Supplementary material

Impaired cholinergic function and an enhanced glymphatic component in the nucleus basalis of Meynert in individuals with Prader-Willi syndrome

Contents

Supplementary Information for Methods

Nissl staining

Sections were mounted on superfrost+ glass slides and dried on a 37°C heating plate. After 48 hours, the sections were deparaffinized in 100% xylene, rehydrated in grading ethanol (100% - 50%) and rinsed in distilled water. Next, the sections were submerged in a 0.5% thionine solution for 5 minutes and rinsed in water. After dehydration in graded ethanol (50%-100%) and xylene, sections were coverslipped using Entellan® (Sigma-Aldrich, 107960, dried by air and ready for analysis).

Heat-induced epitope retrieval

Sections were submerged in citrate buffer at pH 6.0 for ChAT, Iba1, GFAP, Aβ and in citrate buffer at pH 9.0for GM130, BDNF, AChE. The sections were then heated in a microwave oven at 700 W for 15 minutes and cooled for 30 minutes.

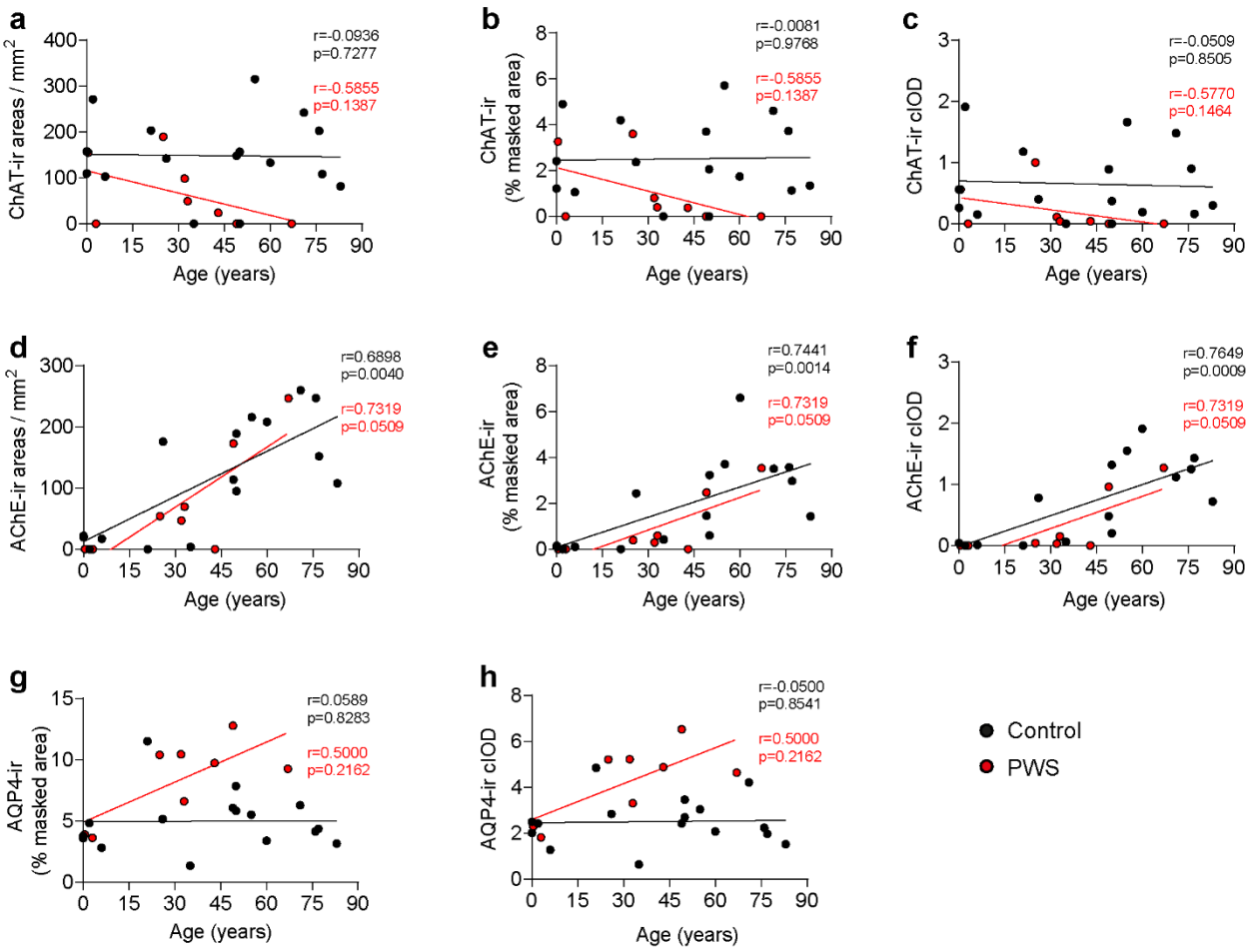
Immunohistochemistry

After overnight primary antibody incubation, sections were rinsed in TBS and incubated for 60 minutes with biotinylated secondary antibody (1:400, Vector Laboratories). The sections were then incubated for 60 minutes with avidin-biotin complex (1:800, Vectastain Elite ABC