Supporting Information

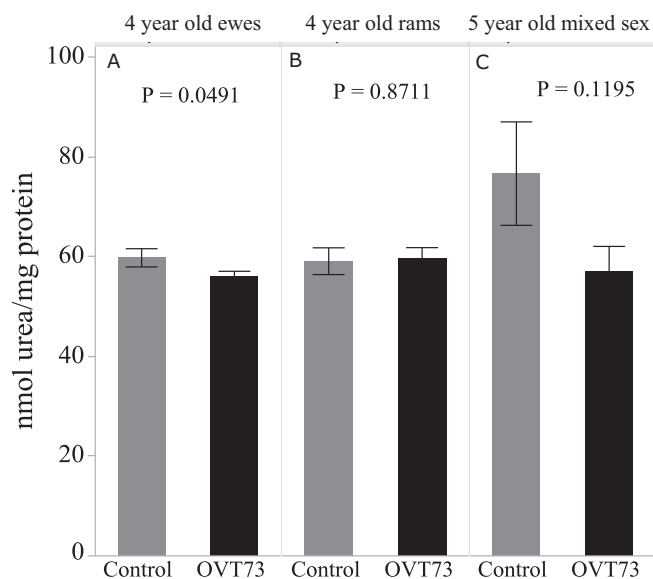## Handley et al. 10.1073/pnas.1711243115

Fig. S1. Urea concentration in *OVT73* sheep serum. Serum urea was quantified in three separate sheep cohorts: (*A*) 4-y-old ewes (23 OVT73, 16 control), (*B*) 4-y-old rams, and (*C*) mixed-sex 5-y-olds (six *OVT73*, six control). Average urea concentration (nanomole per milligram of protein ± SEM) is presented for *OVT73* and control groups. The *OVT73* 4-y-old ewes show modestly lower serum urea concentration on average compared with controls (*$P < 0.05$; two-way Student's *t* test). SEM, standard error of the mean.

Table S1. NanoString analysis of 24 genes of interest in the *OVT73* sheep striatum

Gene name	RNA-seq template	Anterior striatum, DM					Anterior striatum, DL				
		Control, count ± SEM	OVT73, count ± SEM	Fold change	P	Adjusted P	Control, count ± SEM	OVT73, count ± SEM	Fold change	P	Adjusted P
AP2S1	Transcriptome	2,306.2 ± 70	2,413.5 ± 75.4	1.05	0.32	0.41	2,717.2 ± 289.6	2,106.2 ± 149.2	0.78	0.09	0.47
AVPR1A	Gene Models	72.6 ± 26.7	26.4 ± 5.5	0.36	0.14	0.22	78.3 ± 20.5	46.6 ± 12.4	0.59	0.21	0.47
CBS	Transcriptome	456.0 ± 18.5	546.0 ± 24.7	1.20	**0.02**	0.06	336.5 ± 25.1	380.1 ± 24.2	1.13	0.24	0.47
CHST8	Transcriptome	233.7 ± 29.9	193.6 ± 16.4	0.83	0.27	0.36	314.1 ± 54.0	214.0 ± 30.7	0.68	0.14	0.47
CMTM5	Transcriptome	1,223.5 ± 180	893.7 ± 98.7	0.73	0.10	0.18	1,819.6 ± 295.8	2,042.7 ± 223.5	1.12	0.56	0.75
CNTN2	Transcriptome	1,994.3 ± 229.4	1,680.4 ± 281.9	0.84	0.41	0.49	2,435.0 ± 327.4	2,977.6 ± 293.5	1.22	0.25	0.47
CPAMD8	Gene Models	42.4 ± 8.1	22.0 ± 4.0	0.52	**0.05**	0.11	17.5 ± 6.2	19.9 ± 5.5	1.14	0.63	0.76
ENS11790	Transcriptome	1.4 ± 0.1	1.3 ± 0.2	0.90	0.48	0.52	1.6 ± 0.1	1.4 ± 0.1	0.86	0.26	0.47
ETV5	Gene Models	1,780.4 ± 192.6	2,651.7 ± 252.6	1.49	**0.02**	0.07	1,333.6 ± 70.7	1,459.0 ± 215.3	1.09	0.59	0.75
FEZF2	Both	215.6 ± 71.4	1,116.4 ± 17.7	0.54	0.16	0.22	203.0 ± 33.7	196.7 ± 27.1	0.97	0.89	0.97
HPN	Gene Models	44.7 ± 10.3	19.9 ± 2.6	0.45	0.07	0.13	43.9 ± 7.9	41.6 ± 7.4	0.95	0.84	0.96
HSD17B12-like	Both	43.8 ± 10.7	81.6 ± 14.2	1.86	0.06	0.13	23.1 ± 6.1	52.8 ± 14.3	2.29	0.09	0.47
ITGB4	Transcriptome	94.2 ± 8.3	60.2 ± 8.1	0.64	**0.02**	0.06	77.3 ± 20.2	101.2 ± 18.7	1.31	0.40	0.57
MYL4	Transcriptome	211.1 ± 43	250.5 ± 31.2	1.19	0.48	0.52	149.8 ± 26.8	113.4 ± 15.3	0.76	0.27	0.47
OTX2	Transcriptome	16.3 ± 3.5	18.8 ± 3.5	1.16	0.61	0.64	7.6 ± 2.5	7.5 ± 2.2	0.99	0.99	0.99
OXTR	Gene Models	50.5 ± 13.5	110.3 ± 14.6	2.18	**0.01**	0.06	90.0 ± 21.0	60.8 ± 7.5	0.68	0.22	0.47
PRKG1	Transcriptome	336.3 ± 26.4	343.8 ± 22.5	1.02	0.83	0.83	359.9 ± 60.5	248.0 ± 29.4	0.69	0.13	0.47
RHCG	Transcriptome	80.7 ± 11.4	118.6 ± 12.3	1.47	**0.05**	0.11	38.9 ± 6.5	50.6 ± 11.9	1.30	0.40	0.57
SIAH3	Both	167.2 ± 10.6	230.5 ± 9.3	1.38	**0.001***	**0.03**	94.1 ± 15.4	150.3 ± 39.2	1.60	0.37	0.57
SLC14A1	Gene Models	483.6 ± 82.1	1,061.5 ± 149.9	2.20	**0.01**	0.06	164.4 ± 62.7	892.1 ± 196.6	5.43	**0.01**	**0.13**
SLC5A7	Both	300.4 ± 29.7	458.5 ± 37.9	1.53	**0.01**	0.06	165.1 ± 43.4	295.8 ± 104.7	1.79	0.27	0.47
SMOC2	Transcriptome	62.2 ± 13.3	113.4 ± 14.8	1.82	**0.03**	0.08	67.1 ± 12.6	107.0 ± 22.2	1.59	0.12	0.47
TF	Transcriptome	19,506.9 ± 3,860.4	11,825.1 ± 1951.9	0.61	0.11	0.18	32,959.1 ± 7668.9	32,399.3 ± 3842.8	0.98	0.95	0.99
ZNF804A	Transcriptome	706.2 ± 75.4	849.1 ± 52.3	1.20	0.15	0.22	626.6 ±	481.3 ± 84.8	0.77	0.23	0.47

