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BRIEF COMMUNICATION

Interaction between sex and neurofilament light chain on brain structure and clinical severity in Huntington's disease

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Abstract

Female Huntington's disease (HD) patients have consistently shown a faster clinical worsening than male, but the underlying mechanisms responsible for this observation remain unknown. Here, we describe how sex modifies the impact of neurodegeneration on brain atrophy and clinical severity in HD. Cerebrospinal fluid neurofilament light chain (NfL) levels were used as a biological measure of neurodegeneration, and brain atrophy was assessed by structural magnetic resonance imaging. We found that larger NfL values in women reflect higher brain atrophy and clinical severity than in men ($p < 0.05$ for an interaction model). This differential vulnerability could have important implications in clinical trials.

Introduction

Huntington's disease (HD) is an autosomal-dominant neurological disorder caused by a CAG repeat expansion in the *HTT* gene driving a cascade of pathological processes leading to massive neuronal death. HD patients will experience progressive motor, cognitive, and behavioral symptoms resulting in a total loss of functional independence.

Female HD patients have consistently shown a faster clinical worsening than male.[1–3] However, the origin of this clinical observation is not well understood and needs to be investigated further, as it could shed light on novel pathological mechanisms. Crucially, a well-established gender difference in HD should have important implications for the design and interpretation of clinical trials.

In vivo biomarker-by-sex interactions on clinical severity and brain atrophy have contributed to better understand the origin of sex-related differences in other neurodegenerative diseases. Among the available biomarkers of neurodegeneration, cerebrospinal fluid (CSF) neurofilament light chain (NfL) levels have shown the best monitoring and prognostic performance in HD.[4]

In this work, we hypothesize that sex differences in the clinical worsening of HD patients could be driven by a differential impact of the underlying neurodegenerative processes on brain atrophy. Women would be then more vulnerable to the naturally occurring neurodegeneration as measured by NfL. To test this hypothesis, we performed an NfL-by-sex interaction model on structural neuroimaging data.

Materials and Methods

Sample and assessments

A total of 41 HD patients were included in this study from the outpatient clinic of the Movement Disorders Unit at Hospital de la Santa Creu i Sant Pau (Barcelona, Spain). All of them were confirmed gene mutation carriers (CAG length ≥39). This study was approved by the appropriate ethics committee and all participants provided signed informed consent.

The following clinical indicators were considered in this study. The disease burden score (DBS), defined as age × (CAG-35.5), as an index of pathological burden due to lifetime exposure to mutant huntingtin. Total functional capacity (TFC) and motor symptoms (TMS) were recorded from the Unified Huntington's Disease Rating Scale (UHDRS). The following cognitive and neuropsychiatric indicators, known to be sensitive to HD progression with minimal medication-related alterations,[5,6] were included: Symbol Digit Modality Test (SDMT), Stroop-word task, and the apathy score of the Problem Behavior Assessment (PBA). Finally, composite UHDRS (cUHDRS) scores were computed, given its improved performance as a measure of disease progression in HD.[6]

Biomarker and neuroimaging procedures

CSF samples were collected, processed, aliquoted in polypropylene tubes, and frozen at −80°C until analysis according to international consensus recommendations.[7] NfL levels were measured with the NF-light Advantage kit (Cat# 103186) using the Single Molecule Array (Simoa) technology (Simoa; Quanterix, Lexington, MA, USA) in the SR-X Biomarker detection system by following the manufacturer's instructions. A logarithmic transformation (base 10) was applied to work with normally distributed CSF NfL data.

Given that tau accumulation rate is greater in females, we also measured CSF total Tau levels in order to discard that sex differences in our sample were related to an increase in Tau pathology in the female group. CSF Tau was measured with the Neurology 3-Plex Advantage kit (Cat# 101995) using the Simoa technology as described above.

Specific details on the acquisition and preprocessing steps of 3-Tesla T1-MRI images are available in our previous work.[8] It is important to note that cortical thickness (Cth) analyses are not significantly affected by differences in total intracranial volume or morphology.

