective cognitive tests in healthy controls, including age, level of education and gender. It demonstrated that the language in which the neuropsychological

tests are administered systematically influences performance. Therefore, the raw values obtained from the neuropsychological test need to be converted to the z-scores to reduce the impact of the confounders, as mentioned above. In the final step to consider disease-associated alterations at a single patient level and for each assessment, a web-based normative calculator was designed to enable this functioning. This normative calculator is freely accessible to any clinician or researcher to improve clinical care and evaluation of the neuropsychologic tests.

Employing neuropsychological tests in clinical practice or clinical studies provides an added value if scores can be interpreted in a meaningful and appropriate way (Strauss et al., 2006). To this end, a participant's score is compared to scores derived from a healthy control population with comparable matched characteristics (e.g. gender, age, education level, language) by applying normative values for diagnostic purposes. In addition, normative values may allow continuous monitoring of the disease's natural progression and may help demonstrate the potential benefits and side effects of clinical interventions of different kinds. Applying the standardized performance scores controlled for the influence of identified confounding factors instead of raw values helps define and quantify the actual, i.e., disease-associated alterations in cognitive performance.

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V. List of Publications

1. Publications in the Context of this Dissertation

Mühlbäck, A., Frank W., Klempířová O., Bezdíček O., Schmitt L., Hofstetter N., Klempíř J. (2020). Validation study of the German Enroll HD cognitive battery: Relationship between cognitive performance, functional decline and genetic load, Disease: Relationship Between Cognitive Performance, Functional Decline, and Disease Burden. *Archives of Clinical Neuropsychology, 36*(1), 74-86. doi:10.1093/arclin/acaa038. IF: 2,2.

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2. Publications Covering Broader Aspects of Huntington Disease and Movement Disorders

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