NanoString nCounter quantification of 24 target genes of interest identified through RNA-seq analysis confirmed that 10 transcripts were altered in *OVT73* anterior striatum RNA (DM portion), with one transcript (*SLC14A1*) also differentially expressed in a DL portion of anterior striatum ($P < 0.05$; two-way Student's t test). Mean normalized counts ± SEM for control ($n = 6$) and *OVT73* ($n = 6$), fold change (*OVT73*/control), and P values (*P*; nonadjusted, and false-discovery rate adjusted using the Benjamini–Hochberg technique) are shown for each transcript in each tissue. Gene names and P values for the 10 validated transcripts are shown in bold typeface. *Significant ($P < 0.05$) after false-discovery rate adjustment. Means and SEM shown are back-transformed (from $\log_{10}$ data) where appropriate. Transcript counts were normalized to the geometric mean of four reference genes before statistical analysis: *CANX*, *SHMT2*, *TRIM27*, and *TRIP11*. The RNA-seq analysis mapping approach through which each gene of interest was identified is indicated (Gene Models, de novo transcriptome, or both).

Table S2. Differential pairwise correlations of gene expression in the *OVT73* sheep striatum

Gene 1	Gene 2	Control correlation, *r*	Control *P* value	*OVT73* correlation, *r*	*OVT73 P* value	Fisher *r*-to-*z* *P* value	BH-adjusted *P* value
ITGB4	*RHCG*	−0.93	0.01	0.47	0.35	0.01*	0.49
RHCG	*SLC14A1*	−0.29	0.57	0.90	0.01	0.03*	0.77
SIAH3	*SMOC2*	−0.13	0.80	0.89	0.02	0.06	0.79
CBS	*SLC14A1*	0.81	0.05	−0.28	0.59	0.08	0.79
RHCG	*SLC5A7*	0.75	0.08	−0.33	0.53	0.11	0.79
CPAMD8	*SMOC2*	−0.68	0.14	0.42	0.40	0.12	0.79
CPAMD8	*SIAH3*	−0.51	0.30	0.53	0.28	0.16	0.79
OXTR	*RHCG*	0.49	0.32	−0.54	0.27	0.16	0.79
SIAH3	*SLC14A1*	0.13	0.80	0.85	0.03	0.16	0.79
ETV5	*SLC14A1*	0.35	0.49	−0.63	0.18	0.17	0.79
CPAMD8	*RHCG*	−0.27	0.60	0.65	0.16	0.20	0.80
CPAMD8	*SLC14A1*	−0.080	0.89	0.74	0.09	0.20	0.80
RHCG	*SMOC2*	−0.34	0.51	0.50	0.31	0.27	0.82
ETV5	*RHCG*	0.43	0.40	−0.42	0.41	0.27	0.82
SLC5A7	*SMOC2*	−0.30	0.57	0.52	0.29	0.28	0.82
ITGB4	*SIAH3*	−0.56	0.25	0.24	0.65	0.28	0.82
OXTR	*SMOC2*	0.30	0.57	−0.46	0.36	0.33	0.90
CBS	*SIAH3*	0.57	0.24	−0.10	0.85	0.36	0.94
OXTR	*SIAH3*	−0.13	0.80	−0.65	0.17	0.44	0.95
SLC14A1	*SMOC2*	0.40	0.43	0.78	0.07	0.46	0.95
ETV5	*SMOC2*	0.16	0.76	−0.41	0.42	0.46	0.95
CBS	*RHCG*	0.16	0.77	−0.39	0.45	0.49	0.95
CPAMD8	*ETV5*	−0.14	0.80	−0.57	0.24	0.53	0.95
ITGB4	*SLC14A1*	−0.02	0.96	0.44	0.38	0.54	0.95
