

**ORIGINAL ARTICLE**

High Levels of Mutant Huntingtin Protein in Tear Fluid From Huntington's Disease Gene Expansion Carriers

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ABSTRACT

Objective Huntington's disease (HD) is an autosomal dominant, fully penetrant, neurodegenerative disease that most commonly affects middle-aged adults. HD is caused by a CAG repeat expansion in the *HTT* gene, resulting in the expression of mutant huntingtin (mHTT). Our aim was to detect and quantify mHTT in tear fluid, which, to our knowledge, has never been measured before.

Methods We recruited 20 manifest and 13 premanifest HD gene expansion carriers, and 20 age-matched controls. All patients underwent detailed assessments, including the Unified Huntington's Disease Rating Scale (UHDRS) total motor score (TMS) and total functional capacity (TFC) score. Tear fluid was collected using paper Schirmer's strips. The level of tear mHTT was determined using single-molecule counting SMCxPRO technology.

Results The average tear mHTT levels in manifest ($67,223 \pm 80,360$ fM) and premanifest patients ($55,561 \pm 45,931$ fM) were significantly higher than those in controls ($1,622 \pm 2,179$ fM). We noted significant correlations between tear mHTT levels and CAG repeat length, "estimated years to diagnosis," disease burden score and UHDRS TMS and TFC. The receiver operating curve demonstrated an almost perfect score (area under the curve [AUC] = 0.9975) when comparing controls to manifest patients. Similarly, the AUC between controls and premanifest patients was 0.9846. The optimal cutoff value for distinguishing between controls and manifest patients was 4,544 fM, whereas it was 6,596 fM for distinguishing between controls and premanifest patients.

Conclusion Tear mHTT has potential for early and noninvasive detection of alterations in HD patients and could be integrated into both clinical trials and clinical diagnostics.

Keywords Tear fluid; Huntington's disease; Huntingtin; Biomarker.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant heri-

table neurodegenerative disease caused by an expansion of the CAG trinucleotide in the *HTT* gene.¹ A CAG repeat of 40 or more, resulting in an extended polyglutamine (poly-Q) tract in

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the huntingtin protein, known as mutant huntingtin (mHTT), inevitably leads to HD. HD is characterized by motor symptoms, cognitive decline and behavioral changes.² There is currently no cure for HD. However, several potential huntingtin-lowering drugs are currently undergoing human trials.³⁻⁵

Currently, the clinical diagnosis of HD is based on established signs and symptoms that occur well after the disease process begins.⁶ Biomarkers play important roles in determining patient prognosis and diagnosis, monitoring disease progression and assessing the efficacy of disease-modifying treatments in clinical trials.

Brain atrophy and other disease-related changes have been reported in HD gene expansion carriers (HDGECs) up to 10 years before estimated clinical disease onset.⁷ Biofluid markers are even more promising for the early detection of HD pathology because they are elevated in plasma and cerebrospinal fluid (CSF) approximately 24 years before expected clinical disease onset.⁸ These markers, neurofilament light (NfL) and mHTT protein, are reliable biomarkers for disease onset and progression in HD patients and are detectable in both plasma and CSF.⁹⁻¹¹ Whereas NfL is a nonspecific marker of neuronal damage, mHTT is a pathogenic protein and is only detectable in HDGECs.

Currently, plasma and CSF are used as the main sources of biofluid markers in HD. Plasma is an easily accessible fluid for biomarkers. NfL concentrations in plasma are closely associated with clinical features, brain volume and disease progression⁹; they are, however, less closely associated with estimated years to onset than NfL in CSF.⁸ The quantification of mHTT in CSF is difficult and requires ultrasensitive methods, and mHTT is often barely detectable in premanifest HDGECs.¹¹ Detection of mHTT in blood is more challenging than detection of mHTT in CSF because mHTT in CSF is present in its soluble form, whereas plasma mHTT resides intracellularly.^{12,13} Although CSF is the most reliable source for biomarkers related to HD, it must be obtained by lumbar puncture, which is an invasive procedure.

