



HAL
open science

Abnormal spindle-like microcephaly-associated (ASPM) mutations strongly disrupt neocortical structure but spare the hippocampus and long-term memory

Sandrine Passemard, Alain Verloes, Thierry Billette de Villemeur, Odile Boespflug-Tanguy, Karen Hernandez, Marion Laurent, Bertrand Isidor, Corinne Alberti, Nathalie Pouvreau, Séverine Drunat, et al.

► To cite this version:

Sandrine Passemard, Alain Verloes, Thierry Billette de Villemeur, Odile Boespflug-Tanguy, Karen Hernandez, et al. Abnormal spindle-like microcephaly-associated (ASPM) mutations strongly disrupt neocortical structure but spare the hippocampus and long-term memory. *Cortex*, 2016, 74, pp.158-176. <10.1016/j.cortex.2015.10.010>. <hal-02324797>

HAL Id: hal-02324797

<https://hal.science/hal-02324797v1>

Submitted on 29 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1
2
3 Abnormal spindle-like microcephaly-associated (*ASPM*) mutations strongly
4
5
6
7
8
9
10
11
12 disrupt neocortical structure but spare the hippocampus and long-term memory
13
14

15 Sandrine Passemard (1-4), Alain Verloes (1-3), Thierry Billette de Villemeur (1,5-6), Odile
16 Boespflug (1,2,4), Karen Hernandez (3), Marion Laurent (7), Bertrand Isidor (8), Corinne
17 Alberti (2,9), Nathalie Pouvreau (3), Séverine Drunat (1, 3), Bénédicte Gérard (3), Vincent El
18 Ghouzzi (1,2), Jorge Gallego (1,2), Monique Elmaleh-Bergès (1,2,10), Wieland B. Huttner
19 (11), Stephan Eliez (12), Pierre Gressens* (1,2,4,13), Marie Schaer* (12,14).

20 * These authors contributed equally to this work

21 (1) Inserm, U1141, Hôpital Robert Debré, Paris, France

22 (2) Université Paris Diderot – Sorbonne Paris Cité, Paris, France

23 (3) Département de Génétique, Hôpital Robert Debré, AP-HP, Paris, France

24 (4) Service de Neuropédiatrie, Hôpital Robert Debré, AP-HP, Paris, France

25 (5) Sorbonne Universités, UPMC Université Paris 06, Paris, France

26 (6) Service de Neuropédiatrie, Hôpital Trousseau, AP-HP, Paris, France

27 (7) Service de Psychopathologie, Hôpital Robert Debré, AP-HP, Paris, France

28 (8) Service de Génétique, Hôpital Mère-Enfant, Nantes, France

29 (9) Inserm CIC-EC1426, Hôpital Robert Debré, AP-HP, Paris, France

30 (10) Service de Radiopédiatrie, Hôpital Robert Debré, AP-HP, Paris, France

31 (11) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

32 (12) Office Médico-Pédagogique, Département de psychiatrie, Université de Genève,
33
34

35 Suisse

36 (13) Center for Developing Brain, King's College, St. Thomas' Campus, London, United
37
38

39 Kingdom

40 (14) Stanford Cognitive & Systems Laboratory, Stanford University School of Medicine,
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Palo Alto, California, USA

Corresponding author:

Sandrine Passemaid

Address: Inserm U1141, Hôpital Robert Debré, 48 boulevard Sérurier, 75019 Paris, France

sandrine.passemaid@inserm.fr

Phone: +33 (0)1 40 03 36 91, Fax: +33 (0)1 40 03 22 77

Running title: Brain structure and memory in ASPM microcephaly

ABSTRACT

1
2
3
4
5 Autosomal recessive primary microcephaly results from abnormal brain development linked
6
7 to proliferation defects in neural progenitors. The most frequent form, caused by *ASPM*
8
9 mutations, is usually defined by a reduced brain volume and is associated with intellectual
10
11 disability. Although many *ASPM* cases have now been reported, structural brain abnormalities
12
13 and their link with cognitive disabilities have been rarely investigated. In this study, we used
14
15 high resolution T1-weighted magnetic resonance imaging in seven patients with *ASPM*
16
17 mutations and 39 healthy age-matched controls to quantify regional volumes, thickness,
18
19 surface area, gyrification index and white matter volumes of 30 cortical regions. We observed
20
21 a consistent reduction of 50% or more in the volume and surface area of all cortical regions
22
23 except for the hippocampus and surrounding medial temporal structures, which were
24
25 significantly less reduced. Neuropsychologic assessment indicated significant impairments of
26
27 cognitive abilities. However, these impairments were associated with normal mnemonic abilities,
28
29 in keeping with the relative preservation of the hippocampus and medial temporal structures.
30
31 These results show that, contrary to current opinion, the cortical volume and surface area of
32
33 patients with *ASPM* mutations is reduced depending on a regionally specific fashion and their
34
35 cognitive profile reflects this heterogeneity. The precise characterization of the cortical map
36
37 and cognitive abilities of patients with *ASPM* mutations should allow developing more
38
39 focused reeducative interventions well-suited to their real abilities.
40
41
42
43
44
45
46
47
48
49
50
51
52
53

Keywords:

54
55
56
57 Brain development, human cerebral cortex, hippocampus, MCPH, memory
58
59
60
61
62
63
64
65

1
2
3 **Abbreviations:**
4
5

6
7 ANOVA: Analysis Of Variance
8

9 aRGcs: apical Radial Glial cells
10

11 APs: apical progenitors
12

13 ASPM: Abnormal Spindle Microcephaly associated
14

15 bRGcs: basal Radial Glial cells
16

17 BPs: basal progenitors
18

19 CMS: Children's Memory Scale
20

21 CTRL: Control group
22

23 CSF: Cerebrospinal Fluid
24

25 FSIQ: Full scale IQ
26

27 IQ: Intelligence Quotient
28

29 IGI: local Gyrfication Index
30

31 MCPH: Autosomal recessive primary microcephaly
32

33 MEM: Wechsler Memory Scale-III
34

35 NECs: Neurepithelial cells
36

37 OFC: Occipitofrontal circumference
38

39 ROIs: Regions-Of-Interest
40

41 SD: Standard Deviation
42

43 SEM: Standard Error of the Mean
44

45 SVZ: SubVentricular Zone
46

47 VZ: Ventricular Zone
48

49 WAIS-III: Wechsler Adult Intelligence Scale-III
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

WISC IV: Wechsler Intelligence Scale for Children-IV

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

1. Introduction

1
2
3 Microcephaly represents the most common clinical sign of various developmental disorders
4 of the brain (Barkovich, Kuzniecky *et al.*, 2005), with a prevalence of about 2% worldwide
5 (Van Den Bosch, 1959). Microcephaly is characterized by a decrease in brain size that is
6 indirectly reflected by a decrease of occipitofrontal circumference (OFC) that is more than
7 two standard deviations (SD) below normal values for age and sex, and is closely associated
8 with intellectual disability (Passemar, Kaindl *et al.* 2013). Genetic forms of primary
9 microcephaly (collectively known as MicroCephal Primary Hereditary or MCPH), are very
10 rare, genetically heterogeneous disorders with a recessive inheritance, and considered model
11 diseases for impaired brain growth. The incidence of MCPH is estimated at 1-3/100 000
12 births in Western countries, and is probably ten times higher in the Indian subcontinent
13 (Szabo, Pap *et al.*, 2010; Woods, Bond *et al.*, 2005). MCPH patients usually display normal or
14 slightly delayed motor development, mild-to-moderate intellectual disability, and in a
15 minority of cases, mild pyramidal signs and late onset epilepsy (Kaindl, Passemar *et al.*,
16 2009). Microcephaly, always present at birth, may already be detectable by the second
17 trimester of gestation.

18
19
20 MCPH is caused by a reduction in the number of neural progenitors undergoing proliferation
21 possibly associated with increased apoptosis among these progenitors (Gilmore and Walsh,
22 2013; Kaindl, Passemar *et al.*, 2009; Thornton and Woods, 2009; Verloes, Drunat *et al.*,
23 2013). In mouse models of MCPH, this decrease results in a variable reduction in the volume,
24 surface area and/or thickness of the cerebral cortex, depending on the MCPH gene involved
25 (Gruber, Zhou *et al.*, 2011; Lizarraga, Margossian *et al.*, 2010; Passemar, El Ghouzzi *et al.*,
26 2011; Pulvers, Bryk *et al.*, 2010).

27
28
29 In humans, MCPH can result from mutations in at least thirty genes that nevertheless explain
30 less than half the cases in Western countries (for review, see (Verloes, Drunat *et al.*, 2013). Of

1 these, mutations in the *ASPM* (Abnormal spindle-like, microcephaly associated) gene cause
2 MCPH5, the most frequent genetic form of MCPH, especially in Pakistan (Bond, Roberts et
3 al., 2002). *ASPM* is known to contribute to the genetic basis of brain evolution in humans and
4 other primates (Evans, Anderson et al., 2004; Kouprina, Pavlicek et al., 2004; Mekel-Bobrov,
5 Gilbert et al., 2005; Montgomery and Mundy, 2013). Mouse *Aspm* is expressed in the
6 ventricular zone (VZ) of the neocortex during neurogenesis (Bond, Roberts et al., 2002; Fish,
7 Kosodo et al., 2006). It encodes a protein associated with the centrosome (Bond et al., 2002)
8 and the midbody (Paramasivam, Chang et al., 2007) that, by participating in spindle pole
9 positioning in apical progenitor cells, allows them to maintain symmetric divisions (Fish,
10 Kosodo et al., 2006).

11 *ASPM* mutations cause a global reduction in brain volume in humans that is usually not
12 associated with major cerebral malformations. So far, MRI data have been published for only
13 12 among the 200 reported families with *ASPM* mutations (Bond, Roberts et al., 2002; Desir,
14 Cassart et al., 2008; Passemaid, Titomanlio et al., 2009). Hypoplastic frontal lobes have been
15 described in a single patient with an *ASPM* mutation (Desir, Cassart et al., 2008), suggesting
16 that the reduction in brain volume may be non-homogeneous, with some regions being more
17 affected than others. But no quantitative description, according to region of the cortical
18 reduction in volume/thickness and surface area has been reported in these microcephalic
19 patients. It is thus still unclear whether the cortical map is maintained in patients with *ASPM*
20 mutations, or if some cortical regions are less preserved than others.