SIAH3	*SLC5A7*	0.65	0.17	0.29	0.57	0.57	0.95
SLC14A1	*SLC5A7*	0.33	0.52	−0.10	0.85	0.59	0.95
RHCG	*SIAH3*	0.42	0.40	0.71	0.11	0.59	0.95
CPAMD8	*ITGB4*	0.51	0.31	0.75	0.09	0.62	0.95
CBS	*ITGB4*	−0.50	0.31	−0.74	0.09	0.62	0.95
ETV5	*OXTR*	0.31	0.55	−0.08	0.88	0.62	0.95
CBS	*CPAMD8*	−0.54	0.27	−0.22	0.67	0.64	0.95
CPAMD8	*SLC5A7*	−0.18	0.74	−0.48	0.33	0.67	0.95
CPAMD8	*OXTR*	−0.53	0.28	−0.73	0.10	0.67	0.95
ETV5	*SLC5A7*	0.52	0.29	0.25	0.63	0.70	0.95
ITGB4	*SLC5A7*	−0.85	0.03	−0.74	0.09	0.72	0.95
CBS	*SMOC2*	0.45	0.37	0.19	0.72	0.73	0.95
OXTR	*SLC5A7*	−0.09	0.87	0.13	0.81	0.79	0.95
ETV5	*SIAH3*	−0.09	0.87	−0.28	0.59	0.81	0.95
CBS	*SLC5A7*	0.63	0.18	0.57	0.24	0.90	0.98
ITGB4	*SMOC2*	0.05	0.93	−0.05	0.93	0.91	0.98
CBS	*OXTR*	−0.24	0.65	−0.16	0.77	0.92	0.98
ETV5	*ITGB4*	−0.52	0.29	−0.46	0.36	0.92	0.98
OXTR	*SLC14A1*	−0.58	0.23	−0.55	0.26	0.95	0.98
CBS	*ETV5*	0.39	0.44	0.42	0.41	0.97	0.98
ITGB4	*OXTR*	−0.42	0.41	−0.40	0.43	0.98	0.98

Pairwise (Pearson) correlations are shown for all possible pairings of the 10 differentially expressed genes in the *OVT73* anterior striatum (*OVT73*/control, *P* < 0.05; refer to Fig. 1), as quantified by NanoString nCounter analysis. Correlation coefficients (*r*) and associated *P* values are shown for each gene pairing, within *OVT73* (*n* = 6) and control (*n* = 6) groups. **P* < 0.05, Fisher *r*-to-*z* transformation. No differences were significant after Benjamini–Hochberg (BH) adjustment of the *P* values.

Table S3. Expression of key urea cycle enzymes quantified in RNA-seq data from sheep striatum tissue

Animal	Status	Genes of the urea cycle						
		NAGS	*CPS1*	*OTC*	*ASS1*	*ASL*	*ARG1*	*ARG2*
1	Control	0.148	0.000	0.000	4.226	2.867	0.646	0.327
2	Control	0.128	0.005	0.000	3.374	2.464	0.814	0.417
3	Control	0.149	0.008	0.009	4.062	2.132	0.564	0.428
4	Control	0.233	0.004	0.000	3.820	1.819	0.550	0.524
5	Control	0.229	0.000	0.000	6.339	2.267	0.507	0.377
6	Control	0.112	0.008	0.000	2.754	1.384	0.649	0.438
7	*OVT73*	0.208	0.009	0.000	5.055	2.546	0.749	0.341
8	*OVT73*	0.227	0.018	0.000	6.324	2.662	0.565	0.463
9	*OVT73*	0.212	0.000	0.000	5.807	2.251	0.476	0.533
10	*OVT73*	0.166	0.000	0.000	6.914	2.072	0.550	0.397
11	*OVT73*	0.160	0.000	0.000	3.584	1.606	0.604	0.558
12	*OVT73*	0.184	0.004	0.000	3.574	2.177	0.459	0.472
Mean control		0.167	0.004	0.002	4.096	2.156	0.622	0.419
Mean *OVT73*		0.193	0.005	0.000	5.210	2.219	0.567	0.461
P value (OVT73 vs. control)		0.296	0.768	0.341	0.173	0.812	0.399	0.348