Statistical analysis

The following interaction model was applied for each of the considered clinical or neuroimaging variables M:

$$M = \beta_0 + \beta_1 * \text{sex} + \beta_2 * \text{logNfL} + \beta_{\text{int}} * [\text{sex} * \text{logNfL}] + \beta_4 * \text{age} + \beta_5 * \text{DBS}$$

Using this model, we investigated the presence of a significant NfL-by-sex interaction term (β_{int}) independent of age and disease burden. For clinical variables and striatal volumes, significance of β_{int} was set at $p < 0.05$. For the vertexwise Cth analyses, to be consistent with our previous works,[8] surface maps were smoothed using a Gaussian kernel of 15 mm full-width-at-half-maximum to increase the signal-to-noise ratio; and only clusters surviving $p < 0.05$ and family-wise error (FWE) correction for multiple-comparison by a Monte Carlo simulation with 10,000 repeats were considered significant. Effect sizes for the interaction model were assessed by Cohen's f^2.

Finally, aiming to investigate the clinical translation of the imaging findings, we performed the following exploratory analyses. In the set of brain regions showing a significant interaction effect, we computed its Cth/volume values. Then, using Pearson's correlation coefficients, we studied the association of these imaging alterations with the worsening of clinical indicators, for which a value of $p < 0.05$ was considered significant.

Results

Table 1 summarizes the sample's sociodemographic, clinical, and biomarker characteristics. No significant sex-related differences in terms of these variables were observed in this sample.

However, significant NfL-by-sex interactions were observed on: cUHDRS (interaction $\beta = 3.0$, $p = 0.026$, $f^2 = 0.19$; NfL-correlation: males $r = -0.44$, $p = 0.050$; females $r = -0.74$, $p < 0.001$), SDMT (interaction $\beta = 3.2$, $p = 0.034$, $f^2 = 0.16$; NfL-correlation: males $r = -0.36$, $p = 0.122$; females $r = -0.68$, $p = 0.001$) and Stroop-word (interaction $\beta = 4.1$, $p = 0.007$, $f^2 = 0.25$; NfL-correlation: males $r = -0.23$, $p = 0.328$; females $r = -0.68$, $p = 0.001$). No significant tau-by-sex interactions on clinical severity were observed.

The right caudate volume also showed a significant NfL-by-sex interaction: interaction $\beta = 3.0$, $p = 0.035$, $f^2 = 0.15$; NfL-correlation: males $r = -0.50$, $p = 0.023$; females $r = -0.65$, $p = 0.001$. This interaction remained significant after further controlling for total intracranial volume. The vertexwise NfL-by-sex interaction model on Cth revealed two significant clusters where NfL-related cortical thinning was more pronounced in the female group (Figure 1). In the right hemisphere, the fronto-temporal cluster mainly included middle-frontal, insular, and anterosuperior-temporal regions (interaction $\beta = 0.34$, $p = 0.006$, $f^2 = 0.25$). In the left hemisphere, the frontal

Table 1. Sample characteristics.

	HD	HD-Female	HD-Male	*p*-value
n	41	21	20	
Age [years]	44.2 ± 11.4	43.6 ± 10.7	44.9 ± 12.4	0.71
Education [years]	12.6 ± 4.1	12.7 ± 4.1	12.5 ± 4.2	0.89
CAG length	43.4 ± 2.6	43.5 ± 3.0	43.3 ± 2.2	0.70
DBS	419.1 ± 103.6	416.4 ± 102.9	421.8 ± 107.0	0.87
TFC	12.2 ± 1.6	12.0 ± 2.1	12.3 ± 1.1	0.51
cUHDRS	14.4 ± 4.5	14.2 ± 5.0	14.6 ± 4.0	0.79
UHDRS-TMS	12.8 ± 18.3	11.8 ± 15.8	13.8 ± 20.9	0.73
SDMT	43.6 ± 20.0	42.5 ± 22.8	44.6 ± 17.2	0.74
Stroop-word	86.6 ± 27.2	85.1 ± 32.2	88.1 ± 21.4	0.72
PBA – Apathy item	2.2 ± 2.5	2.5 ± 2.4	1.9 ± 2.6	0.39
CSF NfL [pg/mL]	1934.9 ± 1225.4	1810.3 ± 1305.7	2065.8 ± 1153.9	0.51
CSF Tau [pg/mL]	98.1 ± 40.1	88.7 ± 39.3	108.5 ± 39.3	0.12