21 In addition to the scarcity of information on cerebral and cortical structures in MCPH
22 patients, the impact of MCPH mutations on cognitive development remains obscure. Mild to
23 severe cognitive or behavioral deficits have been identified in patients with *ASPM* mutations
24 in previous studies (Bond, Roberts et al., 2002; Bond, Scott et al., 2003; Desir, Cassart et al.,
25 2008; Hu, Suckow et al., 2014; Kumar, Blanton et al., 2004; Muhammad, Mahmood Baig et
26

1 al., 2009; Nicholas, Swanson et al., 2009; Sajid Hussain, Marriam Bakhtiar et al., 2013; Shen,
2 Eyaid et al., 2005; Tan, del Gaudio et al., 2013). However, the systematic cognitive
3 evaluation of these patients has not been reported so far. The magnitude of their cognitive
4 impairments is thus still undetermined and the possibility that MCPH patients with *ASPM*
5 mutations may have conserved cognitive abilities in some domains despite the overall
6 reduction in brain volume should be considered.
7

8
9
10
11
12
13
14 Here, we aimed to determine how these small brains are organized structurally and to what
15 extent they are functionally impaired in a genetically homogeneous cohort of patients with
16 mutations in the *ASPM* gene. To this end, we combined quantitative neuroimaging using T1-
17 weighted anatomical imaging with neuropsychological assessment. We used a region-based
18 approach to measure changes in cortical volume, surface area and thickness, white matter
19 volume, as well as gyrification index in seven patients ranging in age from 7 to 24 years, to
20 test the hypothesis that patients with *ASPM* mutations would display regional differences in
21 brain volume reduction and in cortical area patterning. In addition, we asked whether any
22 structural differences revealed by neuroimaging would differentially impact cognitive
23 outcome.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 2. MATERIALS and METHODS

44 45 **2.1 Participants**

46 47 *Patients:*

48
49
50
51
52 Seven microcephalic male patients with *ASPM* mutations, ranging in age from 7 to 24 years,
53 were recruited in this study. All patients were right-handed. Clinical and mutational data are
54 summarized in Table 1. OFCs of the patients are given in the Supplementary Figure 1A.
55
56
57
58
59
60
61
62
63
64
65

1 Patients, and their parents if they were younger than 18 years old, provided informed consent
2 to participate. The protocol was favorably reviewed by the ethical committee of Paris.
3
4

5 *Healthy controls:*

6

7
8 Age-matched controls (CTRL) included 39 healthy right-handed male subjects, aged from 6
9 to 25 years and paired by age with patients. The distribution of the subjects according to age
10 is shown in Supplementary Figure 1B. Control participants had no past or present history of
11 neurological or psychiatric diseases. The control group had an average full scale IQ of 109.8
12 ± 12.34 SD. Written informed consent was obtained from all subjects, as well as the parents of
13 subjects younger than 18 years of age, in accordance with protocols approved by the
14 Institutional Review Board of the Geneva University School of Medicine.
15
16
17
18
19
20
21
22
23
24

25 **2.2 Imaging**

26

27
28 Cerebral magnetic resonance images were acquired with a T1-weighted 3D volumetric pulse
29 sequence on a 1.5 Tesla Philips scanner (Gyrosan Intera 1.5 T, Philips Medical Systems,
30 Best, The Netherlands) at the time of cognitive evaluation (or within a few weeks). The MRI
31 dataset consisted of a series of 124 coronal slices with a voxel size of 0.94 x 0.94 x 1.5 mm
32 (TR = 35ms, TE = 6ms, flip angle = 45°). Image processing was carried out using published
33 algorithms included in the FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu>). Briefly,
34 the processing consisted of the exclusion of non-brain tissue, the automatic segmentation of
35 subcortical grey matter structures, and the extraction of cortical surfaces (Fischl, Liu et al.,
36 2001). Both intensity and continuity information from the entire 3D MR volume were used in
37 segmentation and deformation procedures, thus generating an accurate representation of
38 cortical thickness and volumes. These procedures have been validated with histological
39 studies (Rosas, Liu et al., 2002) and shown to be reliable across scanner manufacturers and
40 field strengths (Han, Jovicich et al., 2006). At the end of the reconstruction process, the
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 following volumes were available: estimated intracranial [cerebral and cerebellar tissue and
2 ventricles], supratentorial [total intracranial volume except the cerebellum and brain stem]
3 and infratentorial [cerebellum (grey matter and white matter) and brain stem], total cerebral
4 grey [including cortical, subcortical and cerebellar grey matter] and white matter [cerebral
5 and cerebellar white matter], brain stem, cerebellum [grey and white matter], and the volumes
6 of subcortical structures including the thalamus, putamen, pallidum and caudate nucleus, as
7 well as the amygdala, nucleus accumbens, ventral diencephalon, substantia nigra and
8 hippocampus.
9

20 **2.3 Regional cortical volumes**

21
22 After cortical reconstruction, the cortex was also subdivided into cortical gyral regions as
23 described by Desikan *et al.* (Desikan, Segonne *et al.*, 2006). This parcellation method, based
24 on major sulci, has been shown to be both valid and reliable, with high intra-class correlation
25 coefficients between the manual and automated procedures for both cortical volume estimates
26 and regional boundaries. Parcellation produces 34 regions subdivided into 11 frontal regions,
27 9 temporal regions, 5 parietal regions, 4 occipital regions, 4 regions of the cingulate cortex,
28 and the insula. The frontal pole and the banks of the superior temporal sulcus were excluded
29 from statistical analyses, as both are small regions with relatively poor reliability. Cortical
30 volume was therefore estimated for 31 regions-of-interest (ROIs) in each hemisphere and for
31 each subject (10 frontal regions, 8 temporal regions, 5 parietal regions, 4 occipital regions, 4
32 regions of the cingulate cortex) and the insula. We carefully checked the cortical
33 reconstructions for each participant. Even for the smallest patients' brain, a voxel size of 0.94
34 x 0.94 x 1.5 mm allowed an adequate delineation of the anatomic curvature of the cortex. The
35 inter-subject registration and parcellation into gyral regions was also checked for each
36 participant. The pattern of cerebral sulci in patients with *ASPM* mutations being mostly
37 simplified but with the preservation of the primary sulci, the delineation of the 32 cortical
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 regions was not particularly affected by the absence of some secondary or tertiary sulci (see
2 some examples of cortical parcellation in Supplementary Figure 2).
3
4

5 Lobar volumes were computed by summing the volumes of the cortical regions corresponding
6 to each of the four main lobes (frontal, parietal, temporal, occipital) and to the regions
7 corresponding to the cingulate cortex.
8
9

10 **2.4 Cortical thickness, surface area and cortical gyrification**

11
12
13
14
15
16 Cortical thickness was measured in the native space of the images, as the shortest distance
17 between the white (grey-white boundary) and the pial (grey-CSF interface) surfaces. As a
18 result, cortical thickness values with submillimeter accuracy were available for more than 150
19 000 points in each hemisphere. Finally, based on the outer cortical surface reconstruction (pial
20 surface), local Gyrification Index (IGI) was measured at thousands of points across each
21 hemisphere using previously validated algorithms (Schaer, Cuadra *et al.*, 2008). IGI is a
22 surface-based measurement of the degree of cortical folding that iteratively quantifies the
23 amount of cortex buried within the sulcal folds in the surrounding circular region.
24
25
26
27
28
29
30
31
32
33
34
35

36 **2.5 Regional white matter volumes**

37
38
39 The parcellation of the cortex was subsequently used to subdivide the underlying white matter
40 volume as described in (Salat, Greve *et al.*, 2009). Briefly, a Voronoi diagram was created in
41 the white matter voxels based on the distance to the nearest parcellation label, using a distance
42 constraint of 5 mm. At the end of this process, regional white matter volumes were available
43 for the 30 regions corresponding to the aforementioned cortical labels.
44
45
46
47
48
49
50

51 **2.6 Cognitive assessment**

52
53
54 Intellectual abilities of patients and healthy controls were assessed using the recommended
55 Wechsler scale at the time of assessment.
56
57
58
59
60
61
62
63
64
65

Patients

1
2
3 The Intelligence Quotient (IQ) was assessed between 2009 and 2013 using the Wechsler
4
5 Adult Intelligence Scale-III (Wechsler, 1997) or the Wechsler Intelligence Scale for Children-
6
7 IV (Wechsler, 2003) according to age (WISC IV: 6 to 16 years, n=2; WAIS-III after the age
8
9 of 16 years, n=3). Two patients (Patients 6 and 7) could not participate in the
10
11 neuropsychological assessment. The pregnant mother of patient 7 had learned of the
12
13 recurrence of microcephaly in her fetus and did not wish her son to contribute to the
14
15 evaluation at that time. Patient 6 was too hyperkinetic and restless to be assessed with the
16
17 Wechsler scales.
18
19
20

21
22 Full Scale IQ (FSIQ) was calculated from four scores: a verbal score (Verbal Comprehension
23
24 Index, VCI), a visual or visuospatial scale with the use of materials (Perceptual Organisation
25
26 Index, POI), a score of mental manipulation of data (Working Memory Index, WMI) and a
27
28 score of graphic processing speed (Processing Speed Index, PSI). The 4 scores are
29
30 independent and involve specific cognitive abilities such as lexical stock, general knowledge,
31
32 verbal comprehension and verbal reasoning (VCI); visuomotor and visuospatial skills to
33
34 examine a problem, organize thoughts and find solutions using cubes or pictures (POI); short-
35
36 term memory, concentration abilities, mental manipulation, planning abilities, cognitive
37
38 flexibility, arithmetic reasoning (WMI) and finally performance speed in graphic realization
39
40 and visual discrimination (PSI).
41
42
43
44
45
46

47
48 Memory was assessed by the Wechsler Memory Scale-III (Wechsler, 1998) and the Children's
49
50 Memory Scale (Cohen, 1997) depending on age (CMS: 5 to 16 years, n=2; MEM after the age
51
52 of 16 years, n=3). These scales consist of different memory mechanisms: immediate memory
53
54 and delayed memory with respect to both verbal and visual modalities, and the process of
55
56 learning and delayed recognition.
57
58
59

Healthy controls

1 The Intelligence Quotient (IQ) was assessed between 2002 and 2009 using the Wechsler
2 Adult Intelligence Scale-III (Wechsler, 1997) or the Wechsler Intelligence Scale for Children-
3 III (Wechsler, 1991) according to age (WISC III: 6 to 16 years, n=29; WAIS-III after the age
4 of 16 years, n=10). Full scale IQ (FSIQ), Verbal Comprehension Index and Perceptual
5 Organisation Index were calculated from the subtests as recommended for all subjects.
6
7 Working memory IQ and processing speed IQ were available for subjects who were evaluated
8 using the WAIS-III scale. Memory was assessed as for patients.
9

10 **2.7 Statistical analysis:**

11 *Imaging analysis:*

12 Based on histological and structural-MRI studies that provided information on dynamic
13 regional and age-related changes in the volume and thickness of grey matter and in the
14 volume of white matter in developing children and adolescents (Chouinard-Decorte, McKay
15 et al., 2014; Franke, Ziegler et al., 2010; Giedd, Blumenthal et al., 1999; Giedd, Stockman et
16 al., 2010; Gogtay, Giedd et al., 2004; Lenroot and Giedd, 2006; Raznahan, Lerch et al., 2011;
17 Sowell, Thompson et al., 2001), we divided our participant samples into 3 age groups (6-11.9,
18 12-17.9 and 18-25 years). The raw data for intracranial and cerebral cortex volumes are
19 indicated in Supplementary Figure1 C-D. To take into account age-related differences in
20 cortical volumes as detailed below, all volumes in both groups were expressed as a percentage
21 of the average of the controls of the corresponding age group \pm SEM.
22

23 We compared the differences in the volume data between microcephalic patients with *ASPM*
24 mutations and controls. To investigate whether the decrease in cortical volume was similar in
25 the different lobes, we analyzed the volume of the frontal, parietal, occipital and temporal
26 lobes and cingulate cortex. Within each hemisphere, we measured the volumes of 31 specific
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 cortical and underlying white matter regions and, finally, the thickness and surface area of 30
2 cortical regions as previously defined (Desikan, Segonne et al., 2006).
3

