Speech biomarkers in Huntington's disease: A cross-sectional study in pre-symptomatic, prodromal and early manifest stages

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Abstract

Background and purpose: Motor speech alterations are a prominent feature of clinically manifest Huntington's disease (HD). Objective acoustic analysis of speech can quantify speech alterations. It is currently unknown, however, at what stage of HD speech alterations can be reliably detected. We aimed to explore the patterns and extent of speech alterations using objective acoustic analysis in HD and to assess correlations with both rater-assessed phenotypical features and biological determinants of HD.

Methods: Speech samples were acquired from 44 premanifest (29 pre-symptomatic and 15 prodromal) and 25 manifest HD gene expansion carriers, and 25 matched healthy controls. A quantitative automated acoustic analysis of 10 speech dimensions was performed.

Results: Automated speech analysis allowed us to differentiate between participants with HD and controls, with areas under the curve of 0.74 for pre-symptomatic, 0.92 for prodromal, and 0.97 for manifest stages. In addition to irregular alternating motion rates and prolonged pauses seen only in manifest HD, both prodromal and manifest HD displayed slowed articulation rate, slowed alternating motion rates, increased loudness variability, and unstable steady-state position of articulators. In participants with premanifest HD, speech alteration severity was associated with cognitive slowing ($r = -0.52$, $p < 0.001$) and the extent of bradykinesia ($r = 0.43$, $p = 0.004$). Speech alterations correlated with a measure of exposure to mutant gene products (CAG-age-product score; $r = 0.60$, $p < 0.001$).

Conclusion: Speech abnormalities in HD are associated with other motor and cognitive deficits and are measurable already in premanifest stages of HD. Therefore, automated speech analysis might represent a quantitative HD biomarker with potential for assessing disease progression.

KEYWORDS
acoustic analysis, Huntington's disease, hyperkinetic dysarthria, prodromal biomarker, speech
INTRODUCTION

Huntington’s disease (HD) is a progressive autosomal dominant neurodegenerative disease caused by a cytosine-adenine-guanine (CAG) expansion in the huntingtin (HTT) gene [1]. Expression of mutant huntingtin gene products results in neuronal dysfunction and premature brain atrophy [2], leading to characteristic motor signs regarded as important criteria for the clinical diagnosis of HD [3], and cognitive and behavioural abnormalities. No disease-modifying treatment is currently available, although new therapeutic approaches aimed at lowering levels of mutant huntingtin gene products are currently in development and are being tested in randomized controlled clinical trials [3, 4]. These steps towards disease-modifying therapies in HD emphasize the importance of highly sensitive biomarkers that can quantify subtle disease-associated changes in early stages of HD because interventions in early HD are likely to be more promising for the purpose of delaying disease progression [5]. Neuroimaging, measurements of neurofilament light protein in blood and cerebrospinal fluid, and quantitative motor and cognitive assessments have been explored for their potential to serve as markers that precede the traditionally defined clinical onset of HD by many years [5, 6].

Speech represents one of the most complex, yet quantifiable motor functions sensitive to damage to neural circuits [7]. Imprecise consonants, variable rates, mono-pitch, harsh voice and inappropriate silences are considered distinctive characteristics of hyperkinetic dysarthria associated with chorea syndromes based on two independent perceptual studies using the Mayo Clinic dysarthria rating scale [8, 9]. In addition, findings based on objective acoustic analysis showed increased phonatory instability [10, 11], subharmonics [11, 12], syllable repetition instability [13, 14] and intensity variability [15] in manifest HD (mHD). Many of these speech abnormalities are likely to be a reflection/manifestation of involuntary movement patterns that typically predominate in the initial and middle clinical stages of adult-onset HD. However, early in the disease process, abnormal patterns of voluntary movements, such as subtle problems with planning, initiating, smoothly executing and terminating intended movements, emerge in parallel [16]. In addition, premanifest HD gene expansion carriers (HDGECs) perform significantly worse on a range of cognitive measures [5]. How cognitive dysfunction and the voluntary motor abnormalities described above affect speech in premanifest stages of HD is not well established. Only a limited number of studies with relatively small group sizes [13, 17–19] have sought to identify the patterns of subtle speech dysfunction in genetically confirmed prodromal HD (proHD) and reported mostly phono-tary and timing abnormalities. Possible speech abnormalities of HDGECs across the full spectrum of early HD (pre-symptomatic [preHD], proHD, and early mHD), covering all stages of the newly proposed HD Integrated Staging System (HD-ISS) [20], have not yet been investigated.