From the viewpoint of patients, minimally invasive sources of biomarkers in HD patients would be desirable, especially for longitudinal follow-up for disease progression and/or trial drug effectiveness. Since the eye is closely connected to the brain via the optic nerve and both have an embryological origin in the developing neural tube, tear fluid could be a noninvasive source of biofluid markers in HD.¹⁴ Tear fluid is composed of close to 1,500 proteins, as well as small-molecule metabolites and lipids, making it a noteworthy biomarker source.¹⁵ Tear biomarkers have been identified for multiple ocular disorders but also for neurological diseases such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.^{15,16} To our knowledge, there has been little research on noninvasive sources of HD biomarkers. Recently, saliva was reported to be a successful source for quan-

tifying mHTT comparable to plasma mHTT.¹⁷

Here, we report the results of the first study on HD biomarkers in the tear fluid of premanifest and manifest HDGECs compared to healthy controls (HCs).

MATERIALS & METHODS

Study design and participants

The study was approved by the Institutional Review Board (IRB #2021-2503-A-3). All participants signed written informed consent. The study followed the tenets of the Declaration of Helsinki 2013.

Participants were recruited from Huntington's Expertise Centre and social media. The inclusion criteria for manifest HD patients were age 18 years or older, Unified Huntington's Disease Rating Scale (UHDRS) total motor score (TMS) > 5 (maximum score 124), diagnostic confidence level of 4 (motor abnormalities that are unequivocal signs of HD [$\geq 99\%$ confidence]), CAG repeat length ≥ 36 , and total functional capacity (TFC) score > 2 (maximum score 13). The inclusion criteria for premanifest HD patients were age 18 years or older, a CAG repeat length ≥ 36 , and no clinical signs or symptoms of HD. Furthermore, the age of onset of HD and the duration of HD were documented. The inclusion criteria for HCs were age 18 years or older and no history of neurodegenerative disorders. HCs had either a family history of HD but a negative genetic test ($n = 5/20$) or no known family history of HD. The general baseline characteristics of all participants, such as age, sex, comorbidities, and medication use, were collected. The exclusion criteria for all participants were mental incapacity and brain injury (e.g., brain tumor, epilepsy, encephalitis, or cerebrovascular accident) (< 2 years before participation).

Clinical assessments

Participants were assessed using the UHDRS TMS, which is a reliable clinical rating scale used to evaluate clinical performance in HD patients.^{18,19} A higher TMS indicates a greater degree of motor impairment. In addition, the TFC was measured. This scale is a clinician checklist assessing overall functioning.^{18,20} Higher scores correspond to better functional performance. TFC scores were translated into a 5-stage disease classification that corresponds to disease severity: stage 1 (11–13); stage 2 (7–10); stage 3 (3–6); stage 4 (1–2); and stage 5 (0).^{19,21} For each participant, the disease burden score [(CAG repeat length – 35.5)*age],²² 5-year onset probability²³ and estimated time to diagnosis²³ were calculated. Premanifest patients were subdivided into early (disease burden score < 250) and late (disease burden score > 250) groups⁷ and into preA (estimated

time to diagnosis < 17.5 years) and preB (estimated time to diagnosis > 17.5 years) groups.^{7,22} Assessments were conducted by an experienced and certified neurologist or trained and certified researcher.

Tear fluid sampling and preprocessing

Tear fluid was collected from both eyes via Schirmer's paper strips (TEAR strips; Contacare Ophthalmics and Diagnostics, Gujarat, India). The paper strips were placed at the junction of the middle and lateral thirds of the lower eyelid without topical anesthesia. After 5 min, the strips were removed from the eyelid, the migration lengths (part of the strip that was wetted with tear fluid) were recorded, and the samples were stored at -80°C. Tear fluid was extracted from the Schirmer's strips by agitating small pieces of these strips in 150 µL of phosphate-buffered saline, 1% Tween 20 and cOmplete™ Protease Inhibitor Cocktail (Roche, Basel, Switzerland) at 4°C for 1.5 hours.²⁴ Subsequently, the tear fluid was eluted by centrifugation and stored at -80°C until further use.

mHTT analysis in tear samples was performed in duplicate by investigators who were blinded to the groups. Tear fluid samples from one eye only (left or right) were used for mHTT analysis and randomized. A pilot experiment showed that undiluted and 5-fold diluted tear fluid samples yielded mHTT signals within the linear range of the standard curve (data not shown). Subsequently, all the samples were diluted 5-fold and measured in technical duplicates.