4
5 All variables were analyzed by a repeated-measures two-way ANOVA (GraphPad Prism 5.0),
6
7 with genotype (controls versus patients) as a between-subject factor, and lobes or regions as
8
9 within-subject factors (since volume, thickness and area are measured in the same subjects
10
11 (Lew, 2007)). Topographical differences in the effects of the *ASPM* mutations were
12
13 investigated through genotype-by-lobe or genotype-by-region interaction analyses. A
14
15 significant genotype-by-region interaction indicates/means that differences between patients
16
17 and controls depend on the region. Whenever ANOVA revealed statistically significant
18
19 interactions for lobes or specific regions, partial pairwise comparisons were conducted with a
20
21 post hoc test (using the Bonferroni correction for multiple comparisons) in order to identify
22
23 regional differences in patients as compared to controls. In all analyses, a statistical threshold
24
25 of $p < 0.05$ indicated significance (uncorrected for multiple comparisons).
26
27
28
29
30

31 *Cognitive analysis:*

32
33 Within the group of patients, general intellectual abilities (Full Scale IQ, Verbal IQ,
34
35 Performance IQ, Processing Speed IQ and Working Memory IQ) and mnemonic abilities
36
37 (Immediate or Delayed Auditory/Verbal Memory and Immediate or Delayed Visual Memory)
38
39 were compared to search for specific learning disorders using a one-way ANOVA followed
40
41 by a Bonferroni post hoc test if specific differences were observed among scores. In all
42
43 analyses, a statistical threshold of $p < 0.05$ indicated significance.
44
45
46
47
48

49
50 The results of the Wechsler tests in controls were used merely to establish the validity of this
51
52 group for structural analyses (i.e. to ensure that they were normal) and were not compared to
53
54 those of patients.
55
56
57
58
59
60
61
62
63
64
65

3. Results

3.1 Brain volume is reduced by half in patients with *ASPM* mutations and this reduction differentially affects brain regions

Mean values \pm SEM of global brain volumes are presented in Supplementary Table 1. The intracranial volume was strongly reduced in patients as compared to controls (~45% of that in controls, Figure 1A, i.e. a 55% volume reduction). This effect was supported by a significant reduction of supratentorial and infratentorial volumes ($p < 0.0001$ for both comparisons, Figure 1A). However, the volume reduction was stronger in the supratentorial compartment than in the infratentorial compartment in patients as compared to controls (~45% vs 67 % of that in controls, respectively, i.e. a 55% vs 33% volume reduction) as supported by the significant genotype-by-region interaction ($F = 75.45$, $dF = 2;88$, $p < 0.0001$) and the partial pairwise comparisons shown in Figure 1A. Within the infratentorial compartment, the reduction in volume was not uniform in patients as compared to controls (Figure 1B), with the cerebellar volume significantly better preserved than that of the brainstem (~70% vs 58% of that in controls respectively, i.e. a 30% vs 42% volume reduction), as supported by the significant genotype-by-region interaction ($F = 21.96$, $dF = 1;44$, $p < 0.0001$) and the partial pairwise comparisons (Figure 1B). Similarly, the reduction in volume in patients was higher in the total white matter of the brain than in the total grey matter, as shown by the significant genotype-by-region interaction ($F = 9.74$, $dF = 1;44$, $p = 0.0032$) and the partial pairwise comparisons (Figure 1C). Finally, the cortical grey matter was more affected than the subcortical or cerebellar grey matter in patients as compared to controls (~45%, 64% and 70% of that in controls respectively, i.e. a 55%, 36% and 30% volume reduction) as supported by the significant genotype-by-region interaction ($F = 57.2$, $dF = 2;88$, $p < 0.0001$) and the partial pairwise comparisons (Figure 1E). In contrast, *ASPM* mutations caused a similar volume

1 reduction on the cortex and on the underlying white matter (genotype-by-region interaction:
2 ns, Figure 1E).
3

4
5 Taken together, these data indicate that, in our series, *ASPM* deficiency strongly reduces the
6 cerebral cortical grey and underlying white matter volumes by over 50% and has a lesser
7 impact on the subcortical grey matter and cerebellum.
8
9

10 11 12 **3.2 Neocortical areas - but not the hippocampus - are strongly reduced in patients** 13 14 **with *ASPM* mutations** 15

16
17
18 Within each lobe, the cortical volume was strongly reduced in patients as compared to
19 controls (Figure 2A-B). However, this reduction was not similar across lobes in the right
20 hemisphere, as supported by the significant genotype-by-region interaction (Supplementary
21 Table 2A) and the pairwise comparisons (Figure 2B). The reduction in volume was
22 significantly higher in the right cingulate cortex than in the right frontal, parietal and temporal
23 cortices in patients as compared to controls. To determine whether this reduction in the
24 cortical volume was similar in all cortical regions in patients as compared to controls, we
25 carried out a more detailed analysis within each lobe. Mean values \pm SEM of the 31 regional
26 cortical volumes are presented in Supplementary Table 2B.
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 ***Parietal, occipital and cingulate regions*** 42

43
44 In both hemispheres, *ASPM* mutations caused a similar volume reduction in regions of
45 parietal, occipital lobes and cingulate cortex (genotype-by-region interaction: ns,
46 Supplementary Table 2B).
47
48
49
50

51 ***Frontal regions*** 52

53
54 We observed a significant regional effect of the *ASPM* mutations on frontal cortical volumes
55 as supported by the significant genotype-by-region interaction (Supplementary Tables 2A)
56 and the partial pairwise comparisons (Supplementary Figure 3). In the left hemisphere, the
57
58
59
60
61
62
63
64
65

1 volume of the paracentral cortex (premotor cortex and supplementary motor cortex, in orange
2 in Figure 2C) or pars opercularis (Broca's area, in yellow in Figure 2C) was better preserved
3
4 than that of the caudal middle frontal (posterior part of the dorsolateral prefrontal cortex) or of
5
6 the rostral middle frontal cortices (anterior part of the dorsolateral prefrontal cortex, in red in
7
8 Figure 2C). Within the right hemisphere, pars opercularis was significantly higher in volume
9
10 than the prefrontal regions (caudal/rostral middle frontal, lateral or medial orbitofrontal
11
12 cortices (Figure 2C, Supplementary Figure 3C-D).
13
14
15
16

17 ***Temporal regions***

18
19 We observed a regional effect of *ASPM* mutations on temporal cortical volumes, as supported
20
21 by the significant genotype-by-region interaction (Supplementary Tables 2A) and the partial
22
23 pairwise comparisons (Figure 3). The cortical volume of the medial temporal structures
24
25 including the entorhinal and parahippocampal cortices and the hippocampus was significantly
26
27 better preserved than that of the five other temporal regions (Figure 2C and Figure 3A-B).
28
29 This difference was highly significant in the bilateral parahippocampal cortex and
30
31 hippocampus and in the right entorhinal cortex when compared to other regions of the
32
33 temporal lobes (Figure 3C-F).
34
35
36
37
38
39

40 Together, these data indicate that, in our series, the hippocampus and surrounding medial
41
42 temporal structures display a relatively preserved volume when compared to the extreme
43
44 decrease in the volume of all neocortical areas in both hemispheres in patients with *ASPM*
45
46 mutations.
47
48
49

50 **3.3 The decrease in cortical volume is associated with regional increases in** 51 **cortical thickness** 52 53

54
55 Since the cortical volume is the product of thickness x surface area (Im, Lee et al., 2008), we
56
57 sought to determine whether or not the reduction in cortical volume in the absence of *ASPM*
58
59
60
61
62
63
64
65

1 was due to decreased cortical thickness in the regions analyzed above. We observed a
2 regional effect of *ASPM* mutations on cortical thickness within each lobe, as supported by the
3 significant genotype-by-region interaction (Supplementary Table 2A) and the pairwise
4 comparisons (Supplementary Figures 4 and 5), except for the right temporal lobe and the left
5 cingulum. Mean values \pm SEM of the 30 regional cortical thickness are presented in
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Occipital, temporal and cingulate regions

Only two regions of significant decreased cortical thickness were observed in these lobes in patients as compared to controls. These regions (highlighted in green in Figure 4) were located in the cuneus within the left occipital lobe and in the rostral anterior cingulate cortex within the right cingulate region (see also Supplementary Figure 4A, F). No difference in cortical thickness was observed between patients and controls in the temporal lobes (Figure 4 and Supplementary Figure 4 C-D). A few clusters of increased cortical thickness, shown in dark blue in Figure 4, were observed within the right occipital lobe (pericalcarine cortex and lingual gyrus) and cingulate regions (left caudal anterior cingulate cortex, isthmus regions bilaterally, Supplementary Figure 4 B, E-F) in patients as compared to controls.

Frontal and parietal regions

Surprisingly, several frontal and parietal cortical regions were significantly thicker in patients than in controls in both hemispheres, as supported by the significant genotype-by-region interaction (Supplementary Table 2A) and the pairwise comparisons shown in Supplementary Figures 5. Indeed, significant increased cortical thickness was observed in the pars opercularis bilaterally, in the rostral middle frontal, lateral orbitofrontal, pars orbitalis of the left frontal lobe, as well as in the right caudal middle frontal region (in dark blue in Figure 4, see also Supplementary Figure 5A-D).

1 The cortex was also significantly thicker bilaterally in all parietal regions in patients as
2 compared to controls, except for the left precuneus and the right supramarginal gyrus (in blue
3 green or light blue in Figure 4, see also Supplementary Figure 5 E-H).
4
5

6
7 Together, these data indicate that the observed decrease in cortical volume in patients with
8 *ASPM* mutations, in our series, is not due to a decrease in cortical thickness. On the contrary,
9 the cerebral cortex is thicker in the prefrontal cortex, inferior frontal gyrus and parietal
10 regions lining the Sylvian fissure and the postcentral sulcus, especially in the left hemisphere.
11
12
13
14
15
16

17 **3.4 The reduction in cortical volume in patients with *ASPM* mutations is** 18 **accounted for by a decrease in surface area** 19 20 21

22
23 As the reduction in cortical volume was not related to a decrease in cortical thickness in most
24 regions examined, we investigated whether this reduction in volume could be due to
25 decreased surface area. We measured the surface area of the same regions as above. We
26 observed an extreme and homogeneous reduction of the surface area of all lobes in patients as
27 compared to controls in both hemispheres. In the right hemisphere, this reduction was not
28 similar across lobes as supported by the genotype-by-region interaction (Supplementary Table
29 2A) and the pairwise comparisons shown in Figure 5B. The reduction in cortical surface area
30 was significantly higher in the cingulate cortex than in the frontal, parietal and temporal lobes
31 in patients as compared to controls (Figure 5B). Mean values \pm SEM of the 30 regional
32 cortical surface area are presented in Supplementary Table 2B.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 ***Frontal, parietal, occipital and cingulate regions*** 49

50
51 The cortical surface area of most regions of the frontal, parietal, occipital and cingulate lobes
52 in both hemispheres depicted in Figure 5 as was strongly reduced in patients as compared to
53 controls. We did not find any genotype-by-region interaction for the parietal, occipital and
54 cingulate lobes and the right frontal lobe (Supplementary Table 2A). *ASPM* mutations had a
55
56
57
58
59
60
61
62
63
64
65