METHODS

Study design

From 2020 to 2021, we enrolled HDGECs and healthy controls at the Huntington Center Ulm, Department of Neurology, Ulm University. All participants in the HD group underwent a genetic test confirming ≥36 CAG repeats in one of the HTT alleles. The exclusion criteria for HDGECs were history of communication or significant neurological disorders unrelated to HD, being a non-native German language speaker, and severe intellectual impairment that would interfere with study protocol. For healthy controls, exclusion criteria included history of neurological or communication disorder and being a non-native German language speaker.

Clinical examination of HDGECs included demographics, medical history, past and current medication, the Unified Huntington’s Disease Rating Scale (UHDRS) total motor score [21] and the Symbol Digit Modalities Test (SDMT) [22]. The CAG-age-product (CAP) score was calculated using the following formula: age × (CAG−30)/6.49 [23]. Years to disease onset were estimated for participants with premanifest HD using the formula: 21.54+exp(9.556−0.46×CAG) [24].

The HDGECs were divided into three groups: preHD, proHD and mHD based on the proposed criteria by Ross et al. [3]. In the proHD group, gene carriers had a diagnostic confidence level (DCL) equal to 0 or 1 without presence of motor and cognitive signs and symptoms. In the proHD group, gene carriers had a DCL of 2 or 3 and presented with subtle motor signs, typically with some cognitive alteration compared to matched normal controls. In the mHD group, participants had a DCL of 4 and were classified using the UHDRS Total Functional Capacity Rating Scale scores as having early HD (score ≥7). In addition, following the publication of the HD-ISS [20], HDGECs were divided into four groups: Stage 0 (far from onset with CAG ≥40 only); Stage 1 (altered biomarkers of pathogenesis using striatal atrophy as landmark); Stage 2 (displaying a clinical phenotype using total motor score and SDMT as landmark); and Stage 3 (displaying a decline in function using Total Functional Capacity and Independence Scale as landmarks).

The study was approved by the Ethics Committee of the University of Ulm, Germany (approval number: 381/18) and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants provided written, informed consent prior to their inclusion.

Speech recordings

The speech recordings were made in a room with low ambient noise level with a professional head-mounted condenser microphone (Beta 53; Shure, Niles, Illinois, USA) [25]. The audio data were sampled at 48kHz with 16-bit quantization. There were no time constraints
imposed on the recordings. Each participant performed a sustained phonation of the vowel /a/ per one breath for as long and steadily as possible and fast /ta/ syllable repetition for at least 5 s, and read a standardized passage composed of 199 words. Each task was performed twice.

**Acoustic speech analysis**

We selected 10 speech variables representing distinct aspects of hyperkinetic dysarthria that are feasible to evaluate using quantitative objective acoustic analysis. These variables correspond to the perceptual description of the main patterns of hyperkinetic dysarthria associated with chorea based on the Mayo Clinic dysarthria rating scale [8, 9], and were in addition tested in previous pilot studies on acoustic speech abnormalities in proHD and mHD [9–17, 26].

"Unstable steady-state position of articulators" was assessed using standard deviation of power spectral density (stdPDS), "harsh voice" via harmonics-to-noise ratio (HNR) and "pitch breaks" using the proportion of subharmonic intervals via a sustained phonation paradigm. "Imprecise consonants" were assessed using the voice onset time, "slow alternating motion rates" were assessed using the diadochokinetic rate (DDKR), "irregular alternating motion rates" through diadochokinetic irregularity (DDKI), and "increased loudness variability" using standard deviation of speech intensity envelope (stdPWR) via the fast syllable repetition. "Prolonged pauses" using the duration of pause intervals (DPI), "monopitch" was assessed using standard deviation of pitch contour (stdFO) and "slow articulation rate" through the net speech rate (NSR) via reading passage.

Final speech values used for the statistical analysis were calculated as the mean of the two repetitions to provide greater speech assessment stability [25]. A list of used variables and their detailed description can be found in Table 1. Comprehensive algorithmic details on individual acoustic measures were reported previously [27]. In addition, accuracy of the algorithms for the identification of temporal intervals, pitch sequences, and glottal cycles has been thoroughly tested in previous studies [27–29]. All analyses were performed in MATLAB® (MathWorks, Natick, Massachusetts, USA).