mHTT quantification on SMCxPRO

The mHTT assay employs single-molecule counting (SMC™; Merck Millipore, Darmstadt, Germany) technology, which affords ultrasensitivity and a wide linear detection range. mHTT was specifically detected in this assay by immunoreaction with the capture antibody 2B7 and the detection antibody MW1. The 2B7 antibody recognizes the N-terminus of mouse and human HTT (residues 7–13), and the MW1 antibody binds to the poly-Q-expanded domain of the mHTT protein. Importantly, this assay also detects nonmutant HTT, which also contains CAG repeats but typically has a 1,000× lower signal. Prior to sample measurements, both the capture and detection antibodies were labeled and stored until further use. The 2B7 antibody (Mouse anti HTT, clone 2B7, Purified Monoclonal IgG1; Thermofisher Scientific, Waltham, MA, USA) was labeled, coated with magnetic particles (MPs) and stored at 2°C–8°C according to the manufacturer's instructions (SMC™ Capture Labeling Kit; #03-0077-02). The MW1 antibody (Merck Millipore) was labeled and stored at 2°C–8°C according to the manufacturer's instructions (SMC™ Capture Labeling Kit; #3-0076-02).

The protein standards and samples were analyzed as follows:

50 µL/well of D2X buffer + protease inhibitor (PI; 5×) was added to the assay plates (96-well V-plates). Then, 120 µL/well of standard (HTT-Q73, aa 1-573) or sample diluted 1:5 in artificial CSF (150 mM NaCl, 3 mM KCl, 1.4 mM CaCl₂, 0.8 mM MgCl₂, 0.8 mM Na₂HPO₄, 0.2 mM NaH₂PO₄) + 1% Tween-20 + PI was added to the plates in the required wells. As the samples were analyzed on two different plates, an interplate quality control (QC) was prepared by pooling 20 µL of sample from six randomly selected donors. The QC sample was diluted 1:5, similar to the study samples, and added to each plate in duplicate. A volume of 100 µL of 2B7 antibody (500 ng/mL; in assay buffer, Merck Millipore) was added to each well, and the plates were sealed and incubated under shaking (400 rpm) at room temperature. After 1.5 h, the plates were first placed on a magnetic stand and then washed once with 200 µL of wash buffer (Merck Millipore). All washing steps were performed with a Hydro-Flex™ microplate washer (Tecan, Männedorf, Switzerland). After washing, 20 µL/well of 0.22 µm-filtered MW1 detection antibody (1000 ng/mL; in assay buffer, Merck Millipore) was immediately dispensed into each well and incubated under shaking (700 rpm) at room temperature for 1 h. Each assay plate was washed, and the MPs were transferred to a new 96-well plate. After 4 rounds of washing the assay plates, 12 µL of elution buffer (Merck Millipore) was added to the wells. The plates were incubated for 6 min under shaking (700 rpm) followed by incubation on a magnetic stand for 2 min. The eluate was then transferred to a 384er read-out plate (containing neutralizing buffer D, Merck Millipore, 10 µL/well), mixed for 1–2 min on a shaker (450 rpm) and centrifuged for 2 min (1,500 rpm). The plate was then sealed with an adhesive aluminum foil sheet and incubated in the SMCxPRO™ device (Merck Millipore) for 30 min for temperature adjustment before measurement. The assay was performed blinded to the clinical state of the subjects.

The data were analyzed using GraphPad Prism (version 9.5.1; GraphPad Software, Boston, MA, USA). The mHTT concentrations in the sample were obtained by interpolating signals measured in the samples using the five-parameter logistic calibration curve generated with the standards. The back-calculated concentrations obtained from the QC samples were compared, and the variation between the two plates was calculated. A variation below 20% between the QCs was used for the analysis of the samples on the two plates together (Supplementary Table 1 in the online-only Data Supplement).

Prior to the mHTT immunoassay, the collected tear fluid was diluted in extraction buffer. Since the initial tear volume differed between participants, the dilution factor differed as well; to normalize for this, the obtained "raw" concentrations were divided by the migration length (an approximate measure of tear volume²⁴) and multiplied by the extraction buffer volume. The re-

sulting concentrations reflect “real” individual tear mHTT levels.