Expression values are shown for key urea cycle genes quantified in the striatum, as measured in individual sheep ($n = 6$ control, 6 *OVT73*) by RNA-seq. Values presented are normalized by the FPKM method. There was no significant difference in the expression of any of the urea cycle genes between *OVT73* and control groups (*$P < 0.05$, ANOVA). *ARG1*, arginase 1; *ARG2*, arginase 2; *ASL*, arginosuccinate lyase; *ASS1*, arginosuccinate synthase; *CPS1*, carbamoyl phosphate synthase; *NAGS*, *N*-acetylglutamate synthase; *OTC*, ornithine carbomoyltransferase. Expression of *CPS1* and *OTC* in the striatum was absent or negligible in all animals [the median FPKM value in the entire dataset (total RNA) was 0.187].

Table S4. Transgene and sex effects on urea concentration in the *OVT73* sheep

Tissue	Transgene effect				Sex effect	Additive model	
	Control ± SEM	OVT73 ± SEM	Fold difference	*P* value	*P* value	Transgene effect *P* value	Sex effect *P* value
Bladder	242.7 ± 25.3	268.1 ± 16.4	1.10	0.42	0.11	NA	NA
Heart	140.0 ± 19.3	144.1 ± 6.4	1.03	0.83	0.12	NA	NA
Kidney	104.2 ± 13.6	105.3 ± 17.3	1.01	0.97	0.002*	0.45	0.002*
Liver	120.8 ± 13.5	123.5 ± 10.5	1.02	0.87	0.25	NA	NA
Serum	76.7 ± 10.4	57.1 ± 5.0	0.74	0.12	0.01*	0.10	0.01*
Urine	809.7 ± 157.3	682.0 ± 168.0	0.84	0.59	0.005*	0.84	0.01*
Anterior striatum	154.2 ± 8.6	236.7 ± 35.2	1.54	0.02*	0.35	NA	NA
Cerebellum	46.6 ± 19.8	81.4 ± 17.5	1.75	0.22	<0.0001*	0.04*	<0.0001*
Hippocampus	66.9 ± 9.1	54.0 ± 5.8	0.81	0.26	0.19	NA	NA
Motor cortex	52.7 ± 12.5	69.9 ± 10.3	1.33	0.31	0.02*	0.40	0.03*

Urea was quantified by biochemical assay in peripheral (bladder, heart, kidney, liver, serum, and urine) and brain (DM, cerebellum, hippocampus, and motor cortex) tissues dissected from 5-y-old *OVT73* ($n = 6$) and control ($n = 6$) sheep. Mean urea concentration (nanomole per milligram protein) is shown (±SEM) for *OVT73* and control groups and the fold difference (*OVT73*/control). *P* values indicate the statistical significance of the effects on urea concentration of transgene status and sex, respectively (*$P < 0.05$; two-way Student's *t* test). Where a significant effect of sex was observed, an additive statistical model was generated including both transgene status and sex as variables. *P* values for the respective transgene and sex effects within the additive model are shown. Before statistical analysis, urea concentrations for the anterior striatum and kidney were log$_{10}$-transformed to approximate a normal distribution (values shown above are back-transformed). Urea concentration was significantly higher on average in *OVT73* cerebellum compared with controls when variability due to sex was accounted for ($P = 0.04$). NA, not applicable.