**Speech alteration severity analysis**

The primary endpoint was the composite dysarthria index (CDI; measuring the overall severity of speech alteration), which represents a combination of 10 acoustic speech variables associated with hyperkinetic dysarthria in HD. All 10 speech variables were converted to z-scores using the mean and standard deviation of the control group. To ensure correct directionality, the z-scores were reversed for those measures in which lower raw scores were associated with greater severity in speech abnormalities (i.e., HNR, DDKR, stdFO, NSR). We estimated CDI as the mean value from 10 calculated z-scores.

In addition, we created a supporting, rater-based composite score to evaluate elements of speech that might not be captured by acoustic analysis called the "perceptual dysarthria score" (PDS). The PDS was assessed by three independent HD specialists with several years of experience. The perceptual assessment was performed blindly on randomized audio data from all four participant groups using all vocal paradigms. The perceptual criteria for dysarthria outlined by Darley et al. [8] were used to judge the presence and severity of speech abnormalities. The PDS was ranked as: 0 = normal, 1 = slight abnormal signs with at least one distinctive speech dimension affected, 2 = mild dysarthria, 3 = moderate dysarthria, and 4 = severe dysarthria. We estimated the inter-rater reliability using the two-way mixed single score. Intra-class correlation reached a value of 0.75 [30] for HD specialists; thus, the final consensus PDS was calculated as the median value of three perceptual ratings.

**Statistical analysis**

Given the large effect size (Cohen’s $f > 0.4$) observed for overall severity of speech alteration between HD patients and controls in a previous study [15] and considering an error probability of $\alpha$ set at 0.05 and a false-negative rate $\beta$ set at 0.2 (i.e., power of 0.8) for the CDI, the a priori power analysis indicated a recommended minimum overall sample size of 73 for four groups (80 for five groups) [31].

As the Kolmogorov–Smirnov test showed that the acoustic features were normally distributed, we performed analysis of covariance, with age set as a covariate to evaluate group differences. We addressed multiple comparisons via Bonferroni adjustment and determined thresholds of $p < 0.05$ for the primary endpoint CDI (and the PDS) and $p < 0.005$ (0.05/10) for individual speech variables. Post hoc comparisons using Fisher’s least-squares differences were applied only for significant measures on the omnibus test.

Informed by primary hypothesis results, we performed a binary logistic regression followed by a leave-one-subject-out cross-validation to assess the ability of a combination of acoustic features to distinguish between groups (i.e., accuracy, sensitivity, and specificity). As an overall indication of diagnostic accuracy, we reported the area under the curve (AUC) obtained from the receiver-operating characteristic curve. We iterated through all possible feature combinations for the one yielding the highest AUC.

To provide further insights into the features of speech dysfunction in HD and minimize the possibility of Type I errors, speech performance was related to three representative clinical scales including chorea (chorea subscore, composed of the items of the UHDRS chorea subscale), bradykinesia (bradykinesia subscore, composed of the UHDRS finger taps, pronate-supinate hands, bradykinesia and rigidity sub-items), and cognition (processing speed by the SDMT) separately for premanifest (merged preHD and proHD groups) and manifest (mHD group) stages. In addition, we explored the relationship between CAP score and CDI, eliminating the need to bin participants into groups based on clearly defined but somewhat subjective and arbitrary criteria such as DCL. The non-parametric Spearman...
partial correlation coefficient, with age set as covariate, was preferred due to violations of normality of clinical data in premanifest stages. The significance level was set at \( p < 0.05 \) for the primary endpoint CDI (and the PDS) and at \( p < 0.005 \) (0.05/10) for the individual speech variables.

**RESULTS**

**Clinical data**

A total of 69 HDGECs were included in this study, consisting of 29 participants with preHD (10 men) with mean (SD; range) age of 39.0 (10.6; 24–62) years, 15 participants with proHD (seven men) with mean (SD; range) age of 42.1 (11.4; 22–62) years, and 25 participants with mHD (10 men) with mean (SD; range) age of 47.3 (12.5; 22–76) years (Table 2). Out of 69 HDGECs, neuroleptics (quetiapine, olanzapine, zyprexa, promethazine, aripiprazole, sulpiride) were used by four participants in the mHD, two in the preHD and one in the proHD group; antichoreas (tetrabenazine, tiapride) by five in the mHD and one in the preHD group. In addition, a total of 63 HDGECs fulfilled the HD-ISS criteria, consisting of seven participants with Stage 0, 24 with Stage 1, 10 with Stage 2, and 22 with Stage 3 (Table S1); three participants could not be classified by HD-ISS and three had a CAG below 40 and therefore the HD-ISS did not apply. As a healthy control group, 25 participants (10 men) with a mean (SD; range) age of 46.7 (13.5; 27–78) years were recruited.