Statistical analyses

GraphPad Prism 10 (GraphPad Software) was used to visualize the data and calculate descriptive statistics, grouped statistics and correlation analyses. Intergroup differences were analyzed using the Kruskal–Wallis test, Mann–Whitney U test, and χ^2 test. Tear mHTT level duplicates were averaged, and the means were used for further analysis. Data distribution analysis using the Kolmogorov–Smirnov test revealed that the data were not normally distributed; hence, nonparametric statistical tests were used. Group differences were assessed via the Kruskal–Wallis test with Dunn’s multiple comparisons test. The Mann–Whitney test was used to test the difference between males and females and between early and late premanifest expansion carriers. Correlations between mHTT levels and continuous variables were analyzed by Spearman correlation. Receiver operating curves (ROCs), area under the curves (AUCs) and associated values were generated using the Wilson/Brown method. Youden’s index was calculated by deducting 1 from the sum of a test’s sensitivity and specificity expressed not as a percentage but as a part of a whole number: (sensitivity + specificity) – 1. The statistical test used for calculating the significance of each graph is indicated in the figure legend. A p value < 0.05 was considered to indicate statistical significance.

RESULTS

Thirteen premanifest HDGECs and 20 HCs were included. Among the HCs, 5 participants were gene-negative family controls, while the remaining participants were healthy community controls from non-HD families. Premanifest participants were significantly younger ($p = 0.002$) and had lower TMS ($p < 0.001$) and TFC scores ($p < 0.001$) than did manifest participants. The control group was selected to align with the average

age of the HDGECs ($p = 0.818$). No sex differences were detected between the groups ($p = 0.965$). Demographics and clinical characteristics are displayed in Table 1. Three premanifest participants had a TFC below the maximum score of 13 (range 10–12) because of reasons unrelated to HD.

The average tear mHTT levels in manifest ($67,223 \pm 80,360$ fM) and premanifest HD patients ($55,561 \pm 45,931$ fM) were significantly higher than those in HCs ($1,622 \pm 2,179$ fM) ($p < 0.0001$ for both) (Figure 1A). Tear mHTT levels were higher in manifest patients than in premanifest patients, although this difference was not statistically significant. For manifest patients, no differences in tear mHTT levels were observed between disease stages 1, 2, and 3 (Figure 1B). A marked decrease in tear mHTT levels was found in early premanifest patients ($39,283 \pm 29,666$ fM) compared to late premanifest patients ($92,187 \pm 59,231$ fM), but this difference was not statistically significant (Figure 1C). There was no statistically significant difference in tear mHTT levels between preA and preB premanifest patients (Figure 1D). Tear mHTT levels did not differ between males ($n = 21$) and females ($n = 32$) (data not shown). There was no significant correlation between tear mHTT levels and age (Figure 1E). A highly significant correlation between tear mHTT levels and CAG repeat length was detected among all the participants (Figure 1F). However, upon exclusion of HCs from the correlation analysis, the previously observed correlation became less pronounced and not statistically significant. Although reduced penetrance carriers (36–39 CAG repeats, $n = 4$) had lower tear mHTT levels ($38,242 \pm 23,776$ fM) than full penetrance carriers (≥ 40 CAG repeats, $n = 23$) ($70,687 \pm 77,239$ fM), this difference was not significant (Figure 1G).

Within the premanifest HD group, the 5-year onset probability score did not correlate with the tear mHTT level (Figure 2A), while a negative correlation was found between the number of “estimated years to diagnosis” and the tear mHTT level (Figure 2B). In the same patient group, the disease burden score correlated significantly with the tear mHTT levels (Figure 2C). Tear

Table 1. Demographics and patient characteristics of HDGECs and healthy controls

Characteristics	All HDGECs ($n = 33$)	Manifest HDGECs ($n = 20$)	Premanifest HDGECs ($n = 13$)	HC ($n = 20$)	p value for differences		
					HDGEC vs. HC	Manifest vs. premanifest	Manifest vs. premanifest vs. HC
Age	49.0 [41.5–59.0]	53.0 [48.0–61.0]	41.0 [29.5–46.5]	40.0 [35.3–64.5]	0.818 [†]	0.002 [†]	0.027*
Sex (M/F, %M)	13/20 (39)	9/11 (45)	4/9 (31)	8/12 (40)	0.965 [†]	0.414 [†]	0.716 [†]
CAG	42.0 [40.0–43.0]	41.5 [40.3–43.8]	42.0 [39.0–42.5]	19.0 [18.0–23.5]	< 0.001 [†]	0.401 [†]	< 0.001*
TMS	14.0 [3.0–28.0]	25.5 [15.8–37.0]	3 [2–4]	0.0 [0.0–1.0]	< 0.001 [†]	< 0.001 [†]	< 0.001*
TFC score	12.0 [9.0–13.0]	9.0 [8.0–12.0]	13.0 [12.5–13.0]	13.0 [13.0–13.0]	< 0.001 [†]	< 0.001 [†]	< 0.001*

