METHODS

Mouse Genotyping

To genotype the mice, PCR analyses of genomic DNA from tail biopsies were performed. Deletion of PWScr resulted in a PCR product of 300 bp, which was absent in the WT genotype. To genotype mice, PCR analysis of genomic DNA from tail biopsies were performed using the primer pair PWScrF1/ PWScrR2 (5'-AGAATCGCTTGAACCCAGGA and 5'-GAGAAGCCCTGTAACATGTCA, respectively). PCR cycling conditions were as follows: 94°C – 2 min, 35 cycles of 94°C – 30 sec, 55°C – 30 sec, 72°C – 30 sec, followed by a final extension of 72°C – 9 min.

Sleep assessment in mice: wireless EEG system

Mice were anesthetized with ketamine/xylazine, 90-150 K/ 7.5-16 X mg/kg, intra-peritoneally, for the implantation of a transmitter that records EEG and electromyography (EMG) using two biopotential channels (Data Sciences, F20-EET, Gold system). In this study the DSI implantable system was placed subcutaneously on the back. Leads from the transmitter were passed subcutaneously to the skull where holes were drilled to implant the EEG-screw electrodes. Electrodes were placed over the frontal and parietal cortices. the screws were then covered with dental acrylic. EMG's electrodes were attached to the muscle in the nape of the neck. After 10 days of post-surgery recovery, we have continuously recorded the physiological signals for 24 hours (from 7 a.m. to 7 a.m. of the following day). This wireless system facilitates the acquisition of the data (Dataquest A.R.T. software, DSI) while allowing the animals to move freely and be assessed in their home-cage environment. EEG

activity was sampled at 500 Hz with a filter cut-off of 50 Hz, whereas the temperature was detected with a sampling rate of 5 Hz within a range of 34-41 *degrees Celsius* (°C). The EEG and EMG were analyzed offline in epochs of 4 s. Each epoch was scored as W, NREM or REM sleep and was subjected to a Fast Fourier Transformation (FFT) with a resolution of 0,48 Hz using the Hanning window method. Vigilance state scoring was performed, at first, automatically with SleepSign software (Kissei Comtec) and then visually corrected. The spectrum of frequencies of the EEG included for the FFT ranged from 0.5 to 20 Hz. The spectrum was composed by delta (δ, 0.5-5 Hz), theta (ϑ, 5-9 Hz), alpha (α, 9-12 Hz), beta (β, 12-20 Hz) waves. An epoch was labeled as NREM sleep when the EMG showed a low level of activity and the EEG was primarily represented by delta waves; REM sleep when there was a combined occurrence of muscular atonia and EEG theta activity; or W when the EMG was tonic and the EEG featured oscillations with high frequencies (α to β waves). Epochs with artifacts were excluded from the analysis.

The transmitter also contains a sensor that detects temperature. Temperature values averaged across the 24 h for each subject, and the standard deviation (SD) was calculated. Then, we excluded all values greater or lesser than 2 SD from the mean to exclude artifacts. The remaining values were averaged in 3-hour bins and compared between genotypes.

Morphology assessment: Magnetic Resonance Imaging (MRI)

High-resolution morpho-anatomical T2-weighted MR imaging of mouse brains was performed in paraformaldehyde (4% PFA; 100 ml) fixed specimens, a procedure that was employed to obtain high-resolution images with negligible confounding contributions from physiological or motion artefacts [1]. Mice were anesthetized with

an intraperitoneal Avertin injection (375 mg/Kg), and their brains were perfused *in situ* via cardiac perfusion.

The perfusion was performed with phosphate buffered saline followed by paraformaldehyde (4% PFA; 100 ml). Both perfusion solutions were added with a gadolinium chelate (Prohance, Bracco, Milan) at a concentration of 10 and 5 mM, respectively. Brains were imaged inside the intact skull to avoid post-extraction deformations. Prior to imaging, samples were blotted and then immersed in plastic tubes filled with a proton-free susceptibility-matching fluid (Fomblyn, Sigma-Aldrich, Milan).

A four-channel 7.0 Tesla MRI scanner (Bruker Biospin, Milan) was used to acquire the anatomical images of the brain, using a using a 72-mm birdcage transmit coil, a custom-built saddle-shaped solenoid coil for signal reception, and the following imaging parameters: a fast t2-weighted 3D gradient-echo sequence with the following parameters: TR=17 ms, TE=10 ms, RARE factor=8, flip angle=25, echo spacing 10 ms and voxel size of 0.070 mm^3 (isotropic).