1 very mild regional effect on surface area of the left frontal lobe as supported by the genotype-
2 by-region interaction (Supplementary Tables 2A) and the partial pairwise comparisons shown
3
4 in Supplementary Figure 6 C-D. The precentral and pars opercularis regions (depicted in
5 orange in Figure 5) were lightly higher in terms of surface area than the caudal and rostral
6
7 middle frontal regions (depicted in dark red in Figure 5) in patients as compared to controls
8
9 (see also Supplementary Figure 6A-B).
10
11
12
13

14 *Temporal regions*

15
16 We observed a significant regional effect of *ASPM* mutations on temporal cortical surface
17 area as supported by the significant genotype-by-region interaction (Supplementary Table 2A)
18 and the partial pairwise comparisons (Supplementary Figures 6 E-H). As for volume
19 measurements, the surface area of the parahippocampal cortices and of the left entorhinal
20 cortex, depicted in green or yellow in Figure 5, was significantly higher than that of the five
21 other temporal regions (middle, transverse, inferior temporal cortex or fusiform region,
22 depicted in red or dark red in Figure 5) in both hemispheres in patients as compared to
23 controls (see also Supplementary Figure 6 E-H).
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 Taken together, these data suggest that the surface area of various cortical regions is reduced
39 to the same extent as their volume in patients with *ASPM* mutations as compared to controls,
40 except for the parahippocampal and entorhinal cortices, which are significantly more
41 preserved.
42
43
44
45
46
47

48 **3.5 The volumes of the cerebral cortex and the underlying cortical white matter**

49 **are reduced in similar proportions**

50
51

52 To assess the impact of the loss of cortical volume on the underlying white matter, we
53 measured white matter volume in all regions.
54
55
56
57
58
59
60
61
62
63
64
65

1 A strong reduction in the subcortical white matter volume was observed in patients as
2 compared to controls throughout the frontal, parietal, occipital and temporal lobes and
3
4 cingulate regions (Figure 6 A-B), without genotype-by-lobe interaction (Supplementary Table
5
6 3A). However, upon further examination of the regions within each lobe, we detected a
7
8 significant regional effect of the *ASPM* mutations on the temporal lobes, as demonstrated by
9
10 the significant genotype-by-region interaction (Supplementary Tables 3B) and the pairwise
11
12 comparisons (Figure 6 C-D). Mean values \pm SEM of the 30 regional white matter volumes are
13
14 presented in Supplementary Table 3B. Within the temporal lobes, significantly more
15
16 preserved white matter regions were located exclusively in the medial temporal lobes in
17
18 patients as compared to controls. As in the cerebral cortex, the regions with the least reduction
19
20 in the volume of the white matter in patients were in the parahippocampal cortices and in the
21
22 right entorhinal cortex (Figure 6C-D).
23
24
25
26
27
28

29 These data indicate that regions that are more preserved in volume at cortical levels are also
30
31 preserved at the white matter level.
32
33
34

35 **3.6 The Gyrification Index is reduced in patients with *ASPM* mutations and this** 36 37 **reduction differentially affects cortical regions** 38 39

40 Mean values \pm SEM of the IGI of the 30 cortical regions are presented in Supplementary
41
42 Table 4A. The local Gyrification Index (IGI) was significantly reduced in patients as
43
44 compared to controls in all part of the cortex as shown in Figure 7. However, this reduction
45
46 was not similar across regions, as supported by the significant genotype-by-region interaction
47
48 (Supplementary Table 4A), and the pairwise comparisons (Supplementary Tables 4C) except
49
50 for the left parietal lobe. Regions with the higher IGI (Figure 7) were located within the
51
52 frontal lobes (paracentral, superior frontal, medial orbitofrontal regions), the temporal lobes
53
54 (inferior temporal region, entorhinal and parahippocampal cortices) and cingulate regions
55
56
57
58
59
60
61
62
63
64
65

(rostral and caudal anterior cingulate and posterior cingulate regions). At the opposite, pars opercularis and superior temporal bilaterally were regions with the lower IGI (Figure 7).

These data suggest that *ASPM* deficiency disrupts the gyrification with regional variations. Exploratory correlation analysis between IGI and surface area, thickness or volume did not reveal significant relationship.

3.7 Patients with *ASPM* mutations display preserved mnemonic capacities but mildly reduced or borderline intellectual abilities

Neuropsychological tests could be performed in five patients. All patients had mild to moderate intellectual deficiency (mean FSIQ 51.2 ± 6.5), with an impairment of their language skills (Verbal Comprehension Index = 60.6 ± 11.4 , limited lexical stock, but most importantly, difficulties with verbal or conceptual reasoning), and of their visuomotor or visuospatial analysis (Perceptual Organisation Index = 57.2 ± 9.1). They also displayed deficits in the memorization of new information, had significant difficulties with arithmetic and problem solving (Working Memory Index = 54.2 ± 4), and were very slow at performing graphical tasks (Processing Speed Index = 59.4 ± 13.2) (Figure 8 and Supplementary Table 5).

In contrast, and despite this global cognitive disability, in the domain of memory function, all patients had a mean score in the normal range or at the limits of normality for verbal tasks (immediate verbal memory = 82.80 ± 11.86), and more particularly for visual tasks (immediate visual memory = 90.2 ± 12.03). Their abilities in encoding/learning can be mobilized with a low rate of forgetting in delayed memory at recall tasks (delayed verbal memory = 75.6 ± 16.26 and especially delayed visual memory = 85.8 ± 6.4); these differences were very significant ($p < 0.001$ for each of the four comparisons for FSIQ versus immediate (or delayed) verbal (or visual) memory)). No difference was observed between mnemonic

1 processes (immediate and delayed memory) regardless of the type of memory tested
2 (auditory-verbal or visual memory).
3
4

5 These data reveal that patients with *ASPM* mutations in our series have mild intellectual
6 disability, but all of them have preserved memory functions and learning abilities.
7
8
9

10 11 12 13 4. DISCUSSION 14 15

16 Our study demonstrates that *ASPM* loss-of-function mutations in microcephalic patients
17 disrupt cortical and white matter development in a region-specific fashion. The 50% reduction
18 in cortical volume is the result of a decrease in cortical expansion mainly in terms of surface
19 area, although this is partially counterbalanced by a regional increase in cortical thickness.
20
21 The volume of the cerebellum and subcortical grey matter was relatively spared when
22 compared to the cerebral cortex and underlying white matter. Within the cortex, the volume of
23 the hippocampus, surrounding medial temporal structures and the underlying white matter
24 was preserved compared to other regions of the neocortex. In keeping with the relative
25 integrity of the hippocampus, the mnemonic abilities of patients with *ASPM* mutations were
26 preserved despite their intellectual disabilities. These results provide strong evidence for a
27 regional variation in the reduction of brain volume and significant differences in cortical
28 arealization in these patients that could influence their cognitive functions.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 **Global reduction in brain volume** 47 48

49 The brain volume of MCPH patients is reduced by definition, but to our knowledge, no study
50 has quantified this reduction region-wise. Here, we show that the reduction in the brain
51 volume of patients with *ASPM* mutations affects the cortex and the underlying white matter to
52 a greater extent than the subcortical grey matter and cerebellum. Murine *Aspm* gene is
53 expressed in the ventricular zone of the pallium (Bond, Roberts *et al.*, 2002; Fish, Kosodo *et*
54
55
56
57
58
59
60
61
62
63
64
65

1 al., 2006). In humans, recent data have shown that *ASPM* is expressed in the VZ, inner and
2 outer subventricular zone (i and oSVZ) (Fietz, Lachmann et al., 2012), and in apical and basal
3 radial glial cells (Florio, Albert et al., 2012) of the pallium. In line with this pattern of
4 expression, our data show that *ASPM* mutations affect structures derived from the pallium to a
5 greater extent than those derived from the subpallium, the diencephalon, the mesencephalon
6 or the rhombencephalon.
7
8
9
10
11
12

13 **Cortical regionalization**

14
15
16
17
18 Our study highlights a differential reduction in several cortical regions in patients as
19 compared to controls, rather than a homogeneous cortical reduction, suggesting that *ASPM*
20 mutations disrupt cortical and white matter development in a regionally specific fashion.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1 according to region in patients with *ASPM* mutations, it is conceivable that in the absence of
2 *ASPM*, changes in peripheral sensory inputs also regulate the dimensions of cortical areas.
3
4

5 **Volume/surface/thickness/gyrification relationship**

6

7
8 The present study provides evidence that cortical volume, surface area and the gyrification
9 index are significantly decreased in patients with *ASPM* mutations while cortical thickness is
10 maintained or locally increased in some regions. This indicates that in the absence of *ASPM*,
11 there is not enough lateral/tangential cortical expansion and folding, but intact or locally
12 increased radial expansion. The 3D growth in volume of the cerebral cortex is closely related
13 to its surface and thickness components as well as to its folding. The radial expansion (i.e.
14 increase in thickness) and tangential expansion (i.e. increase in surface area) of the future
15 cortex occur concomitantly and depend on the balance between the proliferation and
16 differentiation of the progenitor pool within a limited window of time i.e. neurogenesis
17 (Rakic, 2009). The tangential expansion of the cerebral cortex is the result first of the
18 amplification of neuroepithelial cells (NECs) and apical Radial Glial cells (aRGcs), which
19 undergo symmetric proliferative divisions within the VZ (Florio and Huttner, 2014; Gotz and
20 Huttner, 2005; Stancik, Navarro-Quiroga et al., 2010; Taverna, Gotz et al., 2014), and later
21 during neurogenesis, of the amplification of basal Radial Glial cells (bRGcs). *Aspm* is
22 expressed in apical progenitors (AP; which include the aRGcs) of the VZ and is necessary to
23 maintain their symmetric divisions in mice (Fish, Dehay et al., 2008; Fish, Kosodo et al.,
24 2006). The differential regional amplification of basal progenitors (BPs; including bRGc and
25 basal Intermediate Progenitors or bIPs), which form a large population in the outer SVZ of
26 gyral species such as ferrets, primates and humans (Betizeau, Cortay et al., 2013; Fietz,
27 Kelava et al., 2010; Garcia-Moreno, Vasistha et al., 2012; Hansen, Lui et al., 2010;
28 LaMonica, Lui et al., 2013; Lui, Hansen et al., 2011), is sufficient to drive cortical surface
29 area expansion and gyrification even in lissencephalic species such as mice (Florio, Albert et
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 al., 2015; Nonaka-Kinoshita, Reillo *et al.*, 2013; Rash, Tomasi *et al.*, 2013; Stahl, Walcher *et*
2 al., 2013). The hypothesis put forward by Sun and Hevner in a recent review is that bRGcs
3 may expand the cortical plate tangentially whereas bIPs primarily amplify neuron number to
4 “fill in” the cortical layers that have been thinned out by tangential expansion (Sun and
5 Hevner, 2014). In keeping with this hypothesis, our findings suggest that *ASPM* loss-of-
6 function mutations may lead to a reduction in the amplification not only of the pool of APs
7 but also of BPs (bRGcs rather than bIPs). However, this remains a hypothesis and needs to be
8 verified by studying the pattern of expression of *ASPM* in humans during neurogenesis and
9 analyzing fetuses carrying *ASPM* mutations.
10
11
12
13
14
15
16
17
18
19
20