Disease stage of manifest HDGECs is as follows: stage 1 ($n = 6$, 30%), stage 2 ($n = 12$, 60%), stage 3 ($n = 2$, 10%), and stage 4 and 5 ($n = 0$, 0%). Data represents median [Q1–Q3] or absolute n (%). $p < 0.05$ was considered significant.

Intergroup differences were analyzed using Kruskal–Wallis*, Mann–Whitney U[†], χ^2 statistics[†].

HDGECs, Huntington’s disease gene expansion carriers; HC, healthy controls; M, male; F, female; CAG, CAG repeat length; TMS, total motor score; TFC, total functional capacity.

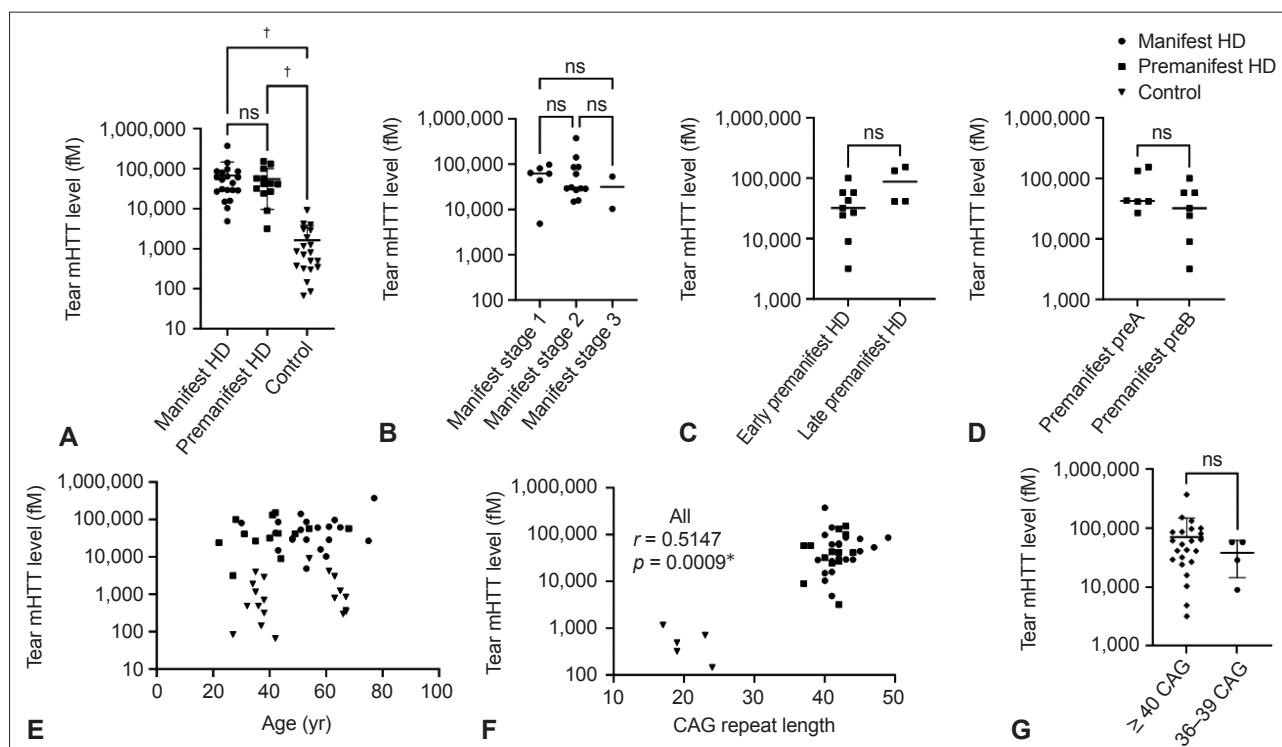


Figure 1. Tear mHTT group differences. A: Tear mHTT levels differed significantly between manifest ($n = 20$) and premanifest ($n = 13$) patients compared to healthy controls ($n = 20$). B: Within the manifest group, no significant differences were found between disease stage 1 ($n = 6$), stage 2 ($n = 12$), and stage 3 ($n = 2$). C and D: Within the premanifest group, there was no statistically significant difference in tear mHTT levels between early ($n = 9$) and late ($n = 4$) premanifest patients (C) or between preA and preB premanifest patients (D). E: There was no correlation between tear mHTT levels and age. F: Significant correlations were found between tear mHTT levels and CAG repeat length for all participants. G: There was no statistically significant difference in tear mHTT levels between carriers with reduced penetrance (36–39 CAG repeats, $n = 4$) and those with full penetrance (≥ 40 CAG repeats, $n = 23$). Group differences were analyzed by the Kruskal–Wallis test with Dunn’s multiple comparisons test. The Mann–Whitney test was used to evaluate the difference between early and late premanifest HD and between preA and preB premanifest HD. All correlations were analyzed by Spearman correlation. The horizontal bars indicate the means $\pm$ standard deviations. * $p < 0.001$; † $p < 0.0001$. HD, Huntington’s disease; ns, not significant; preA, estimated time to diagnosis < 17.5 years; preB, estimated time to diagnosis > 17.5 years.

mHTT levels strongly correlated with UHDRS TMS (Figure 2D) and UHDRS TFC (Figure 2E). However, following the exclusion of HCs and premanifest patients from the correlation analysis, the previously identified correlations were no longer observable.

