

**Title:** Salivary levels of total huntingtin are elevated in Huntington's disease patients

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Figure S1. Western blot of Htt protein in saliva from normal and HD individuals. Saliva supernatants were used as-is (1x) or were concentrated 4-fold by vacuum centrifugation from 4 normal subjects (C5-C8) in (A), and in matched HD and control pairs (n=3) in (B). Supernatant fractions were separated by 7% SDS-PAGE and blotted onto nitrocellulose membranes for immunodetection using MAB2166 antibody (1:1,000) (A) or the 4E10 antibody (1:1,000) and beta-actin (1:1,000) (B). Gel image was acquired using a Fluorochem E imager.

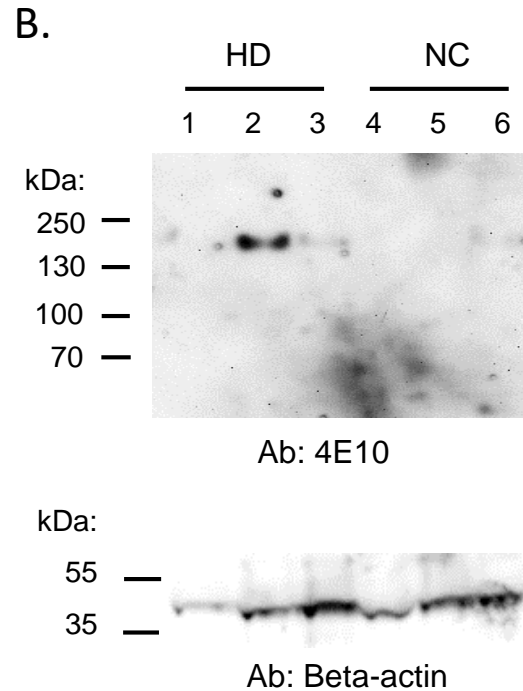
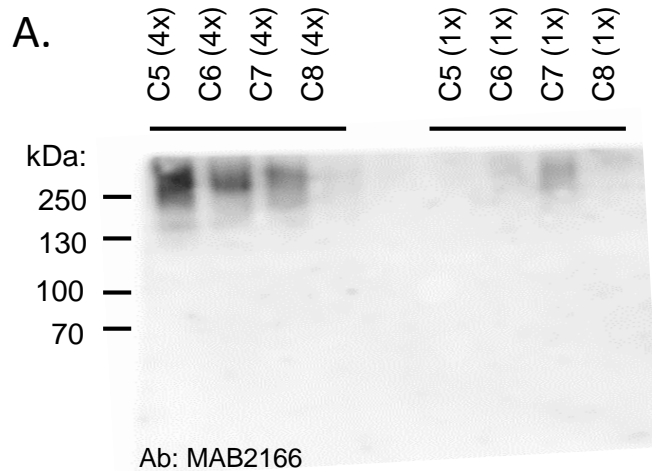


Figure S2. Levels of tHtt in medicated versus unmedicated HD patients. Levels of tHtt were determined by ELISA. The amount of tHtt (ng/ml) reported is relative to a recombinant Htt protein of 139 amino acids per the ELISA assay. We did not observe differences in salivary tHtt in HD patients who were taking anti-depressant or anti-anxiety medications compared to drug-free patients.

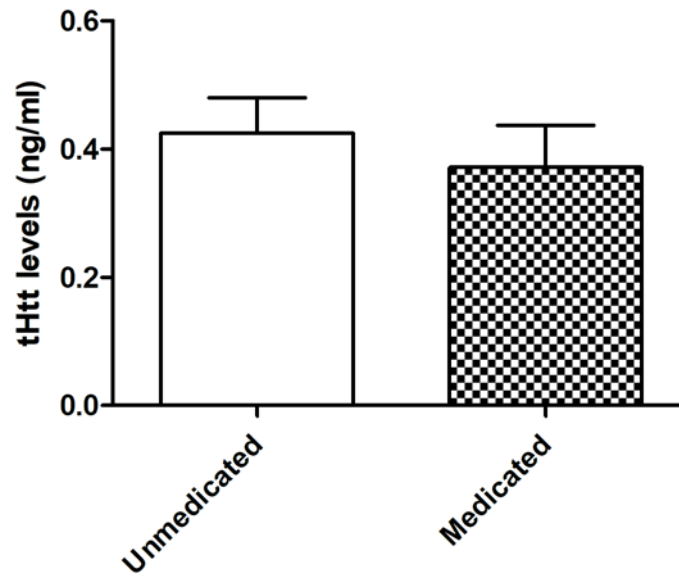


Figure S3. Linearity of dilution of tHtt in saliva. Levels of tHtt were determined by ELISA. The amount of tHtt (ng/ml) reported is relative to a recombinant Htt protein of 139 amino acids per the ELISA assay. The spiked-in recovery for the saliva matrix was 91.2% +/- 4.05% and the  $R^2$  value for linear regression = 0.976.

