Extending the Spectrum of Nonmotor Symptoms with Olfaction in Premotor Huntington’s Disease: A Pilot Study

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Keywords
Huntington’s disease · Mutation carriers · Olfactory dysfunction · Neurodegeneration · Nonmotor symptoms

Abstract
Objective: The aim of this pilot study was to investigate change of olfactory functions in Huntington’s disease (HD).
Background: HD is a neurodegenerative disease characterized by motor, cognitive, and behavioral abnormalities. There are several studies reporting olfactory dysfunction in manifest and some studies in premanifest HD carriers, and a recent neuropathological study demonstrated HD-specific protein aggregation in the anterior olfactory nucleus in HD patients. In this study, we wanted to assess olfactory functions as a possible early nonmotor symptom of HD mutation carriers without disease-specific motor symptoms and HD patients. Methods: All participants had genetic confirmed HD and were prospectively recruited during their routine control in a specialized outpatient clinic of the Medical University of Innsbruck, Department of Neurology, Austria. Healthy controls (HCs) were caregivers from patients. They were only included if they were younger than 70 years, scored more than 24/30 points on the Mini Mental State Examination, and had no other disease compromising olfactory function. Furthermore, all participants were tested on the Sniffin’ sticks 16-items identification test. Results: We included 23 patients with manifest HD, 13 HD mutation carriers, and 19 HCs. Mutation carriers showed significant impaired odor identification compared to HCs (p < 0.001), as well as Huntington’s patients compared with both mutation carriers (p = 0.003) and HCs (p < 0.001). Conclusions: The results of this pilot study suggest that olfactory dysfunction may be an early nonmotor symptom of HD and could be a potential marker to assess disease progression.

Introduction
Huntington’s disease (HD) is an autosomal dominant neurodegenerative disease characterized by motor, cognitive, and behavioral abnormalities caused by a CAG trinucleotides expansion on chromosome 4 Huntington gene resulting in the production of mutant huntingtin protein (mHtt) [1]. There are several studies reporting olfactory dysfunction in manifest HD patients [2, 3] and some studies in premanifest HD carriers [4, 5] which did...
not show significant impairment in olfaction of mutation carriers using the University of Pennsylvania Smell Identification Test (UPSIT). Furthermore, a recent neuropathological study demonstrated HD-specific protein aggregation in the anterior olfactory nucleus which is common in HD [6].

However, olfactory dysfunction in HD carriers without disease-specific motor symptoms assessed by the Sniffin’ sticks test has not been examined so far. In this preliminary study, we wanted to assess if olfactory dysfunction could be an early nonmotor symptom of HD even in asymptomatic carriers by using a more extensive olfactory testing.

**Methods**

The study was approved by local Ethics Committee of the Medical University of Innsbruck (approval reference number AN1979 336/4.19 401/5.10 [4464a], Austria), and all participants provided written informed consent according to the Declaration of Helsinki. Participants were prospectively included during their routine control in a specialized outpatient clinic of the Medical University of Innsbruck, Department of Neurology, Austria. All HD patients had genetic confirmed HD. CAG age product (CAP) scores were computed as described previously [7]. All patients underwent an extensive neurological examination including the total motor score of the Unified Huntington’s Disease Rating Scale (UHDRS) and the Mini Mental State Examination as the screening tool for cognitive impairment [8]. Healthy controls (HCs) were HD-unaffected caregivers (e.g., partners and friends) from patients. Exclusion criteria included age over 70 years, Mini Mental State Examination of equal or below 24, chronic otorhinolaryngeal disease (such as chronic rhinitis, nasal polyps, or sinus disease), head trauma, toxic exposures, upper respiratory infection at time of assessment, previous radiation, or other diseases known to be associated with olfactory disturbances. All participants underwent olfactory testing using the Sniffin’ Sticks identification test (Burghart Medizintechnik, Wedel, Germany) in a modified manner (e.g., not directly smelling the pen) according to a recommendation of the German Society for Otolaryngology regarding the safe and hygienic use of smell tests during the COVID-19 pandemic (https://www.smelltest.eu/en/smell-and-taste/how-to-safely-use-smell-tests-in-relation-to-corona-virus/) [9]. Odor identification was tested using 16 different odors from a single pen by a forced choice from 4 options.