Table S5. Mixed-grade HD case cohort

ID	Status	Sex	Age at death, y	PM delay, h	CAG, short	CAG, long	Grade	Cause of death	Additional studies
1	HD	Male	64	19	18	44	3	Pneumonia	—
3	HD	Male	65	6	18	46	2	Pneumonia	—
5	HD	Male	47	41	19	51	3	Myocardial infarction	—
7	HD	Male	62	19	27	41	0	Pulmonary embolism	
9	HD	Female	50	20	16	46	2	Pneumonia	—
11	HD	Male	71	16	19	42	2	Pneumonia	—
13	HD	Female	53	9	17	53	4	Pneumonia	—
15	HD	Male	48	18	20	45	3	Pneumonia	—
17	HD	Female	45	15	24	43	2	Choking	—
19	HD	Female	43	3.5	21	49	3	Acute renal failure	—
21	HD	Female	57	19	17	44	2	Myocardial infarction	—
23	HD	Male	45	18	20	49	4	Bronchopneumonia	—
25	HD	Female	63	5.5	23	44	3	HD	—
27	HD	Male	72	5	17	41	1	Pneumonia	—
29	HD	Female	67	9	15	42	1	Myocardial infarction	A
31	HD	Female	59	7	23	47	4	Bronchopneumonia	A
32	HD	Male	44	29	20	51	4	Aspiration pneumonia	—
34	HD	Female	91	18	15	40	2	Dehydration (gastro-enteritis)	—
36	HD	Female	62	11	17	45	3	Aspiration pneumonia	A
38	HD	Male	58	14	28	44	2	Bronchopneumonia	A
40	HD	Female	53	12	21	47	2	Pneumonia	—
41	HD	Male	56	16	16	46	2	Pneumonia	—
42	HD	Male	51	15.5	17	48	3	Dehydration	—
43	HD	Male	51	15	10	46	2	Pneumonia	A
44	HD	Male	65	14	17	43	2	Renal failure	A
45	HD	Male	62	9	18	43	2	HD	A
46	HD	Male	83	13	17	42	1	Bronchopneumonia	A
47	HD	Male	64	20.5	27	42	3	Pulmonary thromboembolism	—
48	HD	Male	63	16	22	43	3	Pulmonary embolism	—
49	HD	Male	54	6.5	20	39	2	Pneumonia	—
2	C	Male	81	7	15	18	—	Coronary atherosclerosis	A
4	C	Male	42	14	nd	nd	—	Chest trauma	—
6	C	Male	41	16	nd	nd	—	Heart disease	—
8	C	Male	61	7	17	19	—	Ischemic heart disease	A
10	C	Male	89	19	17	19	—	Probably atherosclerosis	—
12	C	Male	66	15	15	20	—	Ischemic heart disease	A
14	C	Male	53	16.5	nd	nd	—	Ischemic heart disease	—
16	C	Male	73	13	17	23	—	Ischemic heart disease	A
18	C	Female	43	26	17	17	—	Nitrogen poisoning	—
20	C	Male	60	17	10	17	—	Ischemic heart disease	—
22	C	Male	59	24.5	17	18	—	Aortic aneurysm	—
24	C	Female	78	20	18	19	—	Aortic aneurysm	—
26	C	Male	41	16	18	22	—	Asphyxia	—
28	C	Female	72	19	17	19	—	Ruptured myocardial infarction	—
30	C	Male	56	23	nd	nd	—	Asphyxia	—
33	C	Male	48	23	17	20	—	Ischemic heart disease	—
37	C	Female	63	16	14	16	—	Dissecting aortic aneurysm	—
39	C	Female	77	13	nd	nd	—	Ischemic heart disease–coronary atherosclerosis	—
Averages	HD		58.9	14.7	19.3	44.9			
	Non-HD		61.3	16.9	16.1	19.0			

Cerebellum and SFG tissue was obtained from 30 postmortem HD cases (19 male, 11 female) and 18 non-HD individuals (13 male, 5 female) matched for age at death and postmortem delay. Also shown is the number of CAG units within *HTT* exon 1 as determined for each allele (short, long), Vonsattel neuropathological grade at postmortem examination, and cause of death as stated on death certificate. nd, not disclosed . Where a sample was previously assessed for urea quantification by Patassini et al. (21), this is indicated by an "A." Note that study ID refers only to the coded ID of a sample for this study and not to the ID of the sample where it was used in additional studies.

Table S6.   Urea is elevated in brain tissue from a large HD case cohort using two methods of quantification

Method of quantification	Tissue	New cases only, 22 HD, 14 control			Full dataset, 30 HD, 18 control		
		Control, CI	HD, CI	P value	Control, CI	HD, CI	P value
Biochemical assay	Cerebellum	40.6, 124.2	179.5, 422.7	0.0002*	58.9, 126.4	274.6, 565.7	<0.0001*
Biochemical assay	SFG	63.2, 171.6	207.4, 473.2	0.003*	74.7, 157.1	298.0, 613.9	<0.0001*
GC–MS	Cerebellum	4.0, 8.3	10.1, 19.7	0.002*	4.7, 8.1	13.4, 23.9	0.0001*
GC–MS	SFG	3.0, 6.5	7.6, 15.6	0.004*	3.6, 6.3	10.4, 19.2	0.0003*

Urea was quantified by biochemical assay (Abcam ab83362) and gas chromatography-mass spectrometry (GC–MS) in cerebellum and SFG tissue isolated from postmortem HD cases and controls, including repeated quantification of four control and eight HD samples that were previously assessed (21). Values shown compare the 95% confidence interval (CI) for urea concentration in control and HD groups and P value. New cases only: data for cases presented for the first time in this study (n = 22 HD, 14 control). Full dataset: data for all samples assessed in this study (n = 30 HD, 18 control). Refer to Table S4 for further sample description.