We analyzed the sensitivity and specificity of tear mHTT by examining its ability to discriminate between manifest HD patients and controls, between premanifest HD patients and controls and between manifest and premanifest HD patients. The ROC curves and associated values are shown in Figure 3. The area under the ROC curve (AUC) between the control and manifest groups was 0.9975, indicating that the test yielded the correct answer 99.75% of the time (Figure 3A). The AUC between controls and premanifest participants was 0.9846, indicating a discriminatory ability of 98.46% or a 98.46% probability of the test giving the correct answer (Figure 3B). There was no significant difference in the ability of tear mHTT to discriminate between the premanifest and manifest disease groups (Figure 3C). The Youden index measures the effectiveness of a diagnos-

tic marker and enables the selection of an optimal threshold value (cutoff point) for the marker. The best cutoff value (= highest Youden index) to distinguish between controls and manifest patients was 4,544 fM, whereas this value was 6,596 fM between controls and premanifest patients (Tables 2 and 3).

DISCUSSION

HD is an autosomal dominant inheritable neurodegenerative disease caused by an expansion of the CAG trinucleotide in the *HTT* gene.¹ This CAG repeat results in an extended poly-Q tract in the huntingtin protein, known as mHTT. CSF is currently the most reliable source of mHTT; however, it requires an invasive procedure for sampling.

We are the first to report the detection and quantification of mHTT in tear fluid. Prior research has primarily focused on CSF and blood, with only a limited number of publications reporting mHTT levels in saliva.^{11,17,25} However, in these three body fluids,

mHTT was difficult to detect in premanifest HDGEs and was undetectable in HCs. Our results showed that mHTT was detectable in tear fluid in all subjects (100%), including all premanifest

HDGEs and all HCs. The detection of mHTT in all HDGEs is beneficial because it allows the quantification of mHTT in each patient. The detection of mHTT in HCs is likely attribut-