kit; Vector Laboratories Inc.) and were subsequently rinsed in TBS. Finally, sections were incubated in 0.5 mg/ml 3,3′-Diaminobenzidine (Sigma Chemical Co., St. Louis, MO, DAB) in TBS. For ChAT, GM130, BDNF, AT8, AChE and Iba1 staining, 0.2% ammonium nickel sulphate and 0.01% H2O2 (Merck, Darmstadt, Germany) were added (DAB/Ni) (BDH; Brunschwig, Amsterdam, The Netherlands).

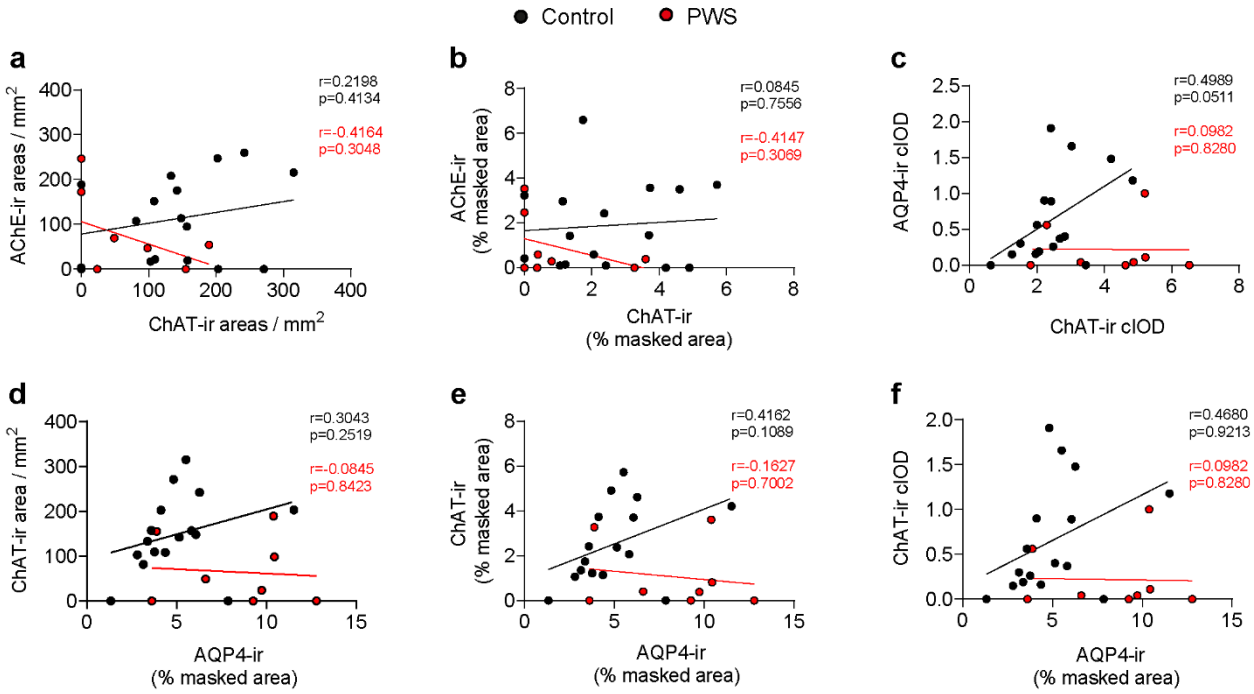
Supplementary Table 1 Antibodies information

Primary antibody	Source	Host	Catalog number	Specificity (PMID)	Dilution
AChE	Thermo	Mouse	MA3-O42	36960685	1:400
Aβ	Proteintech	Rabbit	80007-1-RR	11249427	1:10000
AQP-4	Atlas antibodies	Rabbit	HPA014784	27893874	1:2000
AT8	ThermoFisher	Mouse	MN1020	26792551	1:500
BDNF	Abcam	Rabbit	EPR1292	37190622	1:1000
ChAT	Sigma Aldrich	Goat	AB144P	26792551	1:500
GFAP	DAKO	Rabbit	Z0334	32422642	1:2000
GM130	Netherlands Institute for Neuroscience	Rabbit	α-HG-130	11222994	1:4000
Iba1	Synaptic Systems	Rabbit	234003	32814716	1: 500

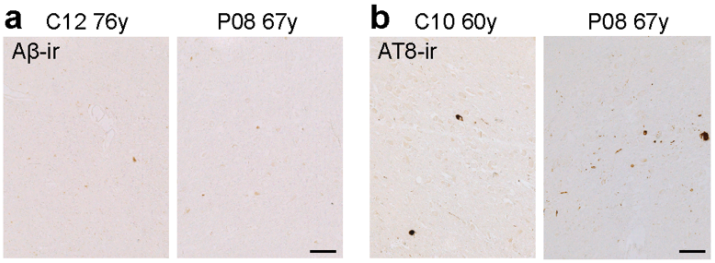
AChE: Acetylcholinesterase; Aβ: Amyloid-beta; AQP4: Aquaporin-4; BDNF: Brain-derived neurotrophic factor; ChAT: Choline acetyltransferase; GFAP: Glial fibrillary acidic protein; GM130: Golgi membrane protein 130; Iba1: Ionized calcium-binding adapter molecule 1; PMID: PubMed Identifier.