Table S7.   Low-grade HD case cohort

ID	Status	Sex	Age at death, y	Age at onset, y*	PM delay, h	CAG, short	CAG, long	Grade	Cause of death	Additional studies
1	HD	Male	62	52	19	27	41	0	Pulmonary embolism	B
3	HD	Male	32	31	14	17	47	1	Submandibular squamous cell carcinoma	
5	HD	Female	67	47	9	15	42	1	Myocardial infarction	A, B
7	HD	Male	83	40	13	17	42	1	Bronchopneumonia	A, B
9	HD	Male	72	40†	5	14	41	0/1	Pneumonia	B
11	HD	Male	41	35	11	19	39	1	Renal failure	
13	HD	Female	70	35	5	17	40	0	Septicaemia	
2	Non-HD	Male	41	—	16	18	22	—	Asphyxia	B
4	Non-HD	Female	72	—	99	15	17	—	Emphysema	
6	Non-HD	Male	83	—	14	19	24	—	Ruptured abdominal aortic aneurysm	
8	Non-HD	Male	32	—	13	nd	nd	—	Pulmonary embolism postsurgery	
10	Non-HD	Male	73	—	13	17	23	—	Ischemic heart disease	A, B
12	Non-HD	Male	61	—	7	17	19	—	Ischemic heart disease	A, B
14	Non-HD	Male	61	—	15	nd	nd	—	Ischemic heart disease	
Average HD			61	40	10.9	18.0	41.7			
Average non-HD			60	—	25.3	17.2	21.0			

Cerebellum tissue was obtained from seven postmortem HD cases (five male, two female) and seven non-HD individuals (six male, one female), matched for age at death and postmortem delay. Also shown is the number of CAG units within *HTT* exon 1 as determined for each allele (short, long), Vonsattel neuropathological grade at postmortem examination, and cause of death as stated on death certificate. nd, not disclosed. Sample ID relates to the numbered lanes in Fig. 5. Where a sample was also used for the quantification of urea in another HD patient cohort, this is indicated [A, used in mixed-grade HD patient cohort by Patassini et al. (21); B, used in the mixed-grade HD case cohort within this publication (refer to Table S1)].
*Age at onset is a best estimate based on available information and was determined by Lynette Tippet and Virginia Hogg.
†Difficulty in determining age at onset.