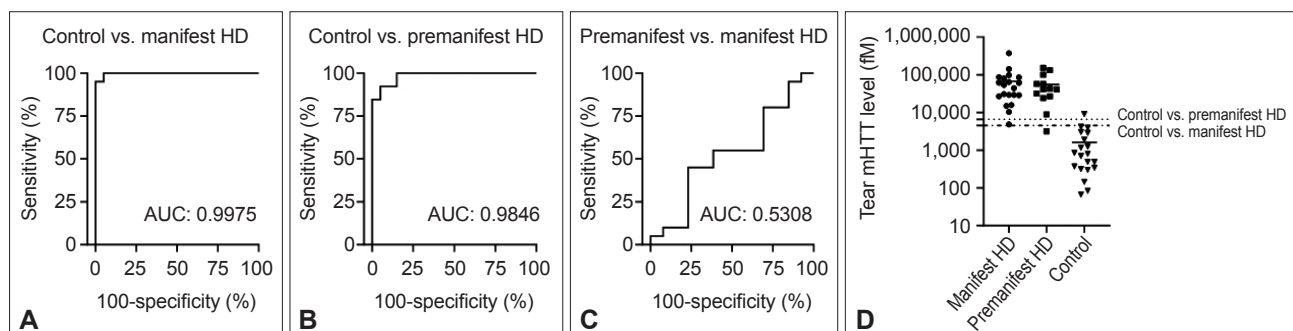
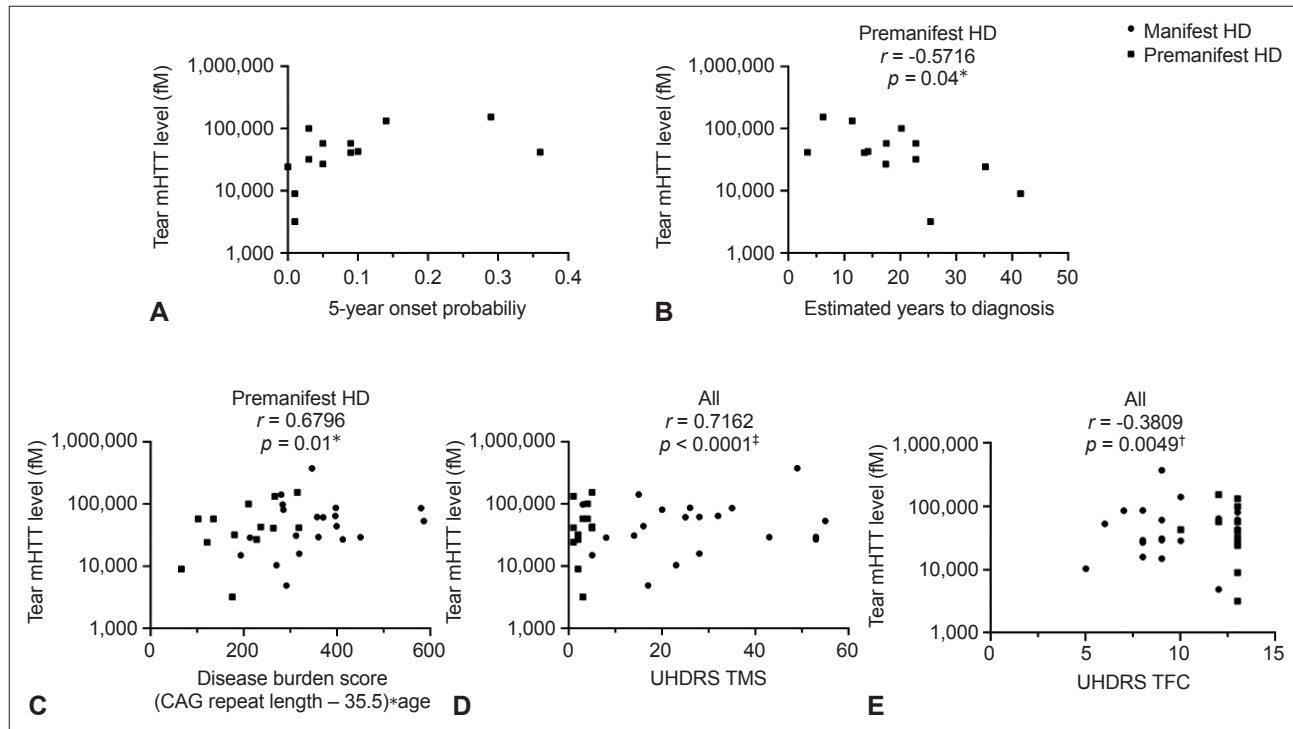


Table 2. Results of the ROC curves

	AUC	SD	Lower 95% CI	Upper 95% CI	p value
Control vs. manifest HD	0.9975	0.004	0.9893	1.0000	< 0.0001
Control vs. premanifest HD	0.9846	0.016	0.9535	1.0000	< 0.0001
Premanifest vs. manifest HD	0.5308	0.106	0.3223	0.7392	0.7682

HD, Huntington’s disease; ROC, receiver operating curve; AUC, area under the curve; SD, standard deviation; CI, confidence interval.