21 **Cognition**

22
23
24
25 Five of the seven patients were assessed using Wechsler neuropsychological tests. Our results
26 show that despite their mild-to-moderate intellectual disabilities, patients with *ASPM*
27 mutations have preserved mnemonic abilities. These findings are consistent with the protection of
28 the volume of their hippocampus and medial temporal structures (entorhinal and
29 parahippocampal cortices). Both the grey and white matter of medial temporal regions are
30 significantly protected compared to other neocortical regions in patients with *ASPM*
31 mutations, suggesting possible interconnections between these structures and other cortical
32 regions required for memory retrieval. Our data demonstrate objectively and quantifiably
33 what other studies have previously suggested based on either partial, or non homogeneous
34 neuropsychological evaluations (Desir, Cassart *et al.*, 2008; Kumar, Blanton *et al.*, 2004;
35 Passemar, Titomanlio *et al.*, 2009) the absence of formal assessments (Bond, Roberts *et al.*,
36 2002; Bond, Scott *et al.*, 2003; Desir, Cassart *et al.*, 2008; Hu, Suckow *et al.*, 2014; Kumar,
37 Blanton *et al.*, 2004; Muhammad, Mahmood Baig *et al.*, 2009; Nicholas, Swanson *et al.*,
38 2009; Sajid Hussain, Marriam Bakhtiar *et al.*, 2013; Shen, Eyaid *et al.*, 2005; Tan, del Gaudio
39 *et al.*, 2013). As a rule, higher IQ is associated with higher grey and white matter volumes in
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 all lobes (Haier, Jung et al., 2004; Posthuma, De Geus et al., 2002). Typically, the strongest
2 correlations with IQ involve frontal grey matter volumes (Haier, Jung et al., 2004; Sowell,
3 Thompson et al., 2001), which are strongly reduced in our patients. Intelligence is linked to
4 dynamic changes in the thickness and surface area of the human cortex during childhood and
5 adolescence: children and adolescents with higher IQ show cortical thinning and surface
6 expansion (Karama, Colom et al., 2011; Schnack, van Haren et al., 2014). This is concordant
7 with the reverse observation in our patients. A large number of studies support the role of
8 medial temporal structures, most notably the hippocampus, in episodic memory, in concert
9 with the parahippocampal, perirhinal and entorhinal cortices (Eichenbaum, 2004; Quiroga;
10 Squire, Stark et al., 2004; Squire, Wixted et al., 2007). Because long-term memory is still
11 functional in patients with *ASPM* mutations, promoting, maintaining and improving their
12 ability to learn would be of great interest.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 Our study aimed to provide new data concerning brain structure in *ASPM* microcephaly that
33 could help extend our understanding of the role of *ASPM* in human brain development. To
34 our knowledge, regional changes in cortical volume, surface, thickness and gyrification have
35 never been analyzed so far in MCPH patients. Our analysis of variance shows that each *ASPM*
36 mutation significantly reduces brain size, supporting the assumption that *ASPM* plays a role
37 in brain development, and that this effect depends significantly on the region under study, as
38 shown by the genotype-by-region interactions. A limitation of the present study is the small
39 cohort size, although the reported data are all statistically significant. Considering that OFC
40 (ranging from -3.5 to -7.5 SD) appear to be reduced to a lower extent than in other studies
41 (Bond, Scott et al., 2003), one could reasonably ask whether this cohort is representative of
42 patients with *ASPM* mutations. However, it should be remembered that our patients are on
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

average younger than in other cohorts and we expect their OFC to worsen with age (Supplementary Figure 1A and (Passemar, Titomanlio et al., 2009).

5. CONCLUSION

Our findings show that despite the mild intellectual disability caused by the reduction of the volume and surface area of their neocortex, patients with *ASPM* mutations exhibit a relative sparing of the volume/surface area of the hippocampus and surrounding medial temporal structures as well as preserved mnemonic abilities. These results highlight how *ASPM* loss-of-function mutations could affect neocortical and hippocampal development and reveal a link between genetics and cognitive behavior in microcephaly.

6. FUNDING

This work was supported by grants from the « Fondation pour la Recherche Médicale (FRC) », the « Programme Hospitalier de Recherche Clinique (PHRC), grant agreement n° P100128 / IDRCB : 2010-A01481-38” and ERA-NET E-Rare-2 2013, grant agreement n° ANR-13-RARE-0007-01 2013. MS was supported by a grant from the National Center of Competence in Research (NCCR) “SYNAPSY—The Synaptic Bases of Mental Diseases” financed by the Swiss National Science Foundation, and by a fellowship from the Swiss National Foundation of Science (#145760).

7. ACKNOWLEDGEMENTS

The authors would like to thank Dr Catherine Verney for discussions and critical reading of the manuscript, Jeannette Nardelli for discussions and S. Rasika for reading and carefully correcting this manuscript.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

REFERENCES

- 1
2
3 ARAI Y and PIERANI A. Development and evolution of cortical fields. *Neurosci Res*, 2014.
- 4
5 BARKOVICH AJ, KUZNIECKY RI, JACKSON GD, GUERRINI R, and DOBYNS WB. A
6
7 developmental and genetic classification for malformations of cortical development.
8
9
10 *Neurology*, 65: 1873-87, 2005.
- 11
12 BARTLEY AJ, JONES DW, and WEINBERGER DR. Genetic variability of human brain size and
13
14 cortical gyral patterns. *Brain*, 120 (Pt 2): 257-69, 1997.
- 15
16
17 BETIZEAU M, CORTAY V, PATTI D, PFISTER S, GAUTIER E, BELLEMIN-MENARD A,
18
19 AFANASSIEFF M, HUISSOUD C, DOUGLAS RJ, KENNEDY H, and DEHAY C. Precursor
20
21 diversity and complexity of lineage relationships in the outer subventricular zone of
22
23 the primate. *Neuron*, 80: 442-57, 2013.
- 24
25
26
27 BISHOP KM, GOUDREAU G, and O'LEARY DD. Regulation of area identity in the mammalian
28
29 neocortex by *emx2* and *pax6*. *Science*, 288: 344-9, 2000.
- 30
31
32 BOND J, ROBERTS E, MOCHIDA GH, HAMPSHIRE DJ, SCOTT S, ASKHAM JM, SPRINGELL K,
33
34 MAHADEVAN M, CROW YJ, MARKHAM AF, WALSH CA, and WOODS CG. *Aspm* is a
35
36 major determinant of cerebral cortical size. *Nat Genet*, 32: 316-20, 2002.
- 37
38
39 BOND J, SCOTT S, HAMPSHIRE DJ, SPRINGELL K, CORRY P, ABRAMOWICZ MJ, MOCHIDA GH,
40
41 HENNEKAM RC, MAHER ER, FRYNS JP, ALSWAID A, JAFRI H, RASHID Y, MUBAIDIN
42
43 A, WALSH CA, ROBERTS E, and WOODS CG. Protein-truncating mutations in *aspm*
44
45 cause variable reduction in brain size. *Am J Hum Genet*, 73: 1170-7, 2003.
- 46
47
48
49 CHEN CH, FIECAS M, GUTIERREZ ED, PANIZZON MS, EYLER LT, VUOKSIMAA E, THOMPSON
50
51 WK, FENNEMA-NOTESTINE C, HAGLER DJ, JR., JERNIGAN TL, NEALE MC, FRANZ CE,
52
53 LYONS MJ, FISCHL B, TSUANG MT, DALE AM, and KREMEN WS. Genetic topography
54
55 of brain morphology. *Proc Natl Acad Sci U S A*, 110: 17089-94, 2013.
- 56
57
58
59
60
61
62
63
64
65