A study-based template was created by aligning the high-resolution T2W images of the control population to a common reference space via a 12 degrees-of-freedom (DOF) affine alignment, followed by 5 consecutive symmetric diffeomorphic registrations [2]. Individual T2W images of 2 groups of subjects were then nonlinearly registered to the study-based template using diffeomorphic registration.

The Jacobian determinants of the deformation were then used to modulate the gray matter (GM) probability maps calculated during the segmentation step. The modulation compensates for the deformation introduced after the spatial normalization so that there is no variation of the total amount of gray matter, focusing the analysis on the local volumetric variation of the GM instead of the tissue density [3].

The resulting modulated GM probability maps were then smoothed using a Gaussian kernel with a sigma of 3 voxels for voxel-wise statistical parametric comparison. Voxel-wise cross-subject statistic was performed using a non-parametric permutation test with 5000 permutations [4] and using a cluster-based threshold of 0.05.

Genotyping of human patients

Patient 1. FISH studies performed on patient 1 revealed the loss of signal on one chromosome 15 in the region recognized by LSI SNRPN, D15S10 and GABRB3 probes that mapped in a position distal to D15S11. Namely, this latest probe produced signals on both chromosome 15 homologues. Array-CGH showed that the proximal breakpoint was mapped around base 21,813,423 and the distal one around base 28,087,557 of chromosome 15. FISH was used to confirm this atypical proximal breakpoint; RP11-484P15 is the last clone retained before the starting of the deletion, and RP11-622C6 is the first deleted one. The distal breakpoint is located in BP4, and we were not able to confirm this breakpoint by FISH because the region is rich in duplicons.

Patient 2. FISH analysis in patient 2 using commercial probes showed that only the SNRPN probe was deleted on chromosome 15, whereas the others, the proximal D15S11 probe and the two distal D15S10 and GABRB3 probes, were all presented on both homologues of chromosome 15. Both parents had normal karyotype, and they did not exhibit a deletion of the SNRPN probe based on FISH. Array-CGH showed that the proximal breakpoint is at base 22,648,348, and the distal one at base 23,020,695 of chromosome 15. The result was confirmed by FISH with the RP11-455E4 clone that recognized the proximal breakpoint, and it produced a diminished signal on one chromosome 15 indicating that is partially deleted, and with RP11-

777J13 (deleted on one chromosome 15) and RP11-720B15 (retained on both chromosomes 15) clones that identified the distal breakpoint.

Sleep assessment in human subjects: Polysomnography and data analysis

All subjects underwent an adaptation night and then a full-night polysomnography (PSG) in the sleep laboratory at the IRCCS, Istituto Auxologico Italiano, in a quiet room with video monitoring. We calculated the percentage of REM and NREM sleep over the total sleep time and the number of REM-NREM cycles in one night and of REM sleep episodes per hour (REM sleep fragmentation) for each subject. Then, for each parameter, we defined the distribution of controls and outlier intervals. Finally, we allocated the patients in the distribution (Table S2). Both patients and healthy control subjects had been drug free for at least 2 weeks preceding the polysomnography recording. Patients were allowed to maintain their usual sleep habits and timing. The following parameters were recorded: electroencephalography (EEG) using C3-A2, C4-A1, O2-A1, O1-A2 derivations integrated by bipolar montages Fp2-F4, F4-C4, C4-P4, P4-02; Fp1-F3, F3-C3, C3-P3, P3-01; Fz-Cz, Cz-Pz of the 10-20 international placement system (256 Hz sampling, 0.3-50 Hz filters); electrooculogram (EOG, bipolar montage: right ocular cantus-left ocular cantus); respiratory effort by thoracic and abdominal strain gauges; nasal air-flow by nasal cannula, snoring nose by a microphone, and arterial oxyhaemoglobin (SaO_2) using a pulse oximeter with a finger probe; and submental and tibialis anterior muscle electromyograms. Subjects were not allowed to sleep during the daytime prior to the polysomnographic examination.

Each 30-s epoch of the polysomnographic trace was scored for vigilance stages, i.e., wakefulness (W) or Rapid Eye Movements (REM) or non REM (NREM) sleep, and

for arousals, breathing events, oxygen desauturations and limb movements, according to the American Academy of Sleep Medicine (AASM) rules for adults [5], by two independent evaluators experienced in sleep staging. We calculated the percentage of REM and NREM sleep over the total sleep time (TST) and the number of REM sleep cycles and episodes in one night. For each parameter, we defined the distribution of healthy and obese controls and outlier intervals. Then, we allocated the patients in the distribution. Each parameter was calculated based on the values of the two control groups, and the 25^{th} and 75^{th} percentiles (Q_1 and Q_3) were determined.