Odorants are presented in commercially available felt-tip pens. When using this test, the pen’s cap has to be removed by the examiner for approximately 3 s for odor presentation, and then the tip of the pen has to be placed about 1-2 cm in front of the nostrils.

**Statistics**

Statistical analyses were performed using SPSS26.0. To test for normal distribution, the Kolmogorov-Smirnov test was used. Parametric and nonparametric tests were used for statistical analysis depending on the distribution and the scale type of variables (see Table 1). As sensitivity analysis, we performed an ANCOVA for the comparison of odor identification results including age and gender as covariates, as it is widely known that odor identification is influenced by age and gender [10]. The Spearman correlation test was applied to determine correlation between odor identification and CAP scores. The significance level was set at 2-sided p value of <0.05 using a Bonferroni adjustment for group comparisons.

**Results**

We included 23 manifest HD patients, 13 HD carriers, and 19 HCs. Demographic data are given in Table 1. There were no group difference in age (all p values >0.05) or gender (χ² = 1.276, p = 0.528). HD patients showed significant higher scores on the total motor score (p < 0.001) and the CAP score (p < 0.001) than HD carriers. Assessing olfactory function, HD patients showed significantly impaired ability to identify odors compared to HD carriers (p = 0.003) and HCs (p < 0.001). Furthermore, HD carriers also showed significant impairment in odor identification compared to HCs (p < 0.001).

Similarly, results of the sensitivity analysis performed with an ANCOVA including age and gender as covariates revealed a statistically significant main effect from groups on odor identification (p < 0.001). Odor identification values were significantly lower in HD patients (9.6; 95% CI: 8.6–10.7) than in HD carriers (12.2; 95% CI: 10.9–13.6; p = 0.003) and HCs (14.7; 95% CI: 13.5–15.7; p < 0.001). Moreover, there was a significant difference of odor identification values between HD carriers and HCs (p < 0.001).

Furthermore, values of the Spearman correlation coefficient for odor identification and CAP scores were found to be correlated negatively (r = −0.508). Calculating effect sizes for the Sniffin’ sticks performance comparing HD carriers and HCs, we found an effect size (Cohen’s d resp. Hedges’ g) [11] of 1.094 (95% CI: 0.34–1.849).

**Discussion**

Olfactory deficits are seen in a range of neurodegenerative disorders likely due to pathological protein aggregation, neurodegeneration, or neuroinflammation [12]. To our knowledge, this is the first prospective study assessing olfactory dysfunction in preclinical HD stages using the validated Sniffin’ Sticks test.

Most studies on odor identification use either the disposable UPSIT or the reusable Sniffin’ sticks test battery [13]. The most common used test in Europe is the Sniffin’ Sticks identification subtest, as it is easy-to-use, inexpen-
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sive, and a handy tool in clinical routine and has been performed to screen for olfactory dysfunction in patients with parkinsonism [14], REM sleep behavior disorders [15], and multiple sclerosis [16].

There are few studies reporting olfactory dysfunction in manifest HD patients [2, 3], supported by a recent neuropathological study establishing mHtt in the olfactory bulb of HD patients [6]. Furthermore, there are preceding studies assessing olfaction in premanifest HD mutation carriers by using the UPSIT, which did not show significant impairment in HD carriers, whereas manifest HD patients showed impaired odor identification progressive with disease course [4, 5, 17]. This difference could be due to a higher sensitivity and specificity of the Sniffin’ stick test, which has been demonstrated in patients with REM sleep behavior disorders [15] as well as in children [18] possibly due to a decreased number of test items and/or a decreased olfactory fatigue.

The potential pathomechanism of odor dysfunction is still unclear, albeit there is assumption that mHtt aggregates could show seeding activity potentially comparable to alpha-synuclein or tau and may be present in early presymptomatic disease stages. Furthermore, imaging studies [19, 20] showed that clinical impairment in premanifest HD carriers is associated with regional brain atrophy as an early sign of subclinical disease progression.

In our cross-sectional study, we observed an association between olfactory dysfunction and disease stage of manifest and premanifest HD patients and we demonstrated a significant negative moderate-sized correlation between CAP scores and olfactory function, suggesting a linear seeding activity of mHtt and that olfactory deficits increase with the evolution of HD pathology. Nevertheless, these are preliminary data of a small sample size only tested by a subset of olfactory functioning. Our results provide indication that apart from early behavioral and cognitive changes in HD carriers [21], olfactory dysfunction could present another nonmotor symptom of HD present in very early disease stages and that further longitudinal studies are needed.