**Group differences**

Compared to controls, CDI was larger in the mHD \( (p < 0.001) \) and proHD groups \( (p < 0.001) \), but not the preHD group \( (p = 0.30) \) (Figure 1). Similarly, PDS was more pronounced in the mHD \( (p < 0.001) \) and proHD groups \( (p = 0.003) \) but did not differ from controls in the

**Table 1** Overview of used acoustic features.

<table>
<thead>
<tr>
<th>Speech dimension</th>
<th>Acoustic feature (unit)</th>
<th>Definition</th>
<th>Hypothesized pathomechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained phonation</td>
<td>stdPSD (dB)</td>
<td>Standard deviation of power spectral density, defined as the mean value of the standard deviations of different frequency banks.</td>
<td>Involuntary movements cause unstable articulatory stability.</td>
</tr>
<tr>
<td>Harsh voice</td>
<td>HNR (dB)</td>
<td>Harmonics-to-noise ratio, defined as the amplitude of noise relative to tonal components.</td>
<td>Reduced rate of airflow and improper control of vocal folds cause increased turbulent noise.</td>
</tr>
<tr>
<td>Pitch breaks</td>
<td>PSI (%)</td>
<td>Proportion of subharmonic intervals, defined as the ratio of subharmonic intervals per total duration of all voiced segments.</td>
<td>Asymmetry of vocal fold cycles.</td>
</tr>
</tbody>
</table>

**Abbreviations:** DDKI, diadochokinetic irregularity; DDKR, diadochokinetic rate; DPI, duration of pause intervals; HNR, harmonics-to-noise ratio; NSR, net speech rate; PSI, proportion of subharmonic intervals; stdF0, standard deviation of pitch contour; stdPSD, standard deviation of power spectral density; stdPWR, standard deviation of power; VOT, voice onset time.
preHD group ($p = 0.33$). A similar pattern of increasing CDI across Stages 0–3 of the HD-ISS was observed, with a trend toward significance between controls and Stage 1 ($p = 0.09$) as well as significant differences between controls and Stage 2 or 3 ($p < 0.001$; Figure S1). Perceptual and acoustic speech severity analyses were strongly correlated (CDI vs. PDS: $r = 0.77$, $p < 0.001$).

Alterations in the speech of HDGECs were observed for six out of 10 acoustic variables (Table 3, Video 1). Compared to controls, the mHD group showed unstable steady-state position of articulators (stdPSD; $p < 0.001$), slow alternating motion rates (DDKR; $p < 0.001$), irregular alternating motion rates (DDKI; $p < 0.001$), increased loudness variability (stdPWR; $p < 0.001$), prolonged pauses (DPI; $p < 0.001$), and slow articulation rate (NSR; $p < 0.001$). Comparison of the proHD and control groups showed unstable steady-state position of articulators (stdPSD; $p = 0.01$), slow alternating motion rates (DDKR; $p = 0.01$), increased loudness variability (stdPWR; $p = 0.01$) and slow articulation rate (NSR; $p = 0.009$). The comparison between the preHD and control groups did not show individual acoustic parameters to differ significantly.

**Sensitivity analysis**

In the mHD versus the control group, the best discrimination accuracy, with AUC of 0.97 (accuracy 90%, sensitivity 92%; specificity 88%), was detected using a combination of five variables including HNR, DDKR, stdPWR, stdF0, and NSR. For the proHD compared to the control group, the best discrimination accuracy (AUC of 0.92) was achieved using the variables stdPSD and DDKI (accuracy 83%, sensitivity 85%; specificity 79%). To discriminate between preHD and control participants, a combination of HNR and stdF0 yielded the best results, with AUC of 0.74 (accuracy 60%, sensitivity 61%; specificity 60%).
TABLE 3 Results of individual acoustic speech variables.