Values are expressed as mean ± standard deviation. DBS, Disease Burden Score; UHDRS, Unified Huntington's Disease Rating Scale; cUHDRS, composite UHDRS score; TFC, Total Functional Capacity; UHDRS-TMS, Total Motor Score; SDMT, Symbol Digit Modality Test; PBA, Problems Behaviors Assessment; CSF NfL, Cerebrospinal fluid Neurofilament Light Chain.

cluster mainly included the orbitofrontal cortex (interaction $\beta = 0.46$, $p = 0.006$, $f^2 = 0.24$).

Finally, the following set of exploratory clinical-neuroimaging associations were observed in the set of brain regions showing a significant NfL-by-sex interaction. Both the right caudate volume and the average Cth in the right frontotemporal cluster correlated with all the considered clinical measures: cUHDRS $r = 0.54$, $p < 0.001$, and $r = 0.60$, $p < 0.001$, respectively; TFC = 0.44, $p = 0.004$, and $r = 0.49$, $p = 0.001$, respectively; UHDRS-TMS $r = -0.39$, $p = 0.011$, and $r = -0.57$, $p < 0.001$, respectively; SDMT $r = 0.51$, $p = 0.001$ and $r = 0.47$, $p = 0.002$, respectively; Stroop-word $r = 0.48$, $p = 0.002$, and $r = 0.56$, $p < 0.001$, respectively; PBA—Apathy item $r = -0.34$, $p = 0.027$, and $r = -0.35$, $p = 0.026$, respectively.

Discussion

We found a significant NfL-by-sex interaction on brain structure and clinical severity in HD. The impact of increasing NfL levels on brain atrophy and clinical severity was more pronounced in women. This could explain, at least partially, their faster clinical progression previously described in large longitudinal cohorts.

Notably, CSF NfL levels were not significantly increased in the female group compared to males. This suggests that women brains are more vulnerable to the naturally occurring neurodegeneration in HD, that is, the same amount of measured NfL reflects higher damage in women than in men. These findings open up the possibility to consider different CSF NfL reference limits for men and women in HD clinical trials. This would be especially important if longitudinal studies confirm that increases in CSF NfL over time translate into a faster atrophy rate in women.

From a clinical perspective, the NfL-by-sex interaction on cUHDRS is noteworthy. This composite score is a multidimensional measure of progression in HD being used as a primary outcome in clinical trials.[6] Interestingly, direct NfL-by-sex interactions on motor or psychiatric symptoms were not observed in our sample. However, as expected, in an indirect manner, the integrity in the brain regions showing a significant NfL-by-sex interaction correlated with motor, cognitive, and psychiatric symptoms. These results highlight the clinical relevance of the observed interaction.

Importantly, the increased vulnerability observed in the female group appear unrelated to possible differences in tau pathology. Therefore, further research is needed to elucidate the specific pathological mechanisms responsible for this phenomenon. Vascular or hormonal factors could be involved. Sex-related neurodevelopmental differences related to the HTT gene could also underlie the observed effects.[9] Finally, as similar sex-related clinical differences were observed in spinocerebellar ataxia,[10] sex-related alterations in dynamic mutation mechanisms could also be implicated.

Limitations of this work include cross-sectional and a relative low sample size. As this study was motivated by an HD-specific clinical observation with potential implications in clinical trials involving HD patients, we did not include a control group. The inclusion of other risk factors such as the APOE genotype, MAPT haplotype, or cardiovascular profiles could have also contributed to a better understanding of the origin of the observed interaction.