**Table 1. Demographic data**

|                    | HD patients | HD carriers | HCs   | p value* |
|--------------------|-------------|-------------|-------|----------|
| n                  | 23          | 13          | 19    |          |
| Gender (female:male) | 11:12       | 6:7         | 12:7  | 0.528    |
| Age, years         | 48.7±12.4   | 39.9±8.9    | 42.6±9.9 | 0.05     |
|                    |             |             |       | 0.069    |
|                    |             |             |       | 1.0      |
|                    |             |             |       | 0.236    |
| CAP scores         | 554.3±87.7  | 394.7±86.3  | –     | <0.001   |
| MMSE               | 27.8±1.5    | 29.9±0.6    | 29.8±0.4 | <0.001   |
|                    |             |             |       | <0.001   |
|                    |             |             |       | 0.570    |
|                    |             |             |       | <0.001   |
| UHDRS-TMS          | 35.4±24.1   | 2.5±4.3     | –     | <0.001   |
| Sniffin’ Sticks identification | 9.6±2.7 | 12.2±3.3 | 14.7±1.2 | <0.001 |
|                    |             |             |       | 0.003    |
|                    |             |             |       | <0.001   |
|                    |             |             |       | <0.001   |

The significance level is set at p < 0.05. p values of post hoc comparisons are adjusted by Bonferroni correction for multiple comparisons. CAP scores, CAG age products; HCs, healthy controls; HD patients, patients with manifest Huntington’s disease; HD carriers, Huntington’s disease mutation carriers; MMSE, Mini Mental State Examination; n, number; TMS, total motor score of the UHDRS; UHDRS, Unified Huntington’s Disease Rating Scale; ANOVA, one-way analysis of variance. * p value for overall group comparison/HD patients versus HD carrier/HD carrier versus HCs/HCs versus HD patients. a χ² test. b Parametric tests (unpaired t test; univariate ANOVA). c Nonparametric tests (Mann-Whitney U test; Kruskal-Wallis ANOVA).
Conclusion

Our results provide evidence of a simple nonmotor measurement that may be useful early in the course of HD and may offer some insight into disease mechanisms.

Acknowledgments

We thank all patients who volunteered to participate in our study.

Statement of Ethics

The study was approved by the local Ethics Committee of the Medical University of Innsbruck (approval reference number AN1979 336/4.19 401/5.10 (4464a), Austria, and all participants provided written informed consent according to the declaration of Helsinki.

Conflict of Interest Statement

Financial disclosure related to research covered in this article for all authors. The authors have no conflicts of interest to declare. Full financial disclosure for the previous 36 months: Beatrice Heim: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Dora Valent: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Federico Carbone: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Sabine Spielberger: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Florian Krismer: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Atbin Djamshidian: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: Institut de Recherches Internationales Servier, Clarion Healthcare, LLC. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Sabrina Valentin: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Klaus Seppi: stock ownership in medically-related fields: none. Intellectual property rights: none. Consultancies: none. Expert testimony: none. Advisory boards: none. Employment: Medical University Innsbruck. Partnerships: none. Contracts: none. Honoraria: None. Grants: none. Other: none. Michael J. Fox Foundation, International Parkinson and Movement Disorder Society.

Funding Sources

No funding sources have been received.

Author Contributions

B.H. involved in conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, and writing – original draft, review, and editing. D.V. involved in conceptualization, investigation, project administration, resources, software, visualization, and writing – review and editing. F.C. involved in investigation, project administration, resources, software, visualization, and writing – review and editing. S.S. involved in investigation, project administration, resources, software, visualization, and writing – review and editing. K.S. involved in conceptualization, formal analysis, methodology, project administration, resources, supervision, validation, visualization, and writing – review and editing. A.D. involved in data curation, investigation, methodology, supervision, validation, visualization, and writing – review and editing.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