<table>
<thead>
<tr>
<th>Speech feature</th>
<th>controls Mean±SD</th>
<th>preHD Mean±SD</th>
<th>proHD Mean±SD</th>
<th>mHD Mean±SD</th>
<th>ANCOVA p-value</th>
<th>Post hoc significance (least-squares difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stdPSD (dB)</td>
<td>1.9±0.4</td>
<td>2.0±0.4</td>
<td>2.3±0.5</td>
<td>2.8±0.7</td>
<td>&lt;0.001</td>
<td>controls &lt; mHD***, controls &lt; proHD*, preHD &lt; mHD***, proHD &lt; mHD**</td>
</tr>
<tr>
<td>HNR (dB)</td>
<td>21.2±3</td>
<td>20.8±2.7</td>
<td>21.4±3.3</td>
<td>19.5±2.8</td>
<td>0.02</td>
<td>controls &gt; mHD***, controls &gt; proHD*, proHD &gt; mHD***</td>
</tr>
<tr>
<td>PSI (%)</td>
<td>5.6±12.6</td>
<td>3.9±8</td>
<td>3.3±4.8</td>
<td>6.9±14.8</td>
<td>0.85</td>
<td>controls &lt; mHD***, preHD &lt; mHD***, proHD &lt; mHD***</td>
</tr>
<tr>
<td>VOT (ms)</td>
<td>26.0±5.2</td>
<td>27.0±7.5</td>
<td>27.5±7.6</td>
<td>30.8±7.6</td>
<td>0.10</td>
<td>controls &lt; mHD***, controls &lt; proHD*, preHD &lt; mHD***</td>
</tr>
<tr>
<td>DDKR (syll/s)</td>
<td>6.7±1</td>
<td>6.6±0.8</td>
<td>6.0±0.9</td>
<td>4.6±1.1</td>
<td>&lt;0.001</td>
<td>controls &gt; mHD***, controls &gt; proHD*, proHD &gt; mHD***</td>
</tr>
<tr>
<td>DDKI (ms)</td>
<td>22.6±15.0</td>
<td>25.6±15.7</td>
<td>40.1±18.0</td>
<td>82.5±51.0</td>
<td>&lt;0.001</td>
<td>controls &lt; mHD***, preHD &lt; mHD***, proHD &lt; mHD***</td>
</tr>
<tr>
<td>stdPWR (dB)</td>
<td>2.2±0.7</td>
<td>2.4±1.0</td>
<td>3.2±1.1</td>
<td>3.8±1.9</td>
<td>&lt;0.001</td>
<td>controls &lt; mHD***, controls &lt; proHD*, proHD &lt; mHD***</td>
</tr>
<tr>
<td>DPI (ms)</td>
<td>140±36</td>
<td>141±41</td>
<td>155±35</td>
<td>210±87</td>
<td>&lt;0.001</td>
<td>controls &lt; mHD***, preHD &lt; mHD***, proHD &lt; mHD***</td>
</tr>
<tr>
<td>stdF0 (semitones)</td>
<td>2.9±0.9</td>
<td>2.5±0.9</td>
<td>2.3±0.8</td>
<td>2.3±0.7</td>
<td>0.09</td>
<td>controls &gt; mHD***, controls &gt; proHD*, proHD &gt; mHD***</td>
</tr>
<tr>
<td>NSR (syll/s)</td>
<td>5.0±0.6</td>
<td>4.9±0.7</td>
<td>4.4±0.7</td>
<td>3.9±0.8</td>
<td>&lt;0.001</td>
<td>controls &gt; mHD***, controls &gt; proHD*, proHD &gt; mHD***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviations: ANCOVA, analysis of covariance; DDKR, diadochokinetic rate; DDKI, diadochokinetic irregularity; DPI, duration of pause intervals; HD, Huntington’s disease; HNR, harmonics-to-noise ratio; mHD, manifest HD gene expansion carriers; NSR, net speech rate; PSI, proportion of subharmonic intervals; preHD, pre-symptomatic HD gene expansion carriers; proHD, prodromal HD gene expansion carriers; stdPSD, standard deviation of power spectral density; stdPWR, standard deviation of power; stdF0, pitch variability; VOT, voice onset time.