Table S8. Custom CodeSet design for nCounter gene expression analysis

Gene	NCBI accession	Target sequence, 5'–3'
Target genes		
AP2S1	XM_004015771.1	CGAGCAGCAGCAAGACGCGCCTGGCCAAGTGGTACATGCAGTTCGATGATGACGAGAAACAGAAGTTGATCGAGGAGGTCATGCTGTGGTCACTGTCCGAG
AVPR1A	NM_001199792.1	GCGGGAGAAAGCGCCTTGTACCGAGGAGTCCTGCCACGCGCGGTGTGTGAGCGACGTGAAGACCATTCCCCGCCAAGATCCGCACCGTGAAAATGACCT
CBS	XM_004003371.1	CCCACGGTGCTGGACCGCACGGTGGTGGACAAGTGGTCAAGAGCAACGACGAGGAGGCCTTTGCTTTCGCCCGCATGCTGATCGTCGCCTCAAGAGGGGCTGC
CHST8	XM_004015624.1	CCACCCAGCGCCAGGAATAAAGTTCAGCATCAGGCCACAGCAGCCTCCCACCCCAGGGAACTTCCCAGGATGGTGTCCTTTTAAGGCAGCCACG
CMTM5	XM_004010326.1	AGGCCTGAGAAGTCACGTGGACTTGTCTGTGGGGCGGCTAGTCTGGCATTTGATGAAGCTCGGCCAGGGAGGCTGCTTTATGGTCTAAGGGGAGGGTGCAGG
CNTN2	XM_004013552.1	TACTCCTGCCTGGCCACCAGCCACATGGACTTCTTCCACCAAGAGCGTCTTCAGCCAAGTTTGCTCAGCTCAACCTGGCCGCCAGAAGACACCAGGCTCTTTG
CPAMD8	XM_004009178.1	GGCAGCTGTGTGTGTTGCTGCAGTTGACAAGAGCGTTTACCTGCTCAGGTCTGGTTTCGGCTGACTCCCGCCCAGGTTTTCCGGAATTAGAAGATT
ENS11790	Unannotated transcript	CATGGGCACAAGGAAAAACATCCTCTGTGTGGCTCATTGTCCACCTTCTCTCCTCCGCCACCTCCAGTTACAATGGGAGGATTATCATCCGAATATCATCT
ETV5	XM_004003069.1	GCTCATGATTCTGAAGAGTTGTTTCACGATCCAGGATCTCAGTCAACTTCAAGAGAGCCTTTCAGAGGCTTGGTTAGCTGAAGCACAAGTTCCTGATGACGAACAGTTTGTCCCAGATT
FEZF2	XM_004018621.1	GTCTATAACCTGACCTTCCACATGCACCACCACAACGCAAGAAGCCTTTCACGTGCGCCACTTGCGCAAAGGGTTTTGCAGAAACTTTGACTTAAAGA
HPN	XM_004015173.1	GGCTGTGCCCTGGCCCAGAAGCCAGGCGTCTACACCAAAGTCAGTGACTTCCGGGAGTGGATCTTCCAGGCCATAAAGACTCACTCCGAGCCCAGCGGCA
ITGB4	XM_004013496.1	CCAGCCGGATCGTGTCCTTTGAGCAGCTGTCCTCATCAGTCGAGACCTGAGCAAGTTGAAGCACGAGGCAAGGGAGATAGAGCGGCTTTACGGCAAAAGTACACG
HSD17B12-like	XM_004015316.1	TCGCCCGGAGAGGTCTGAACGTCGTCCTCATCAGTCGAGACCTGAGCAAGTTGAAGCACGAGGCAAGGGAGATAGAGCGGCTTTACGACAAGGAGAGCAACGGCACGGT
MYL4	XM_004013009.1	TGCCCATCCTCCAGCACATCTCCCGAAACAAGGAGCAGGGCACCTACGAGGACTTCGTGGAGGGGCTGCGTGTGTTCGACAAGGAGCAACGGCACCGGT
OTX2	XM_004011036.1	CAGAGTCCTATCCCATGACCTATACTCAGGCTTCAGTTATAGTCAAGGATATGCTGCTCAACTTCCTACTTTGGGGGCATGGACTGTGGATCTTTATT
OXTR	OXTR_ex23UTR.1	ACTATATCTTAGAAGTAAGCAGGGATGAATCTTTGTGGCCTTGGACTAGGCCATGGTTCTTAGATACAACACCCAAAACATAAGCCAAGGAAGAATAG
PRKG1	XM_004020002.1	TTAAAAAGCGTTCCAACATTCCAGAGCCTTCCTGAAGAGATCCTCAGTAAGCTTGCTGACGTCCTTGAAGAGACCCCACTATGAAAAATGGGGAATATATCA
RHCG	NM_001199791.1	CTACAGCTCCGTGGCCTTCAACTTTCTGCTCCGGGCGGCTTCCAGTGGCCGTCCATGCAGCGTGGCTTCAATCCTTTGATGGACGCTACATT
SIAH3	XM_004012262.1	TTTCTTCACCCAGTGCTTTGGGCGTTGTTAGATCTCATTCACCTCCGCTTTCAGCACAACCAAGGCTAAACGAGTATCGCGCGCGCGGCCAACTTGTC
SLC14A1	NM_001163054.1	CAATTTTCTCAAGGTGCATTGAAACTCCGTGTTGAGCAAATGGACCTCCCGTGTTCCACTCTGATTTAGGTGCTCGTCTTCCGCAAAACCCATGCGTTCCAAGGGC
SLC5A7	XM_004005919.1	TCCAGATTACGGCTTGGCGTGGCCTCAGGCACCCATTGAATATTCTTAGTCTGATTTTAGGTGCTCGTCTTCCGCAAAACCCATGCGTTCCAAGGGC
SMOC2	XM_004011565.1	GTGGTCCACTGGTATTTCAAACTGCTGGATAAAAACAACACGCGGGACATCGGACATCATCAGGAAATCAAGCCCTCAAGAGATTTCTTCGGAAGAAGTCCA
TF	XM_004003330.1	ATGGGAAGGACCTGCTGTTTAAGGACCTCGCTGATGGGTTTTAAGATTCCTTCAAGATGGATTTCGAGCTGTACTTGGGATATGAATATGTCACTGC
ZNF804A	XM_004004521.1	GAAGGCCAATTTTTTACTGTGAACTCTGTGACAAGCAGTACTACAGCATCAGGAAATTTGACAATCACATTAATTCGTATGACCACGCTCACAAGCAGAGG
Reference genes		
CANX	XM_004008385.1	GGCTCGAGATCATGGAAGGAGAAGTGGTTGCTGTGTATGTTACTGGTCCTTGGAACTGCTCTTGTTCAAGCTCATGATGGACATGACGATGTGATTGA
SHMT2	XM_004006554.1	TCCGGAAAGTTGTAGGCTTTATAGATGAAGGTGCAACATTGGCTTGGAAGTGAAGAGCAAGACCACCAAAACTCCAGGATTTCAAATCTTTCCTGCTCAA
TRIM27	XM_004019024.1	AGGGAGAAGATTGTTTGGGAGTTTGAGCAGCTGTATCACTCCTTGAAGGAGCATGAGTATCGCCTTTGCCCCGTCTCGAGGAGCTAGACTTGGCCATTT
TRIP11	XM_004017952.1	CAGCTGCTTCAGTCTTTACAGGAGCAGAAGAAGAAAGAAATGGATGAGTTTAGATACCAGCATGAAGAAGAAGAAGAAGAAGAAGAAGAGA