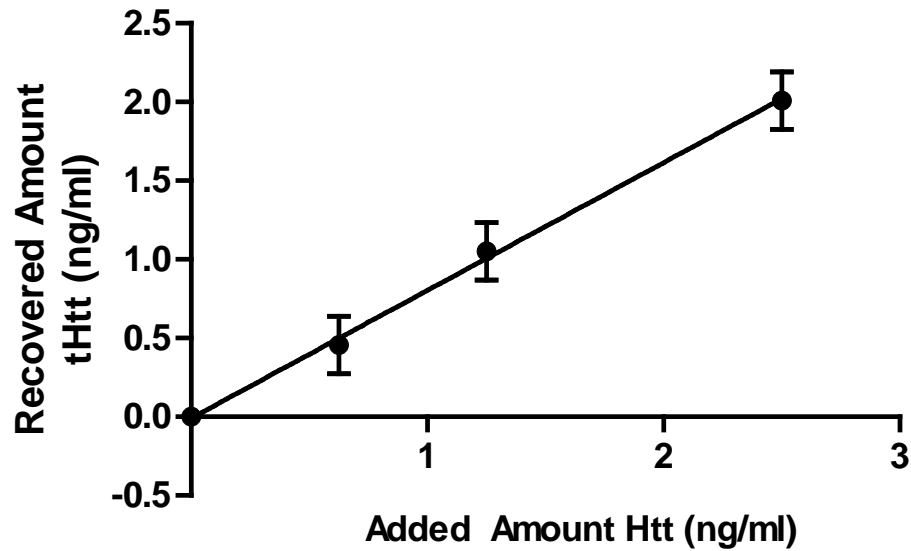


Table S1. Clinical data from the subjects used in this study.

COHORT 1												
		CAG repeat #	Age of Onset (yrs)	PAO (yrs)	Langbehn # (yrs)	CAP	DBS	TFC	MMSE	Motor	DCL	tHtt (ng/ml)
<b>HD</b> <b>N=23</b>	Mean	43.167	48.208	48.696	6.575	484.667	385.000	9.250	26.667	32.083	4.000	0.547
	SD	3.031	11.538	14.294	3.772	85.144	92.240	2.005	2.808	13.227	0.000	0.079
	Min	37.000	22	30	3.930	220.440	99.000	6	20	9	4	0.043
	Max	50.000	68	70	23.000	678.700	577.500	13	30	63	4	1.530
<b>LPM</b> <b>N=9</b>	Mean	42.250	53.625	51.375	7.183	452.285	350.625	12.000	28.250	10.375	3.000	0.425
	SD	2.315	14.667	14.071	1.357	30.338	41.552	1.195	1.832	3.926	0.926	0.112
	Min	40.000	36	38	5.14	396.36	297.00	10	25	6	2	0.048
	Max	47.000	71	70	8.98	485.68	414.00	13	30	17	4	1.240
<b>EPM</b> <b>N=10</b>	Mean	41.222		55.111	18.548	303.876	225.778	12.889	28.111	2.222	0.444	0.436
	SD	3.420		13.941	12.261	116.381	113.274	0.333	1.764	1.716	0.527	0.133
	Min	38.000		32	4.97	165.54	105.00	12	25	0	0	0.149
	Max	48.000		75	37.86	487.56	425.00	13	30	5	1	1.520
<b>AR-</b> <b>N=16</b>	Mean	23.077						13.000	28.813	1.563	0.250	0.368
	SD	6.157						0.000	1.601	1.153	0.447	0.073
	Min	17.000						13	25	0	0	0.021
	Max	33.000						13	30	4	1	0.935
<b>NC</b> <b>N=30</b>	Mean							12.667	28.611	2.444	0.278	0.340
	SD							0.767	2.570	2.684	0.461	0.046
	Min							10	20	0	0	0.028
	Max							13	30	9	1	1.070

Table S1, cont. Clinical data from the subjects used in this study.

COHORT 2												
		CAG repeat #	Age of Onset (yrs)	PAO (yrs)	Langbehn # (yrs)	CAP	DBS	TFC	MMSE	Motor	DCL	tHtt (ng/ml)
<b>HD</b>	Mean	42.250	52.750	58.875	6.231	485.938	376.688	8.500	24.429	33.500	4.000	0.593
<b>N=14</b>	SD	2.816	11.732	13.632	1.268	46.951	58.990	3.505	2.507	11.314	0.000	0.077
	Min	40.000	28	27	3.940	411.040	308.000	2	19	15	4	0.293
	Max	49.000	68	70	8.220	567.580	499.500	13	27	52	4	1.320
<b>LPM</b>	Mean	42.000	38.000	45.000	12.810	341.940	266.500	13.000	27.000	14.000	4.000	0.732
<b>N=1</b>	SD											
	Min											
	Max											
<b>EPM</b>	Mean	41.400		52.667	20.085	323.995	245.733	13.000	28.333	1.933	0.467	0.462
<b>N=16</b>	SD	1.724		12.838	15.452	83.306	75.765	0.000	1.496	1.831	0.640	0.055
	Min	38.000		35	7.16	165.54	108.50	13	25	0	0	0.106
	Max	44.000		75	66.47	448.32	360.00	13	30	6	2	0.881
<b>AR-</b>	Mean	19.800						13.000	28.400	0.800	0.200	
<b>N=13</b>	SD	3.834						0.000	1.517	1.304	0.447	
	Min	17.000						13	26	0	0	
	Max	26.000						13	30	3	1	
<b>NC</b>	Mean							12.909	28.727	1.909	0.182	0.378
<b>N=14</b>	SD							0.302	0.905	1.814	0.405	0.068
	Min							12	27	0	0	0.029
	Max							13	30	5	1	0.729

HD, Huntington's disease; LPM, late pre-manifest; EPM, early pre-manifest; AR-, at-risk, gene negative; NC, normal controls.

PAO, paternal age of onset; CAP, normalized CAG Age Product; DBS, disease burden score; TFC, total functional capacity score.

MMSE, mini-mental state exam; Motor, motor symptom score; DCL, diagnostic confidence level.

"Langbehn #" is based on a formula that estimates years to onset in HD (Langbehn DR et al., Clin Genet. 65:267-77, 2004).