Correlations between speech variables, clinical characteristics and CAP scores

The severity of acoustic speech alteration, as measured by CDI, in premanifest stages (merged preHD and proHD groups) correlated with bradykinesia subscore (r = 0.43, p = 0.004) and SDMT (r = −0.52, p < 0.001), whereas CDI in manifest stages (i.e., in the mHD group) correlated with chorea subscore (r = 0.49, p = 0.02), bradykinesia subscore (r = 0.57, p = 0.005) and SDMT (r = −0.49, p = 0.02). The same significant correlations were observed for severity of the perceptual speech alteration measured using the PDS (Table 4). Considering individual acoustic speech variables, the highest correlations were found for the bradykinesia subscore with increased loudness variability in the premanifest HD group (stdPWR; r = 0.52, p < 0.001), prolonged pauses in the mHD group (DPI; r = 0.57, p = 0.005) and slow articulation rate in the mHD group (NSR; r = −0.62, p = 0.002 [Table 4]). HDGECs with CAP scores of 100 and above (mean CDI 1.95, SD 1.01) compared to HDGECs with CAP scores below 100 (mean CDI 0.63, SD 1.00) showed more severe alterations (p < 0.001); a correlation of CDI with CAP score was also observed (r = 0.60, p < 0.001 [Figure 2]).

DISCUSSION

This study provides the first attempt to characterize speech alterations from preHD to proHD to early-stage mHD, comparing subjective perceptual analysis by experts head-to-head with objective acoustic analysis. Both types of analysis were mutually supportive in demonstrating dysarthria already in the prodromal stage of HD. Our classification analysis yielding an AUC of 0.74 suggests that subclinical speech abnormalities are already present in a pre-symptomatic stage of HD. The correlations observed with clinical, rater-based scales and with CAP scores support the concept that objective acoustic analysis may be used to quantify alterations of brain structure and function driven by the progressive disease process.
in HD. The results demonstrating graded alterations of speech in a stage-dependent manner are consistent with the notion that a fully automated speech assessment method has potential as a quantitative marker of progression of HD, and could inform future clinical trials aimed at disease modification. In addition, objective quantitative acoustic analysis may help refine the definition of landmarks for stage transition in HD [20].

In our study, the main speech dimensions affected in proHD were slow articulation rate, slow alternating motion rates, increased loudness variability and unstable steady-state position of articulators. The receiver-operating characteristic curve analysis yielded a very high AUC of 0.92 for discrimination between proHD and control participants, suggesting the presence of considerable speech alteration already before the emergence of the motor signs currently widely accepted as criteria for a clinical diagnosis of HD [3]. In agreement with the observations presented here, a previous study reported that trained listeners perceived subtle differences in the proHD group [19]. While temporal abnormalities including

<table>
<thead>
<tr>
<th>Deviant speech dimension</th>
<th>Chorea (UHDRS)</th>
<th>Bradykinesia (UHDRS)</th>
<th>Cognitive decline (SDMT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDI</td>
<td>0.26 (0.097)</td>
<td>0.43 (0.004)</td>
<td>−0.52 (&lt;0.001)</td>
</tr>
<tr>
<td>PDS</td>
<td>0.28 (0.071)</td>
<td>0.44 (0.004)</td>
<td>−0.49 (0.001)</td>
</tr>
<tr>
<td>stdPSD</td>
<td>0.05 (0.743)</td>
<td>0.17 (0.281)</td>
<td>−0.33 (0.034)</td>
</tr>
<tr>
<td>HNR</td>
<td>0.09 (0.594)</td>
<td>0.24 (0.132)</td>
<td>−0.17 (0.294)</td>
</tr>
<tr>
<td>PSI</td>
<td>0.05 (0.738)</td>
<td>0.06 (0.722)</td>
<td>0.02 (0.916)</td>
</tr>
<tr>
<td>VOT</td>
<td>−0.39 (0.010)</td>
<td>−0.12 (0.443)</td>
<td>0.01 (0.974)</td>
</tr>
<tr>
<td>DDKR</td>
<td>−0.13 (0.419)</td>
<td>−0.34 (0.029)</td>
<td>0.28 (0.074)</td>
</tr>
<tr>
<td>DDKI</td>
<td>0.04 (0.805)</td>
<td>0.37 (0.017)</td>
<td>−0.30 (0.050)</td>
</tr>
<tr>
<td>stdPWR</td>
<td>0.36 (0.019)</td>
<td>0.52 (&lt;0.001)</td>
<td>−0.32 (0.038)</td>
</tr>
<tr>
<td>DPI</td>
<td>0.38 (0.013)</td>
<td>0.27 (0.090)</td>
<td>−0.21 (0.169)</td>
</tr>
<tr>
<td>stdF0</td>
<td>−0.05 (0.735)</td>
<td>0.02 (0.888)</td>
<td>0.13 (0.409)</td>
</tr>
<tr>
<td>NSR</td>
<td>−0.32 (0.038)</td>
<td>−0.35 (0.024)</td>
<td>0.40 (0.009)</td>
</tr>
</tbody>
</table>