Assays to detect and quantify 24 genes of interest from an RNA-seq analysis of OVT73 sheep anterior striatum, and four reference genes, were custom designed by New Zealand Genomics Ltd. for nCounter expression analysis. Gene names, NCBI accession numbers, and the sequence targeted (100 nt) for nCounter analysis are shown for each target and reference gene. AP2S1, Adaptor Related Protein Complex 2 Sigma 1 Subunit; AVPR1A, Arginine Vasopressin Receptor 1A; CANX, Calnexin; CBS, Cystathione-Beta-Synthase; CHST8, Carbohydrate Sulfotransferase 8; CMTM5, CKLF Like MARVEL Transmembrane Domain Containing 5; CNTN2, Contactin 2; CPAMD8, C3 and PSP Like Alpha-2-Macroglobulin Domain Containing 8; ENS11790, ENSOARG00000011790.1; ETV5, ETS Variant 5; FEZF2, FEZ Family Zinc Finger 2; HPN, Hepsin; ITGB4, Integrin Subunit Beta 4; HSD17B12-like, Hydroxysteroid 17-Beta Dehydrogenase 12-Like; MYL4, Myosin Light Chain 4; OTX2, Orthodenticle Homeobox 2; OXTR, Oxytocin Receptor; PRKG1, Protein Kinase; CGMP-Dependent Type 1; RHCG, Rh Family C Glycoprotein; SHMT2, Serine Hydroxymethyltransferase 2; SIAH3, Siah E3 Ubiquitin Protein Ligase Family Member 3; SLC14A1, Solute Carrier Family 14 Member 1 (Kidd Blood Group); SMOC2, SPARC Related Modular Calcium Binding 2; TF, Transferrin; TRIM27, Tripartite Motif Containing 27; TRIP11, Thyroid Hormone Receptor Interactor 11; ZNF804A, Zinc Finger Protein 804A.

Table S9. Real-time PCR primer sequences for *SLC14A1* quantification in human and sheep tissue

Gene	Forward primer, 5′ to 3′	Reverse primer, 5′ to 3′	Product size, bp	UPL no.	NCBI accession
Sheep					
SLC14A1	tcagtacccaacgtcacctg	cagccatatatctgacccacac	86	96	NM_001163054
CANX	ggaactctgtcagggtggatt	tcatttcatctacctcccattttc	97	142	XM_004008385.1
TRIM27	aagacttaaagaagaggcgaagg	ctgggtcaggctcaagagtt	68	108	XM_004019024
YWHAZ	cgctacttggctgaggttg	aaaagcttcttggtatgcttgc	81	9	NM_001267887.1
Human					
SLC14A1	cttgttcagaacccctggtg	gccatagagcccagatgctat	114	20	NM_001128588
CANX	aggtagaagactcaaaaccagatacc	tgttggaactggagctttgtaa	77	63	NM_001746.3
SDHA	cagaccatctacggagcagag	gatgggcttggagtaatcgt	102	12	NM_004168
TRIP11	caaaatgagctccgacaagaa	caatgaagaggtttctcattaggac	112	42	NM_004239.3

Primer sequences, expected product size (base pairs), and the Universal Probe Library (UPL) probe number are shown for *SLC14A1* and relevant reference genes used in the relative quantification of SC14A1 transcript levels in sheep and human tissues. *CANX*, Calnexin; *GAPDH*, Glyveraldehyde-3-Phosphate Dehydrogenase; *SDHA*, Succinate Dehydrogenase Complex Flavoprotein Subunit A; *SLC14A1*, Solute Carrier Family 14 Member 1 (Kidd Blood Group); *TRIM27*, Tripartite Motif Containing 27; *TRIP11*, Thyroid Hormone Receptor Interactor 11; *YWHAZ*, Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation protein Zeta. Reference genes used for normalization were selected for each tissue analyzed, based on the combination that gave the best stability score in geNorm and NormFinder software. For sheep tissues, the most stable reference gene combinations were *CANX* and *YWHAZ* (Cerebellum) and *CANX* and *TRIM27* (motor cortex, striatum). For human cerebellum, the most stable reference gene combination was *SHDA* and *TRIP11*. The geometric mean of the selected reference gene expression was used to normalize *SLC14A1* expression in each respective tissue.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)