1 Conneally PM, Wallace MR, Gusella JF, Wexler NS. Huntington disease: estimation of heterozygote status using linked genetic markers. Genet Epidemiol. 1984;1(1):81–8.
2 Lazic SE, Goodman AO, Grote HE, Blakemore C, Morton AJ, Hanaan AJ, et al. Olfactory abnormalities in Huntington’s disease: decreased plasticity in the primary olfactory cortex of R6/1 transgenic mice and reduced olfactory discrimination in patients. Brain Res. 2007;1151:219–26.
3 Moberg PJ, Pearson GD, Speedie IJ, Lipsey JR, Strauss ME, Folstein SE. Olfactory recognition: differential impairments in early and late Huntington’s and Alzheimer’s diseases. J Clin Exp Neuropsychol. 1987;9(6):650–64.
4 Tabrizi SJ, Langbehn DR, Leavitt BR, Roos RA, Durr A, Craufurd D, et al. Biological and clinical manifestations of Huntington’s disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. *Lancet Neurol.* 2009;8(9):791–801.
5 Bylsma FW, Moberg PJ, Doty RL, Brandt J. Odor identification in Huntington’s disease patients and asymptomatic gene carriers. *J Neuropsychiatry Clin Neurosci.* 1997;9(4):598–600.
6 Hight B, Dieriks BV, Murray HC, Faull RLM, Curtis MA. Huntingtin aggregates in the olfactory bulb in Huntington’s disease. *Front Aging Neurosci.* 2020;12:261.
7 Ross CA, Aylward EH, Wild EJ, Langbehn DR, Long JD, Warner JH, et al. Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat Rev Neurol.* 2014;10(4):204–16.
8 Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12(3):189–98.
9 Rumeau C, Nguyen DT, Jankowski R. How to assess olfactory performance with the Sniffin’ Sticks test(®). *Eur Ann Otorhinolaryngol Head Neck Dis.* 2016;133(3):203–6.
10 Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the “Sniffin’ Sticks” including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur Arch Otorhinolaryngol.* 2007;264(3):237–43.
11 Lenhard W, Lenhard A. Calculation of effect sizes. *Psychometrika.* Bibergau (Germany); 2015. Available from: http://www.psychometr`ica.de/effect_size.htm. 2015.
12 Doty RL. Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? *Lancet Neurol.* 2017;16(6):478–88.
13 Kronenbuerger M, Pilgramm M. Olfactory testing. *StatPearls.* Treasure Island (FL): StatPearls Publishing Copyright© 2021, StatPearls Publishing LLC.; 2021.
14 Krismer F, Pinter B, Müller C, Mahlknecht P, Nocker M, Reiter E, et al. Sniffing the diagnosis: olfactory testing in neurodegenerative parkinsonism. *Parkinsonism Relat Disord.* 2017;35:36–41.
15 Campabadal A, Segura B, Junque C, Serradell M, Abos A, Uribe C, et al. Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin’ Sticks test in REM sleep behavior disorder. *Parkinsonism Relat Disord.* 2019;65:197–202.
16 Bsteh G, Berek K, Hegen H, Teuchner B, Auer M, Wurth S, et al. Smelling multiple sclerosis: different qualities of olfactory function reflect either inflammatory activity or neurodegeneration. *Mult Scler.* 2020;26(1):57–68.
17 Tabrizi SJ, Reilmann R, Roos RA, Durr A, Leavitt B, Owen G, et al. Potential endpoints for clinical trials in premanifest and early Huntington’s disease in the TRACK-HD study: analysis of 24 month observational data. *Lancet Neurol.* 2012;11(1):42–53.
18 Hugh SC, Siu J, Hummel T, Forte V, Campisi P, Papsin BC, et al. Olfactory testing in children using objective tools: comparison of Sniffin’ sticks and university of Pennsylvania smell identification test (UPSIT). *J Otolaryngol Head Neck Surg.* 2015;44(1):10.
19 Schiulli RI, Hobbs NZ, Say MJ, Rechel N, Henley SM, Hyare H, et al. Clinical impairment in premanifest and early Huntington’s disease is associated with regionally specific atrophy. *Hum Brain Mapp.* 2013;34(3):519–29.
20 Ahveninen LM, Stout JC, Georgiou-Karistianis N, Lorenzetti V, Glikmann-Johnston Y. Reduced amygdala volumes are related to motor and cognitive signs in Huntington’s disease: the IMAGE-HD study. *Neuroimage Clin.* 2018;18:881–7.
21 Heim B, Pehall M, Saft C, von Hein SM, Ellmerer P, Piater JM, et al. Time will tell: decision making in premanifest and manifest Huntington’s disease. *Brain Behav.* 2020;10(11):e01843.