**Early-stage manifest HD (n = 25)**

| CDI                     | 0.49 (0.021)   | 0.57 (0.005)         | −0.49 (0.021)          |
| PDS                     | 0.72 (<0.001)  | 0.73 (<0.001)        | −0.58 (0.004)          |
| stdPSD                  | 0.43 (0.044)   | 0.34 (0.121)         | −0.33 (0.138)          |
| HNR                     | −0.24 (0.282)  | 0.13 (0.580)         | −0.10 (0.675)          |
| PSI                     | 0.39 (0.077)   | 0.21 (0.345)         | −0.34 (0.119)          |
| VOT                     | 0.08 (0.713)   | 0.21 (0.343)         | −0.33 (0.131)          |
| DDKR                    | −0.14 (0.537)  | −0.39 (0.072)        | 0.44 (0.042)           |
| DDKI                    | 0.52 (0.013)   | 0.44 (0.042)         | −0.53 (0.010)          |
| stdPWR                  | 0.23 (0.298)   | 0.27 (0.232)         | −0.19 (0.397)          |
| DPI                     | 0.10 (0.653)   | 0.57 (0.005)         | 0.02 (0.946)           |
| stdF0                   | 0.17 (0.452)   | 0.05 (0.844)         | 0.04 (0.861)           |
| NSR                     | −0.37 (0.093)  | −0.62 (0.002)        | 0.20 (0.381)           |

**TABLE 4** Correlations between speech and clinical metrics for premanifest and manifest stages of Huntington’s disease separately.

Note: Data are represented by correlation coefficient r (p value). Bold values indicate significant differences with p < 0.05 for primary endpoints (CDI and PDS) and p < 0.005 for individual acoustic variables.

Abbreviations: CDI, composite dysarthria index; DDKI, diadochokinesic irregularity; DDKR, diadochokinesic rate; DPI, duration of pause intervals; HD, Huntington’s disease; HNR, harmonics-to-noise ratio; NSR, net speech rate; PDS, perceptual dysarthria score; PSI, proportion of subharmonic intervals; stdPSD, standard deviation of power spectral density; stdPWR, standard deviation of power; stdF0, pitch variability; SDMT, Symbol Digit Modalities Test; VOT, voice onset time.

aPremanifest HD group was defined as joint pre-symptomatic and prodromal HD groups.
bChorea subscore (ranging from 0 to 28) was composed from the items of the chorea subscale.
cBradykinesia subscore (ranging from 0 to 4) was composed of the finger taps, pronate–supinate hands, bradykinesia and rigidity subitems.
slow articulation rate and slow alternating motion rates have already been reported in proHD [13, 17-19], to the best of our knowledge, increased loudness variability and unstable steady-state position of articulators have not been described previously in proHD.

Increased loudness variability during the fast syllable repetition paradigm was found to be associated with the extent of bradykinesia in premanifest HD. A similar increased variability in the execution of movements in HD was observed in a range of motor tasks including tapping [32], grasping [33], tongue protrusion [5], gait [34], and reaching [35]. Therefore, variability in the loudness of repetitive vocalization expands the family of more variable motor coordination variables that appear to be a hallmark of HD [32]. Unstable steady-state position of articulators during sustained phonation has only been reported in manifest stages of HD and was associated with the occurrence of chorea [11]. Although we did not find a definite clinical correlate for this speech phenomenon in our premanifest HD cohort, we suggest that unstable articulatory stability might be considered a precursor of chorea as we observed a trend towards an association between unstable steady-state position of articulators and chorea ($p = 0.04$, uncorrected) in our mHD group.