- 1 CHEN CH, GUTIERREZ ED, THOMPSON W, PANIZZON MS, JERNIGAN TL, EYLER LT,
 2 FENNEMA-NOTESTINE C, JAK AJ, NEALE MC, FRANZ CE, LYONS MJ, GRANT MD,
 3 FISCHL B, SEIDMAN LJ, TSUANG MT, KREMEN WS, and DALE AM. Hierarchical
 4 genetic organization of human cortical surface area. *Science*, 335: 1634-6, 2013.
 5
 6
 7
 8
 9 CHEN CH, PANIZZON MS, EYLER LT, JERNIGAN TL, THOMPSON W, FENNEMA-NOTESTINE C,
 10 JAK AJ, NEALE MC, FRANZ CE, HAMZA S, LYONS MJ, GRANT MD, FISCHL B,
 11 SEIDMAN LJ, TSUANG MT, KREMEN WS, and DALE AM. Genetic influences on
 12 cortical regionalization in the human brain. *Neuron*, 72: 537-44, 2011.
 13
 14
 15
 16
 17
 18
 19 CHO ZH, HAN JY, HWANG SI, KIM DS, KIM KN, KIM NB, KIM SJ, CHI JG, PARK CW, and
 20 KIM YB. Quantitative analysis of the hippocampus using images obtained from 7.0 t
 21 mri. *Neuroimage*, 49: 2134-40, 2010.
 22
 23
 24
 25
 26 CHOUINARD-DECORTE F, MCKAY DR, REID A, KHUNDRAKPAM B, ZHAO L, KARAMA S,
 27 RIOUX P, SPROOTEN E, KNOWLES E, KENT JW, JR., CURRAN JE, GORING HH, DYER
 28 TD, OLVERA RL, KOCHUNOV P, DUGGIRALA R, FOX PT, ALMASY L, BLANGERO J,
 29 BELLEC P, EVANS AC, and GLAHN DC. Heritable changes in regional cortical
 30 thickness with age. *Brain Imaging Behav*, 8: 208-16, 2014.
 31
 32
 33
 34
 35
 36
 37
 38
 39 COHEN. Children's memory scale. . *San Antonio, TX: The Psychological Corporation*, 1997.
 40
 41 DAGER SR, WANG L, FRIEDMAN SD, SHAW DW, CONSTANTINO JN, ARTRU AA, DAWSON G,
 42 and CSERNANSKY JG. Shape mapping of the hippocampus in young children with
 43 autism spectrum disorder. *AJNR Am J Neuroradiol*, 28: 672-7, 2007.
 44
 45
 46
 47
 48
 49 DESIKAN RS, SEGONNE F, FISCHL B, QUINN BT, DICKERSON BC, BLACKER D, BUCKNER RL,
 50 DALE AM, MAGUIRE RP, HYMAN BT, ALBERT MS, and KILLIANY RJ. An automated
 51 labeling system for subdividing the human cerebral cortex on mri scans into gyral
 52 based regions of interest. *Neuroimage*, 31: 968-80, 2006.
 53
 54
 55
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- DESIR J, CASSART M, DAVID P, VAN BOGAERT P, and ABRAMOWICZ M. Primary microcephaly with *aspm* mutation shows simplified cortical gyration with antero-posterior gradient pre- and post-natally. *Am J Med Genet A*, 146A: 1439-43, 2008.
- EICHENBAUM H. Hippocampus: Cognitive processes and neural representations that underlie declarative memory. *Neuron*, 44: 109-20, 2004.
- EVANS PD, ANDERSON JR, VALLENDER EJ, GILBERT SL, MALCOM CM, DORUS S, and LAHN BT. Adaptive evolution of *aspm*, a major determinant of cerebral cortical size in humans. *Hum Mol Genet*, 13: 489-94, 2004.
- FEI JF, HAFFNER C, and HUTTNER WB. 3' utr-dependent, mir-92-mediated restriction of *tis21* expression maintains asymmetric neural stem cell division to ensure proper neocortex size. *Cell Rep*, 7: 398-411, 2014.
- FIETZ SA, KELAVA I, VOGT J, WILSCH-BRAUNINGER M, STENZEL D, FISH JL, CORBEIL D, RIEHN A, DISTLER W, NITSCH R, and HUTTNER WB. Osvz progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat Neurosci*, 13: 690-9, 2010.
- FIETZ SA, LACHMANN R, BRANDL H, KIRCHER M, SAMUSIK N, SCHRODER R, LAKSHMANAPERUMAL N, HENRY I, VOGT J, RIEHN A, DISTLER W, NITSCH R, ENARD W, PAABO S, and HUTTNER WB. Transcriptomes of germinal zones of human and mouse fetal neocortex suggest a role of extracellular matrix in progenitor self-renewal. *Proc Natl Acad Sci U S A*, 109: 11836-41, 2012.
- FISCHL B, LIU A, and DALE AM. Automated manifold surgery: Constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging*, 20: 70-80, 2001.
- FISH JL, DEHAY C, KENNEDY H, and HUTTNER WB. Making bigger brains-the evolution of neural-progenitor-cell division. *J Cell Sci*, 121: 2783-93, 2008.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- FISH JL, KOSODO Y, ENARD W, PAABO S, and HUTTNER WB. Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. *Proc Natl Acad Sci U S A*, 103: 10438-43, 2006.
- FLORIO M, ALBERT M, TAVERNA E, NAMBA T, BRANDL H, LEWITUS E, HAFFNER C, SYKES A, WONG FK, PETERS J, GUHR E, KLEMROTH S, PRUFER K, KELSO J, NAUMANN R, NUSSLEIN I, DAHL A, LACHMANN R, PAABO S, and HUTTNER WB. Human-specific gene *arhgap11b* promotes basal progenitor amplification and neocortex expansion. *Science*, 347: 1465-70, 2015.
- FLORIO M and HUTTNER WB. Neural progenitors, neurogenesis and the evolution of the neocortex. *Development*, 141: 2182-94, 2014.
- FRANKE K, ZIEGLER G, KLOPPEL S, and GASER C. Estimating the age of healthy subjects from t1-weighted mri scans using kernel methods: Exploring the influence of various parameters. *Neuroimage*, 50: 883-92, 2010.
- GARCIA-MORENO F, VASISTHA NA, TREVIA N, BOURNE JA, and MOLNAR Z. Compartmentalization of cerebral cortical germinal zones in a lissencephalic primate and gyrencephalic rodent. *Cereb Cortex*, 22: 482-92, 2012.
- GIEDD JN, BLUMENTHAL J, JEFFRIES NO, CASTELLANOS FX, LIU H, ZIJDENBOS A, PAUS T, EVANS AC, and RAPOPORT JL. Brain development during childhood and adolescence: A longitudinal mri study. *Nat Neurosci*, 2: 861-3, 1999.
- GIEDD JN, STOCKMAN M, WEDDLE C, LIVERPOOL M, ALEXANDER-BLOCH A, WALLACE GL, LEE NR, LALONDE F, and LENROOT RK. Anatomic magnetic resonance imaging of the developing child and adolescent brain and effects of genetic variation. *Neuropsychol Rev*, 20: 349-61, 2010.
- GILMORE EC and WALSH CA. Genetic causes of microcephaly and lessons for neuronal development. *Wiley Interdiscip Rev Dev Biol*, 2: 461-78, 2013.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- GOGTAY N, GIEDD JN, LUSK L, HAYASHI KM, GREENSTEIN D, VAITUZIS AC, NUGENT TF, 3RD, HERMAN DH, CLASEN LS, TOGA AW, RAPOPORT JL, and THOMPSON PM. Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A*, 101: 8174-9, 2004.
- GOTZ M and HUTTNER WB. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol*, 6: 777-88, 2005.
- GRUBER R, ZHOU Z, SUKCHEV M, JOERSS T, FRAPPART PO, and WANG ZQ. Mcph1 regulates the neuroprogenitor division mode by coupling the centrosomal cycle with mitotic entry through the chk1-cdc25 pathway. *Nat Cell Biol*, 13: 1325-34, 2011.
- HAIER RJ, JUNG RE, YEO RA, HEAD K, and ALKIRE MT. Structural brain variation and general intelligence. *Neuroimage*, 23: 425-33, 2004.
- HAN X, JOVICICH J, SALAT D, VAN DER KOUWE A, QUINN B, CZANNER S, BUSA E, PACHECO J, ALBERT M, KILLIANY R, MAGUIRE P, ROSAS D, MAKRIS N, DALE A, DICKERSON B, and FISCHL B. Reliability of mri-derived measurements of human cerebral cortical thickness: The effects of field strength, scanner upgrade and manufacturer. *Neuroimage*, 32: 180-94, 2006.
- HANSEN DV, LUI JH, PARKER PR, and KRIEGSTEIN AR. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature*, 464: 554-561, 2010.
- HOPKINS RO, ABILDSKOV TJ, BIGLER ED, and WEAVER LK. Three dimensional image reconstruction of neuroanatomical structures: Methods for isolation of the cortex, ventricular system, hippocampus, and fornix. *Neuropsychol Rev*, 7: 87-104, 1997.
- HU H, SUCKOW V, MUSANTE L, ROGGENKAMP V, KRAEMER N, ROPERS HH, HUBNER C, WIENKER TF, and KAINDL AM. Previously reported new type of autosomal recessive primary microcephaly is caused by compound heterozygous *aspm* gene mutations. *Cell Cycle*, 13: 1650-1, 2014.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- IM K, LEE JM, LYTTTELTON O, KIM SH, EVANS AC, and KIM SI. Brain size and cortical structure in the adult human brain. *Cereb Cortex*, 18: 2181-91, 2008.
- KAINDL AM, PASSEMARD S, KUMAR P, KRAEMER N, ISSA L, ZWIRNER A, GERARD B, VERLOES A, MANI S, and GRESSENS P. Many roads lead to primary autosomal recessive microcephaly. *Prog Neurobiol*, 2009.
- KARAMA S, COLOM R, JOHNSON W, DEARY IJ, HAIER R, WABER DP, LEPAGE C, GANJAVI H, JUNG R, and EVANS AC. Cortical thickness correlates of specific cognitive performance accounted for by the general factor of intelligence in healthy children aged 6 to 18. *Neuroimage*, 55: 1443-53, 2011.
- KOUPRINA N, PAVLICEK A, MOCHIDA GH, SOLOMON G, GERSCH W, YOON YH, COLLURA R, RUVOLO M, BARRETT JC, WOODS CG, WALSH CA, JURKA J, and LARIONOV V. Accelerated evolution of the *aspm* gene controlling brain size begins prior to human brain expansion. *PLoS Biol*, 2: E126, 2004.
- KUMAR A, BLANTON SH, BABU M, MARKANDAYA M, and GIRIMAJI SC. Genetic analysis of primary microcephaly in indian families: Novel *aspm* mutations. *Clin Genet*, 66: 341-8, 2004.
- LAMONICA BE, LUI JH, HANSEN DV, and KRIEGSTEIN AR. Mitotic spindle orientation predicts outer radial glial cell generation in human neocortex. *Nat Commun*, 4: 1665, 2013.
- LENROOT RK and GIEDD JN. Brain development in children and adolescents: Insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev*, 30: 718-29, 2006.
- LEW M. Good statistical practice in pharmacology. Problem 2. *Br J Pharmacol*, 152: 299-303, 2007.
- LIZARRAGA SB, MARGOSSIAN SP, HARRIS MH, CAMPAGNA DR, HAN AP, BLEVINS S, MUDBHARY R, BARKER JE, WALSH CA, and FLEMING MD. *Cdk5rap2* regulates

centrosome function and chromosome segregation in neuronal progenitors.

Development, 137: 1907-17, 2010.

LOHMANN G, VON CRAMON DY, and STEINMETZ H. Sulcal variability of twins. *Cereb Cortex*,

9: 754-63, 1999.

LUI JH, HANSEN DV, and KRIEGSTEIN AR. Development and evolution of the human

neocortex. *Cell*, 146: 18-36, 2011.

LUKASZEWICZ A, CORTAY V, GIROUD P, BERLAND M, SMART I, KENNEDY H, and DEHAY C.

The concerted modulation of proliferation and migration contributes to the specification of the cytoarchitecture and dimensions of cortical areas. *Cereb Cortex*,

16 Suppl 1: i26-34, 2006.

MALLAMACI A, MUZIO L, CHAN CH, PARNAVELAS J, and BONCINELLI E. Area identity shifts

in the early cerebral cortex of *emx2*^{-/-} mutant mice. *Nat Neurosci*, 3: 679-86, 2000.

MEKEL-BOBROV N, GILBERT SL, EVANS PD, VALLENDER EJ, ANDERSON JR, HUDSON RR,

TISHKOFF SA, and LAHN BT. Ongoing adaptive evolution of *aspm*, a brain size determinant in homo sapiens. *Science*, 309: 1720-2, 2005.

MIYASHITA-LIN EM, HEVNER R, WASSARMAN KM, MARTINEZ S, and RUBENSTEIN JL. Early

neocortical regionalization in the absence of thalamic innervation. *Science*, 285: 906-9, 1999.

MONTGOMERY SH and MUNDY NI. Evolution of *aspm* is associated with both increases and

decreases in brain size in primates. *Evolution*, 66: 927-32, 2012.

MUHAMMAD F, MAHMOOD BAIG S, HANSEN L, SAJID HUSSAIN M, ANJUM INAYAT I, ASLAM

M, ANVER QURESHI J, TOILAT M, KIRST E, WAJID M, NURNBERG P, EIBERG H, TOMMERUP N, and KJAER KW. Compound heterozygous *aspm* mutations in pakistani