We then calculated the intervals that define the strong outliers as follows:

$$[\ Q\_1 - 3(Q\_3 - Q\_1\ )\ ,\ Q\_3 + 3(Q\_3 - Q\_1\ )\ ]$$

and the outliers as follows:

$$[\ Q\_1 - 1.5(Q\_3 - Q\_1\ )\ ,\ Q\_3 + 1.5(Q\_3 - Q\_1\ )\ \ ]$$

We then examined the values of the two patients and labeled them as strong outliers, outliers or trend outliers if their parameters were within the relevant defined ranges.

References:

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4. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 2002;15:1-25.

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Table S1. Consensus diagnostic criteria (Holm et al., 1993), developmental milestones and behaviour of PWS patients 1 and 2

	Consensus diagnostic criteria for PWS	patient 1 39y, M	patient 2 43y, F
MC 1	neonatal and infantile hypotonia	+	+
	feeding problems and/or failure to thrive in infancy	+/+	+/+
	rapid weight gain between 12 months and 6 years, causing central obesity	+	+
	Hyperphagia	+	+
	characteristic facial features	**+**	**-**
	Hypogonadism: genitalia hypoplasia / pubertal deficiency	**+/+**	**-/+**
	developmental delay / mild to moderate mental retardation / multiple learning disabilities IQT / IQP / IQV*	+/+/+ 57/66/58	+/+/+ 71/72/76
mc 0.5	decreased fetal movement, weak cry and infantile lethargy	+/+	+/+
	typical behavior problems	**+**	+
	sleep disturbance/sleep apnea	**+/+**	**+/+**
	short stature by age 15y	+	+
	Hypopigmentation	**-**	**-**
	small hands and feet	**+**	**+**
	narrow hands with straight ulnar border	**+**	**-**
	esotropia, myopia	**-/+**	**-/+**
	thick, viscous saliva	**+**	**+**
	speech articulation defects	**-**	**-**
	skin picking	**-**	**+**
SC	high pain threshold	**+**	**-**
	decreased vomiting	+	+
	scoliosis and/or kyphosis	**+/-**	**+/-**
	early adrenarche	**-**	**-**
	Osteoporosis	**+**	**-**
	unusual skill with jigsaw puzzles	**-**	**+**
	normal neuromuscular studies	nk	nk
	Total score	**11.0**	**10.0**
	Early Development		
	Sat	nk	nk
	walked unaided	21 mo	19 mo
	first words	18 mo	>12 mo
	first phrases	21 mo	wn range
	toilet training	wn range	wn range
	Autism (AD)/ Autism Spectrum (ASD) diagnosis	broader ASD	-

Major criteria (MC) are weighted at one point each; minor criteria (mc) are one-half point each.
SC = supportive findings (they only increase or decrease the level of suspicion of the diagnosis).
+ = present, - = absent, wn = within normal, nk = not known.
*IQ values were obtained with the WAIS-R intelligence scale.

Table S2. Lower and upper ends of the outlier intervals according to the healthy and obese distribution of values. Values above the upper ends and below the lower ends are considered as belonging to one of the outlier categories.

CONTROLS:	HEALTHY		OBESE	
STRONG OUTLIERS (●)				
Interval of values:	LOWER	UPPER	LOWER	UPPER
TOTAL NREM	59.22	100	59.9	96.4
TOTAL REM	3.55	40.77	3.53	40.1
REM-NREM CYCLES	-2.25	10	-4.25	15
REM FRAGMENTATION	0.9	2.42	-0.7	2.45
OUTLIERS (●)				
Interval of values:	LOWER	UPPER	LOWER	UPPER
TOTAL NREM	67.96	91.26	67.73	88.64
TOTAL REM	8.74	32.03	11.36	32.26
REM-NREM CYCLES	-0.375	7.375	-0.125	10.87
REM FRAGMENTATION	-0.19	1.71	-0.025	1.78
TREND OUTLIERS (●)				
Interval of values:	LOWER	UPPER	LOWER	UPPER
TOTAL NREM	76.1	87.3	74.6	85.3
TOTAL REM	12.66	23.86	14.6	25.3
REM-NREM CYCLES	2.99	5.98	3	8.9
REM FRAGMENTATION	0.3	1.2	0.4	1.3