For slowness of speech in premanifest HD we found a trend towards a correlation with bradykinesia ($p = 0.02$, uncorrected) and cognitive decline measured by the SDMT ($p = 0.009$, uncorrected), a widely used cognitive measure of processing speed known for its sensitivity in HD [36]. This observation is consistent with a recent report on proHD [19] and with a previous study showing a strong association between slowed articulation rate and processing speed decline in multiple sclerosis [37]. Reduced speech rate has also been observed in idiopathic rapid eye movement sleep behavior disorder [38], a special case of prodromal parkinsonism which is associated with a higher risk of cognitive impairment. Contrary to the natural speech rate, which reflects a combination of speech motor execution and cognitive-linguistic processing, the diadochokinetic rate measures the motor abilities of the speech articulators and reveals their movement limitations. We may hypothesize that slower alternating motion rates in our premanifest HD patients are mainly related to changes in voluntary motor control, which is partly supported by a trend towards a correlation with the bradykinesia subscore ($p = 0.02$, uncorrected). A previous study showed that reduced maximum speed during oral diadochokinesis in multiple sclerosis was related to greater cerebellar atrophy [39]. Because abnormal cerebellar volume has also been observed in early mHD [40], its contribution to slow oral diadochokinesis in our HD cohort may need to be considered, aside from the impact of marked basal ganglia dysfunction and of striatal atrophy.

An excellent AUC of 0.97 was observed for discriminating between early-stage HD and control speakers, confirming motor speech disorder as common but underappreciated manifestations of HD [15]. Two additional speech abnormalities including irregular alternating motion rates and prolonged pauses were detected, which accords well with previous studies [13-15]. Instability of syllable repetition appears to be influenced by chorea, which is not surprising as sudden choreic movements may influence the timing of syllable production. On the other hand, prolongation of pauses in our mHD cohort likely reflect the extent of bradykinesia, and therefore might be linked to difficulties in the initiation of speech and inappropriate timing. In addition, some prolonged pauses might reflect the effects of chorea as well. For instance, chorea blocks/interrupts voluntary movement, or the speaker waits in anticipation of an abnormal movement or until the abnormal movement ceases. Overall, HD speech alterations appeared to develop in parallel with other motor abnormalities, which is in accordance with recent research demonstrating that acoustic speech features allowed the prediction of individual cognitive, motor, and functional scores [41]. Interestingly, perceptual analysis in mHD was more influenced by the extent of chorea than was acoustic analysis. Probably, this is mainly caused by a bias in the perceptual analysis of experienced clinicians recognizing specific effects of involuntary movements on speech in HD patients.

A limitation of the present study is that the classification of participants in several groups based on criteria that imply judgement calls (e.g., DCL) raises the possibility of some questionable classifications of individual HDGECs. Nevertheless, applying a different
staging system (ID-ISS) did not change the conclusion that the severity of speech alterations is stage dependent. In addition, the clear correlation of speech alterations with CAP scores highlights that the severity of speech alterations is driven by a key biological marker underlying disease progression. Also, some of the HDGECs studied here were subject to pharmacotherapy aimed at symptomatic relief using neuroleptics, antihyperkinetics and sedatives, which may impact vocal performance. Finally, we did not collect subjective self-report of voice alterations (e.g., via the Voice Handicap Index [10]). Therefore, future studies are encouraged to investigate how well the self-expressed awareness of change in speech matches clinical examination, formal perceptual analysis, and acoustic analysis in premanifest HD.

In conclusion, speech deficits are detectable already in premanifest stages of HD and are associated with other motor and cognitive deficits. Automated acoustic analysis provides an inexpensive, non-invasive way to assess HD repeatedly, without sophisticated technical equipment, that is scalable across languages [42]. Future work should focus on extending our findings in a longitudinal design while correlating speech changes with brain structures by magnetic resonance imaging.

AUTHORS CONTRIBUTIONS
Tomas Kouba was responsible for the conception and execution of the research project, data analysis, design of the statistical analysis, and writing of the manuscript. Wiebke Frank was responsible for the conception, organization and execution of the research project and critique of the manuscript. Tereza Tykalova was responsible for the execution of the research project, and review and critique of the manuscript. Alžbeta Muhlbäck, Jiří Klempíř and Katrin S. Lindenberg were responsible for the execution of the research project, securing funding, and review and critique of the manuscript. Jan Rusz was responsible for the conception and execution of the research project, design of the statistical analysis, securing funding, and writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT
The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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