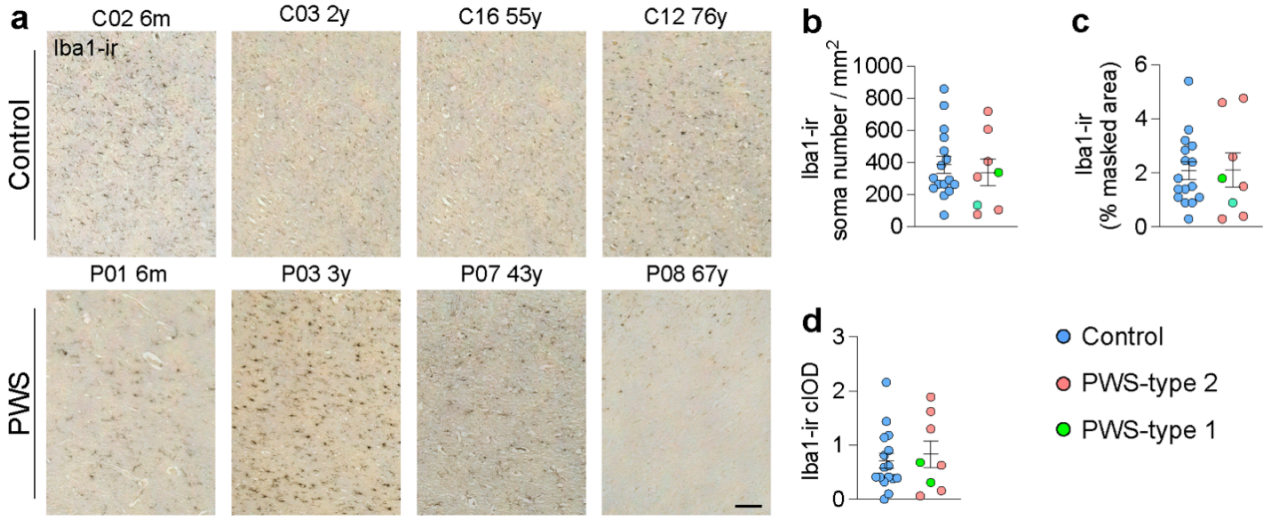
Supplementary Fig. 1 Correlation analysis of ChAT-ir, AChE-ir, and AQP4-ir expression in the NBM of control and PWS subjects in relation to subject age. a, b, c Plots of choline acetyltransferase immunoreactive (ChAT-ir) neurons and age. **d, e, f** Plots of acetylcholine esterase immunoreactive (AChE-ir) neurons and age. Note that there is a positive correlation between the number of AChE-ir neurons and age in control and PWS subjects. **g, h** Plots of aquaporin 4 immunoreactive (AQP4-ir) astrocytes and age.



Supplementary Fig. 2: **Correlation analysis of ChAT-ir neurons with AChE-ir or AQP4-ir expression in the NBM of control and PWS subjects. a, b** Plots of choline acetyltransferase immunoreactive (ChAT-ir) neurons and acetylcholine esterase immunoreactive (AChE-ir) neurons. **c - f** Plots of ChAT-ir expressing neurons and aquaporin-4 immunoreactive (AQP4-ir) astrocytes. Note that there is no significant correlation between AChE-ir or AQP4-ir and ChAT-ir in either control or PWS subjects.



Supplementary Fig. 3: Representative images of Aβ-ir accumulation and AT8-ir in the NBM of controls and PWS subjects. Only minimal immunoreactivity of amyloid-β (Aβ-ir) (**a**) and hyperphosphorylated tau (AT8-ir) (**b**) were observed in the NBM of control subjects (C12 and C10) and PWS subjects (P08). Scale bar: 100µm in **a** and **b**.



Supplementary Fig. 4 Unaltered Iba1-expresing microglia in the NBM of PWS subjects. a Representative images of ionized calcium binding adaptor molecule 1 immunoreactivity **(**Iba1-ir) in the NBM of control and PWS subjects. **b - d** Quantification of Iba1-ir in the NBM of control and PWS subjects. cIOD: corrected integrated optical density. Data are represented as mean ± SEM. Scale bar: 100 µm.