Table 3. Results of the cutoff values

	Cutoff (fM)	Sensitivity (%)	Specificity (%)	Youden index
Control vs. manifest HD	4,544	100	95	95.00
Control vs. premanifest HD	6,596	92.31	95	87.31

HD, Huntington's disease.

able to the fact that the MW1 antibody used in our assay binds not only to elongated poly-Q stretches of mHTT but also to shorter poly-Q stretches, such as those of wild-type HTT.^{10,26} Despite this nonspecific binding, mHTT levels in the tear fluid of HCs were markedly lower than those in HDGECs, and the ability of mHTT in tear fluid to distinguish HCs from manifest and premanifest HDGECs was 99.75% and 98.46%, respectively.

Although tear mHTT levels serve as a clear indicator for distinguishing between HD patients and controls, we did not observe significant differences when subgrouping manifest patients based on disease stage (1/2/3) and premanifest patients based on disease burden score (early/late) or estimated years to diagnosis (preA/preB). Tear mHTT levels were much higher in patients with late premanifest HD than in patients with early manifest HD, but this difference was not statistically significant due to the small sample size of the patient subgroups. Similar to the results of Wild et al.,¹¹ who quantified mHTT in CSF, our study showed that mHTT in tear fluid was significantly correlated with CAG repeat length, disease burden score, estimated years to diagnosis, and the UHDRS TFC and TMS scores. The latter result suggested that tear mHTT levels are also predictive of HD clinical parameters beyond the known predictive ability of age and CAG repeat length.

The tominersen trial (NCT02519036) marks a significant milestone, as it was the first trial of a huntingtin-lowering therapy to successfully demonstrate a reduction in mHTT levels in CSF.²⁷ Our findings may serve as a valuable addition to future disease-modifying therapy trials by providing a noninvasive method for mHTT quantification and, consequently, for assessments of target engagement or drug efficacy; this will create a more participant-friendly approach, eliminating the need for lumbar punctures between dosing days.

Tear fluid also contributes to the cost-efficiency of future patient and drug monitoring. Since tear fluid can be collected in just 5 minutes, it is less time-consuming than performing a lumbar puncture. Moreover, this method can be performed outside academic hospitals or study sites without the need for specifically skilled health professionals, which will minimize health costs for patients. Additionally, this method allows significant reductions in hospital encounters and travel time, especially for less mobile patients and patients living in remote areas; hence, it will reduce the environmental impact of health care.

Previous research has indicated that tear fluid is a valuable source of biomarkers for neurological and neurodegenerative diseases. Our group recently demonstrated that the amyloid-beta peptides A β 38, A β 40 and A β 42, total tau (t-tau) and phosphorylated-tau (p-tau) are detectable in the tear fluid of patients with cognitive decline.^{28,29} Tear fluid has further revealed its potential in the context of other neurodegenerative disorders, including Alzheimer's disease,^{16,28-30} Parkinson's disease^{31,32} and multiple sclerosis.^{33,34} However, it has never been explored before for HD. When comparing the levels of biomarkers in CSF and tear fluid, the absolute biomarker levels in tear fluid were substantially higher than those in CSF. In the present study, we observed approximately 230 times higher levels of mHTT in tear fluid than in CSF in manifest patients and a 600-fold difference between tear fluid and CSF in premanifest HD patients.^{9,11} Higher analyte levels lead to enhanced reliability of the results since the detection signals are well above the limit of detection. In addition, as tear fluid can be collected in a noninvasive, inexpensive and easy way, this presents a promising avenue for screening, diagnosing and monitoring biomarkers for neurological and neurodegenerative diseases beyond their application in HD.

A few limitations of the present study must be considered. First, we did not have paired CSF samples; therefore, our mHTT tear fluid levels can only be compared to mHTT levels in CSF from previous reports. Second, our study did not include patients with advanced disease stages 4 and 5. Finally, longitudinal data on mHTT in tear fluid were not collected.

In conclusion, the detection of high levels of mHTT in tear fluid from HDGECs in our study confirmed that tear fluid is a valuable source of HD biomarkers. Ultimately, tear biomarkers could help the HD community noninvasively measure the effectiveness of disease-modifying drugs and determine the optimal time and dose to start such therapies.

Supplementary Materials

The online-only Data Supplement is available with this article at <https://doi.org/10.14802/jmd.24014>.

Conflicts of Interest

University Maastricht and the Academic Hospital Maastricht, with MG and MO as inventors, have submitted a patent application EP23197746.

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