mcpf families. *Am J Med Genet A*, 149A: 926-930, 2009.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- NICHOLAS AK, SWANSON EA, COX JJ, KARBANI G, MALIK S, SPRINGELL K, HAMPSHIRE D, AHMED M, BOND J, DI BENEDETTO D, FICHERA M, ROMANO C, DOBYNS WB, and WOODS CG. The molecular landscape of aspm mutations in primary microcephaly. *J Med Genet*, 46: 249-53, 2009.
- NONAKA-KINOSHITA M, REILLO I, ARTEGIANI B, MARTINEZ-MARTINEZ MA, NELSON M, BORRELL V, and CALEGARI F. Regulation of cerebral cortex size and folding by expansion of basal progenitors. *EMBO J*, 32: 1817-28, 2013.
- O'LEARY DD, CHOU SJ, and SAHARA S. Area patterning of the mammalian cortex. *Neuron*, 56: 252-69, 2007.
- PARAMASIVAM M, CHANG YJ, and LOTURCO JJ. Aspm and citron kinase co-localize to the midbody ring during cytokinesis. *Cell Cycle*, 6: 1605-12, 2007.
- PASSEMARD S, EL GHOZZI V, NASSER H, VERNEY C, VODJDANI G, LACAUD A, LEBON S, LABURTHE M, ROBBERECHT P, NARDELLI J, MANI S, VERLOES A, GRESSENS P, and LELIEVRE V. Vip blockade leads to microcephaly in mice via disruption of mcph1-chk1 signaling. *J Clin Invest*, 12:3071-3087, 2011.
- PASSEMARD S, KAINDL AM, and VERLOES A. Microcephaly. *Handb Clin Neurol*, 111: 129-41, 2013.
- PASSEMARD S, TITOMANLIO L, ELMALEH M, AFENJAR A, ALESSANDRI JL, ANDRIA G, DE VILLEMEUR TB, BOESPFLUG-TANGUY O, BURGLEN L, DEL GIUDICE E, GUIMIOT F, HYON C, ISIDOR B, MEGARBANE A, MOOG U, ODENT S, HERNANDEZ K, POUVREAU N, SCALA I, SCHAER M, GRESSENS P, GERARD B, and VERLOES A. Expanding the clinical and neuroradiologic phenotype of primary microcephaly due to aspm mutations. *Neurology*, 73: 962-9, 2009.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- POSTHUMA D, DE GEUS EJ, BAARE WF, HULSHOFF POL HE, KAHN RS, and BOOMSMA DI.
The association between brain volume and intelligence is of genetic origin. *Nat Neurosci*, 5: 83-4, 2002.
- PULVERS JN, BRYK J, FISH JL, WILSCH-BRAUNINGER M, ARAI Y, SCHREIER D, NAUMANN R, HELPPI J, HABERMANN B, VOGT J, NITSCH R, TOTH A, ENARD W, PAABO S, and HUTTNER WB. Mutations in mouse *aspm* (abnormal spindle-like microcephaly associated) cause not only microcephaly but also major defects in the germline. *Proc Natl Acad Sci U S A*, 107: 16595-600, 2010.
- QUIROGA RQ. Concept cells: The building blocks of declarative memory functions. *Nat Rev Neurosci*, 13: 587-97, 2012.
- RAKIC P. Specification of cerebral cortical areas. *Science*, 241: 170-6, 1988.
- RAKIC P. Evolution of the neocortex: A perspective from developmental biology. *Nat Rev Neurosci*, 10: 724-35, 2009.
- RASH BG, TOMASI S, LIM HD, SUH CY, and VACCARINO FM. Cortical gyrification induced by fibroblast growth factor 2 in the mouse brain. *J Neurosci*, 33: 10802-14, 2013.
- RAZNAHAN A, LERCH JP, LEE N, GREENSTEIN D, WALLACE GL, STOCKMAN M, CLASEN L, SHAW PW, and GIEDD JN. Patterns of coordinated anatomical change in human cortical development: A longitudinal neuroimaging study of maturational coupling. *Neuron*, 72: 873-84, 2011.
- RONAN L, VOETS N, RUA C, ALEXANDER-BLOCH A, HOUGH M, MACKAY C, CROW TJ, JAMES A, GIEDD JN, and FLETCHER PC. Differential tangential expansion as a mechanism for cortical gyrification. *Cereb Cortex*, 24: 2219-28, 2013.
- ROSAS HD, LIU AK, HERSCH S, GLESSNER M, FERRANTE RJ, SALAT DH, VAN DER KOUWE A, JENKINS BG, DALE AM, and FISCHL B. Regional and progressive thinning of the cortical ribbon in huntington's disease. *Neurology*, 58: 695-701, 2002.

- 1 SAJID HUSSAIN M, MARRIAM BAKHTIAR S, FAROOQ M, ANJUM I, JANZEN E, REZA TOLIAT M,
 2 EIBERG H, KJAER KW, TOMMERUP N, NOEGEL AA, NURNBERG P, BAIG SM, and
 3 HANSEN L. Genetic heterogeneity in pakistani microcephaly families. *Clin Genet*, 83:
 4 446-51, 2013.
 5
 6
 7
 8
 9 SALAT DH, GREVE DN, PACHECO JL, QUINN BT, HELMER KG, BUCKNER RL, and FISCHL B.
 10 Regional white matter volume differences in nondemented aging and alzheimer's
 11 disease. *Neuroimage*, 44: 1247-58, 2009.
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26
 27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37
 38
 39
 40
 41
 42
 43
 44
 45
 46
 47
 48
 49
 50
 51
 52
 53
 54
 55
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65

expansion and folding of the mammalian cerebral cortex by control of radial glial fate.
Cell, 153: 535-49, 2013.

STANCIK EK, NAVARRO-QUIROGA I, SELLKE R, and HAYDAR TF. Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. *J Neurosci*, 30: 7028-36, 2010.

SUN T and HEVNER RF. Growth and folding of the mammalian cerebral cortex: From molecules to malformations. *Nat Rev Neurosci*, 15: 217-32, 2014.

SZABO K, FORSTER A, and GASS A. Conventional and diffusion-weighted mri of the hippocampus. *Front Neurol Neurosci*, 34: 71-84, 2014.

SZABO N, PAP C, KOBOR J, SVEKUS A, TURI S, and SZTRIHA L. Primary microcephaly in hungary: Epidemiology and clinical features. *Acta Paediatr*, 99: 690-3, 2010.

TAN CA, DEL GAUDIO D, DEMPSEY MA, ARNDT K, BOTES S, REEDER A, and DAS S. Analysis of aspm in an ethnically diverse cohort of 400 patient samples: Perspectives of the molecular diagnostic laboratory. *Clin Genet*, 85: 353-8, 2010.

TAVERNA E, GOTZ M, and HUTTNER WB. The cell biology of neurogenesis: Toward an understanding of the development and evolution of the neocortex. *Annu Rev Cell Dev Biol*, 30: 465-502, 2014.

THORNTON GK and WOODS CG. Primary microcephaly: Do all roads lead to rome? *Trends Genet*, 25: 501-10, 2009.

VAN DEN BOSCH J. Microcephaly in the netherlands: A clinical and genetical study. *Ann Hum Genet*, 23: 91-116, 1959.

VERLOES A, DRUNAT S, GRESSENS P, and PASSEMARD S. Primary autosomal recessive microcephalies and seckel syndrome spectrum disorders, 2013.

WECHSLER. Adult intelligence scale-iii. *Psychological Corporation, New York.*, 1997.

WECHSLER. Wais-iii—wms-iii

The Psychological Corporation. Harcourt Brace & Co., London., 1998.

WECHSLER. Wechsler intelligence scale for children-iv. *The Psychological Corporation. Toronto, Ontario, Canada, 2003.*

WOODS CG, BOND J, and ENARD W. Autosomal recessive primary microcephaly (mcph): A review of clinical, molecular, and evolutionary findings. *Am J Hum Genet*, 76: 717-28, 2005.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

FIGURE LEGENDS

Figure 1

Regional differences in brain volume reduction in patients with *ASPM* mutations

(A-E) Quantification of the overall brain volumes in controls and patients. Volumes are expressed as a percentage of the average of the controls. *ASPM* mutations result in regional differences of brain volume reduction between supra and infratentorial compartments (A), brain stem and cerebellum (B), total grey matter and white matter (C), cerebral cortex, subcortical gray matter and cerebellar cortex (D).

Analyses are two-way ANOVAs followed by Bonferroni post hoc tests (# $p < 0.05$; ## $p < 0.01$; ###: $p < 0.001$). Values are group means \pm SEM.

Figure 2

Regional differences in cortical volume reduction in patients with *ASPM* mutations

(A-B) Quantification of the cerebral cortical volume in the different lobes in controls and patients (AB). Volumes are expressed as a percentage of the average of the controls. The volume of the cingulate cortex was more reduced than that of the frontal, parietal and temporal lobes in patients as compared to controls, in the right hemisphere (B).

Analyses are two-way ANOVAs followed by Bonferroni post hoc tests (## $p < 0.01$; ###: $p < 0.001$). Values are group means \pm SEM.

(C) Color-coded representation of the cortical volume in patients with *ASPM* mutations, expressed as a percentage of the average of the controls.

The reduction in volume was not uniform throughout the cerebral cortex. Regions in orange to brown indicate those most reduced in volume, i.e. the occipital and cingulate cortices. In contrast, green and yellow regions are those in which patients showed a relative preservation

1
2 of their cortical volume, i.e. the parahippocampal and entorhinal cortices and the pars
3 opercularis.
4
5
6

7 Figure 3

8 **Hippocampal volume is preserved in patients with *ASPM* mutations**

9
10 (A-B) Example of a 3D "cutaway" view that includes a coronal section and an axial section of
11 the hippocampus in a control subject (A) and in a patient with *ASPM* mutation (B) (as
12 previously shown in (Cho, Han et al.; Dager, Wang et al., 2007; Hopkins, Abildskov et al.,
13 1997; Szabo, Forster et al.), highlighting the volume of the hippocampus in comparison with
14 the rest of the brain (both images are at the same scale).
15
16
17
18
19
20
21
22

23 (C-F) Quantification of the cortical volume in nine regions of the temporal lobes in controls
24 and patients (C, E: bar graph; D, F: individual data presented in a vertical scatter plot).
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Volumes are expressed as a percentage of the average of the controls. *ASPM* mutations result
in regional differences of cortical volume reduction within temporal lobes with preservation
of both hippocampi and parahippocampal cortices as well as of the right entorhinal cortex as
compared to the other temporal regions.

Analyses are two-way ANOVAs followed by Bonferroni post hoc tests (###: $p < 0.001$).
Values are group means \pm SEM.

Figure 4

Cortical thickness is maintained or increased in patients with *ASPM* mutations

Color-coded representation of the cortical thickness in patients with *ASPM* mutations,
expressed as a percentage of the average of the controls, using the same code as for
volumetric data.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Cortical regions depicted in dark blue indicate regions with the thicker cortex in patients with *ASPM* mutations as compared to controls, i.e. regions in the dorsolateral cortex lining the Sylvian fissure and the post central sulcus.

Figure 5

The cortical surface area is the most affected parameter in patients with *ASPM* mutations in all regions except in the hippocampus and surrounding medial temporal cortex

(A-B) Quantification of the surface area of the cerebral cortex in the different lobes in controls and patients (AB). Surface areas are expressed as a percentage of the average of the controls. The surface area of the cingulate cortex was more reduced than that of the frontal, parietal, occipital and temporal lobes in patients as compared to controls, in the right hemisphere (B).

Analyses are two-way ANOVAs followed by Bonferroni post hoc tests (##: $p < 0.01$). Values are group means \pm SEM.

(C) Color code representation of the cortical surface area in patients, expressed as a percentage of the average of the controls. Regions in dark red or brown indicate those most reduced in surface area, i.e. the caudal and rostral middle frontal and rostral anterior cingulate region regions. In contrast, regions in green or yellow are those in which the cortical surface area was more preserved in patients as compared to controls, i.e. the parahippocampal cortices and the right entorhinal cortex.

Figure 6

White matter volume is reduced in similar proportion as the cortex

(A-B) Quantification of the reduction in the volume of the white matter in the different lobes in controls and patients. Volumes are expressed as a percentage of the average of the controls

(C-D) Quantification of the volume of the white matter in the temporal lobes in controls and patients. *ASPM* mutations result in regional differences of white matter volume reduction within the temporal lobe with preservation of the white matter volume of both parahippocampal regions and of the right entorhinal region as compared to the other temporal regions.

Analyses are two-way ANOVAs followed by Bonferroni post hoc tests (#: $p < 0.05$; ##: $p < 0.01$). Values are group means \pm SEM.

Figure 7

Regional differences in Gyrfication Index reduction in patients with *ASPM* mutations

Color code representation of the local Gyrfication Index in patients, expressed as a percentage of the average of the controls. Regions in yellow are those in which the GI was the lowest in patients as compared to controls, i.e. both pars opercularis and superior temporal regions.