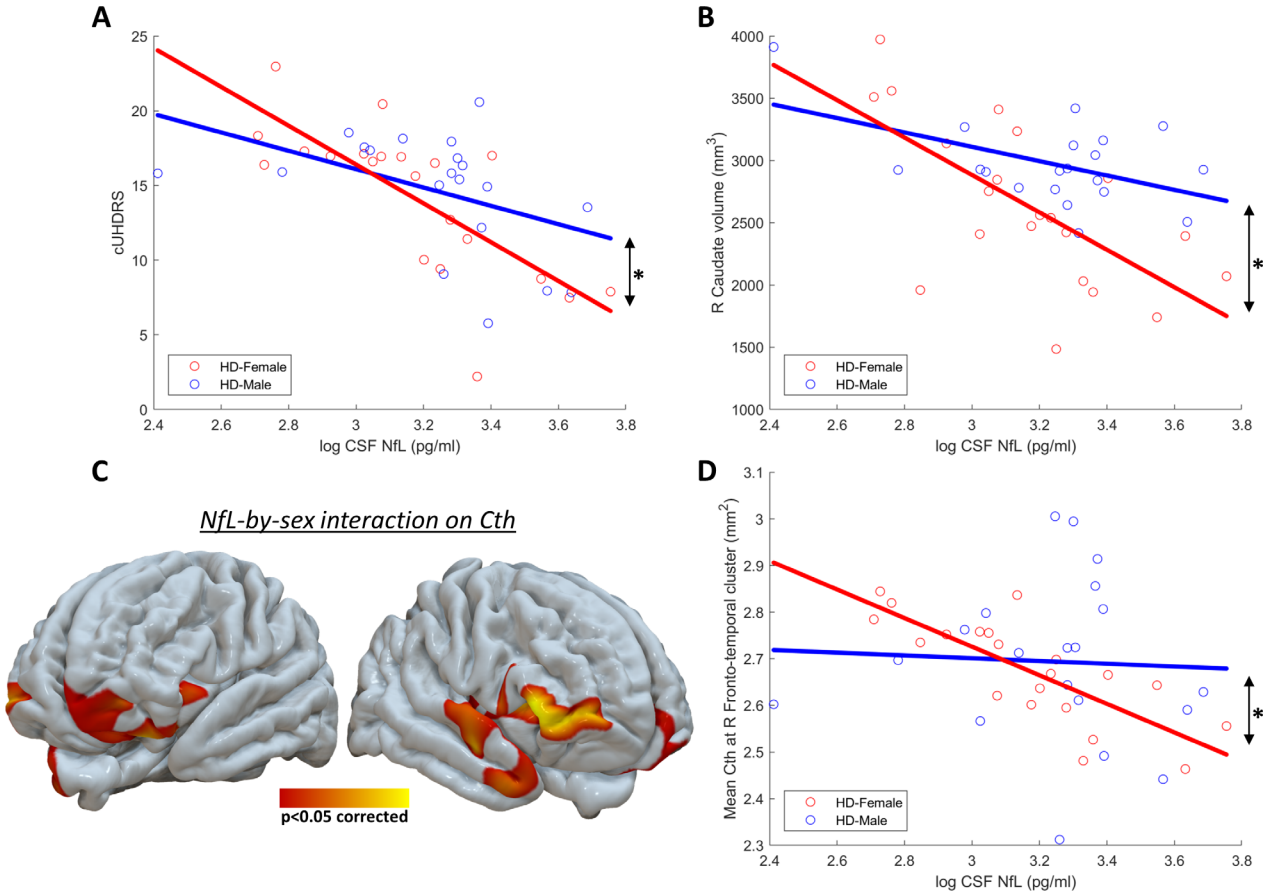

Figure 1. NfL-by-sex interaction on cUHDRS scores (A), right caudate volume (B) and Cth (C). (D) Scatter plot illustrating the direction of the Cth interaction in the right fronto-temporal cluster. * A $p < 0.05$ for the interaction model.

To conclude, we found that sex modifies the impact of HD's neurodegeneration on clinical severity and brain atrophy. Overall, these results suggest that future studies using CSF NfL as a marker of neurodegeneration in HD should take into consideration this NfL-by-sex interaction.

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Conflict of Interest

None of the authors of this manuscript have any potential conflict of interest related to the content of this study. FS, SMH, RPG, AHB, IAB, AC, BGA, JP, and JK have nothing to disclose.

Author Contributions

FS: Data analysis and manuscript writing. SMH, AHB: Project conception and clinical project execution. RPG, AC, IAB: Project execution: blood sample collection and processing. BGA, Project execution - MRI acquisition. JP, Manuscript review and critique. JK: Project organization, manuscript review, and critique.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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