Figure 8

Mnesic abilities of patients with *ASPM* mutations are preserved despite their intellectual deficiency

Cognitive assessment of patients with *ASPM* mutations using Wechsler scales [WAIS III and MEM III (Patients 1 and 2) or WISC IV and CMS (Patients 3 to 5)]. Normal IQ values [115-85] are indicated in dark grey and borderline IQ values [84-70] in light grey. Patients with *ASPM* mutations exhibited mild-to-moderate intellectual disabilities with better performances on long-term memory tests compared to their mean Full scale IQ.

Analyses are one-way ANOVA followed by Bonferroni post hoc tests (***: $p < 0.001$). Values are group means \pm SEM.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

TABLES

Table 1

Molecular and clinical characteristics of patients with *ASPM* mutations

	Referred as in Passemard et al 2009	<i>ASPM</i> mutations	OFC at birth	Age at the time of evaluation	OFC at the time of evaluation	Cortical dysgenesis	Education and/or career (age)
Patient 1 *	1-2	c.7782_7783delGA homozygous	29cm (-4SD)	24y	47.2cm (-7.5 SD)	0	Sheltered employment in an office (24y)
Patient 2 #	7-1	c.9319+C9507_delG	31cm (-3SD)	20y	47.5cm (-6SD)	0	Sheltered employment as gardener (20y) Reads and writes
Patient 3 *	1-1	c.7782_7783delGA homozygous	32cm (-2SD)	14y	48cm (-4SD)	0	Apprentice baker (at 17y) Reads and writes
Patient 4 #	7-2	c.9319+C9507_delG	31cm (-3SD)	17y	46cm (-6SD)	Right extensive polymicrogyria	Occupational activity Reads and writes with difficulty
Patient 5	3-1	c.6651_6654delAACA homozygous	31,5cm (-2.5SD)	13y9m	47.5 cm (-4SD)	0	Middle school with special educational facilities (13y9m). Reads and writes
Patient 6	10-1	c.2389C>T+c.6686_6689 delGAAA	28 cm (-5SD)	7y	42.5cm (-6SD)	Focal parietal cortical dysplasia	Special educational setting
Patient 7	2-1	c.77delG+c.6232T>C	32cm (-2SD)	8y	42.5 cm (-6SD)	0	Elementary school with special educational setting

OFC = Occipitofrontal circumference, SD = standard deviation, y = year, m = months

*, # : siblings

Abnormal spindle-like microcephaly-associated (*ASPM*) mutations strongly disrupt neocortical structure but spare the hippocampus and long-term memory

Sandrine Passemard (1-4), Alain Verloes (1-3), Thierry Billette de Villemeur (1,5-6), Odile Boespflug (1,2,4), Karen Hernandez (3), Marion Laurent (7), Bertrand Isidor (8), Corinne Alberti (2,9), Nathalie Pouvreau (3), Séverine Drunat (1, 3), Bénédicte Gérard (3), Vincent El Ghouzzi (1,2), Jorge Gallego (1,2), Monique Elmaleh-Bergès (1,2,10), Wieland B. Huttner (11), Stephan Eliez (12), Pierre Gressens* (1,2,4,13), Marie Schaer* (12,14).

HIGHLIGHTS

- First study of cortical volume/surface/thickness/gyrification in *ASPM* microcephaly
- *ASPM* mutations disrupt cortical development in a region-specific fashion
- Neocortical volume and surface are reduced by 50%, but with regional variations
- The hippocampus and surrounding medial temporal structures are spared
- There is a corresponding preservation of long-term memory in *ASPM* patients

Figure 1
[Click here to download high resolution image](#)

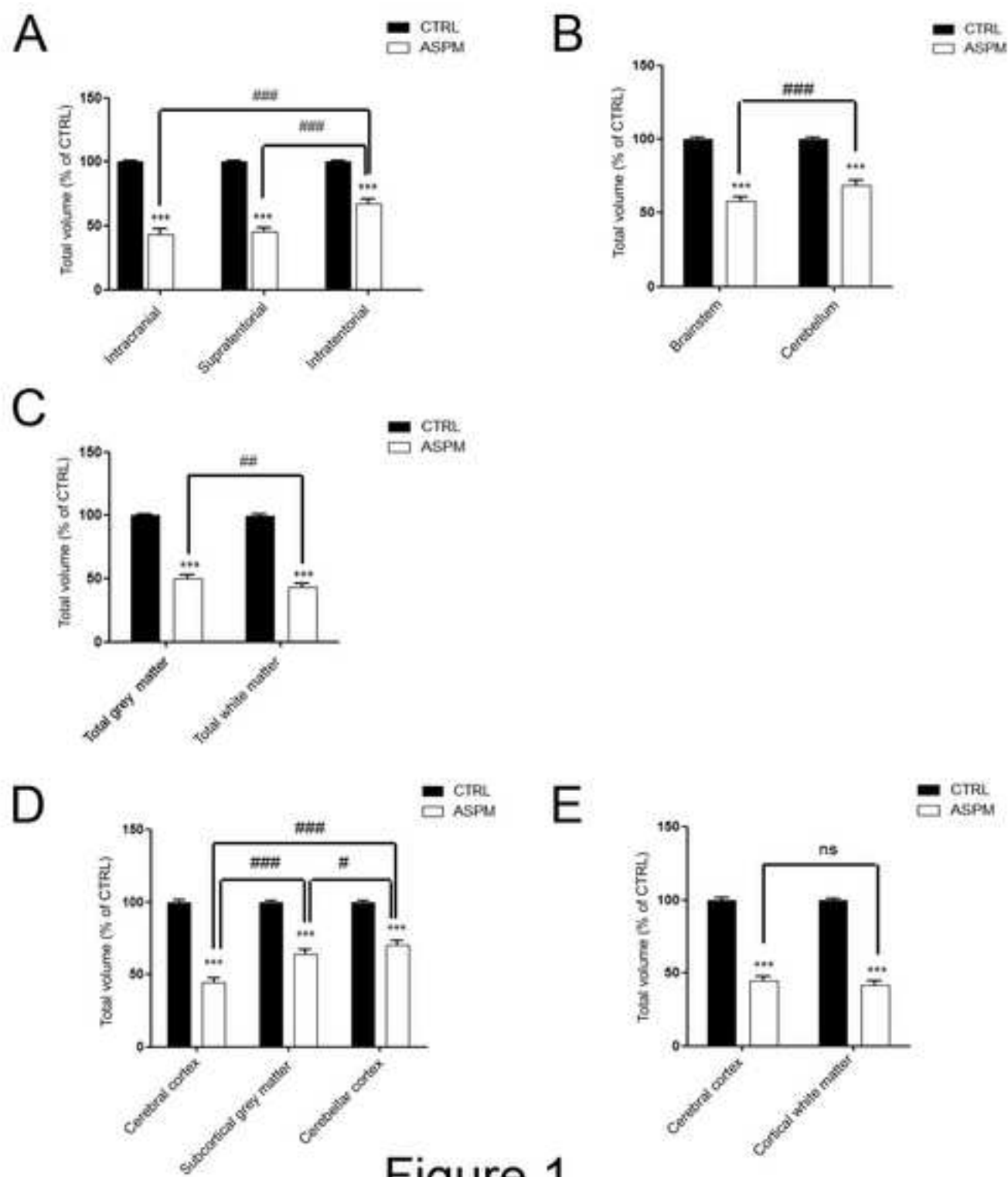


Figure 1

Figure 2
[Click here to download high resolution image](#)

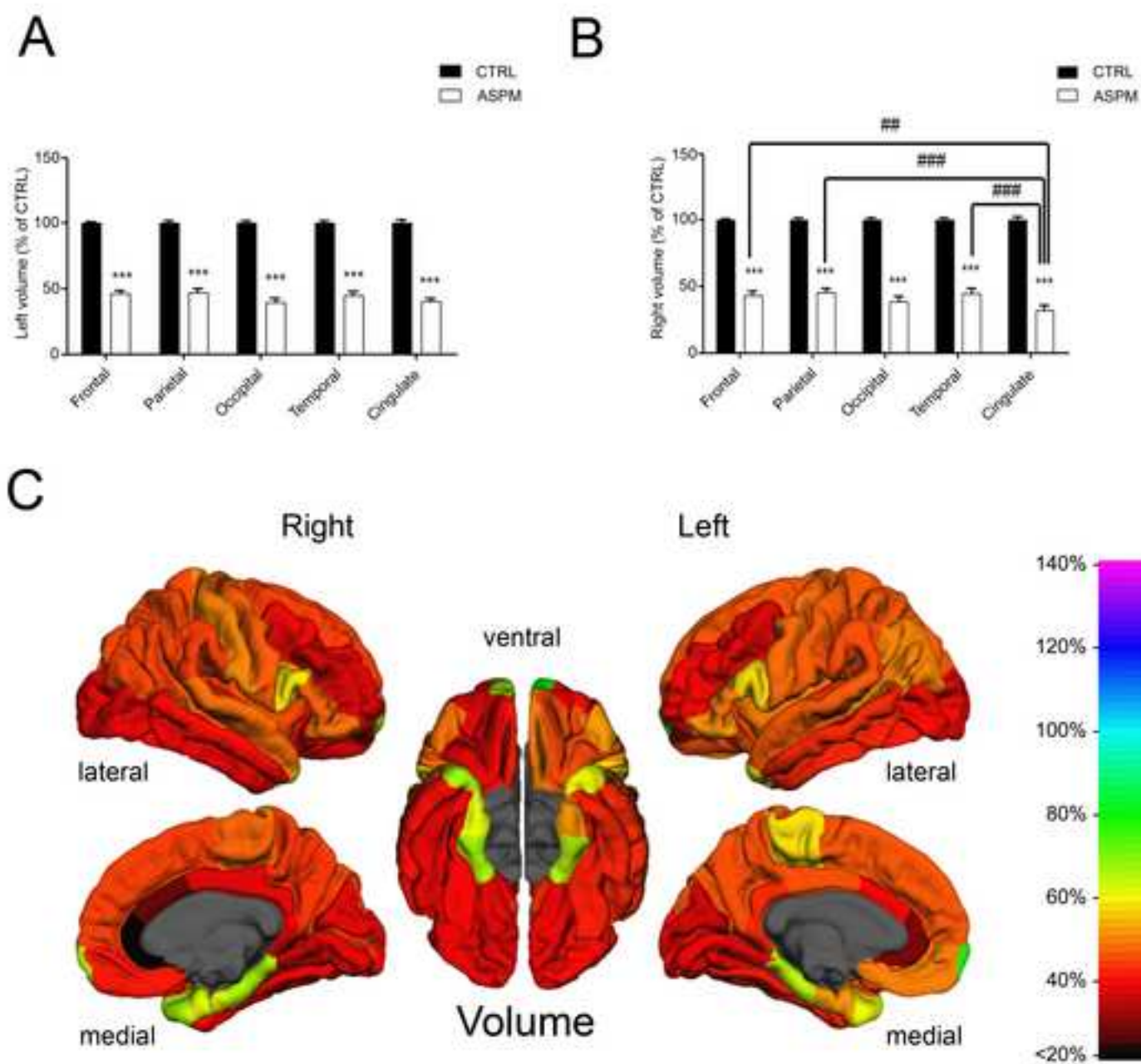


Figure 2

Figure 3
[Click here to download high resolution image](#)

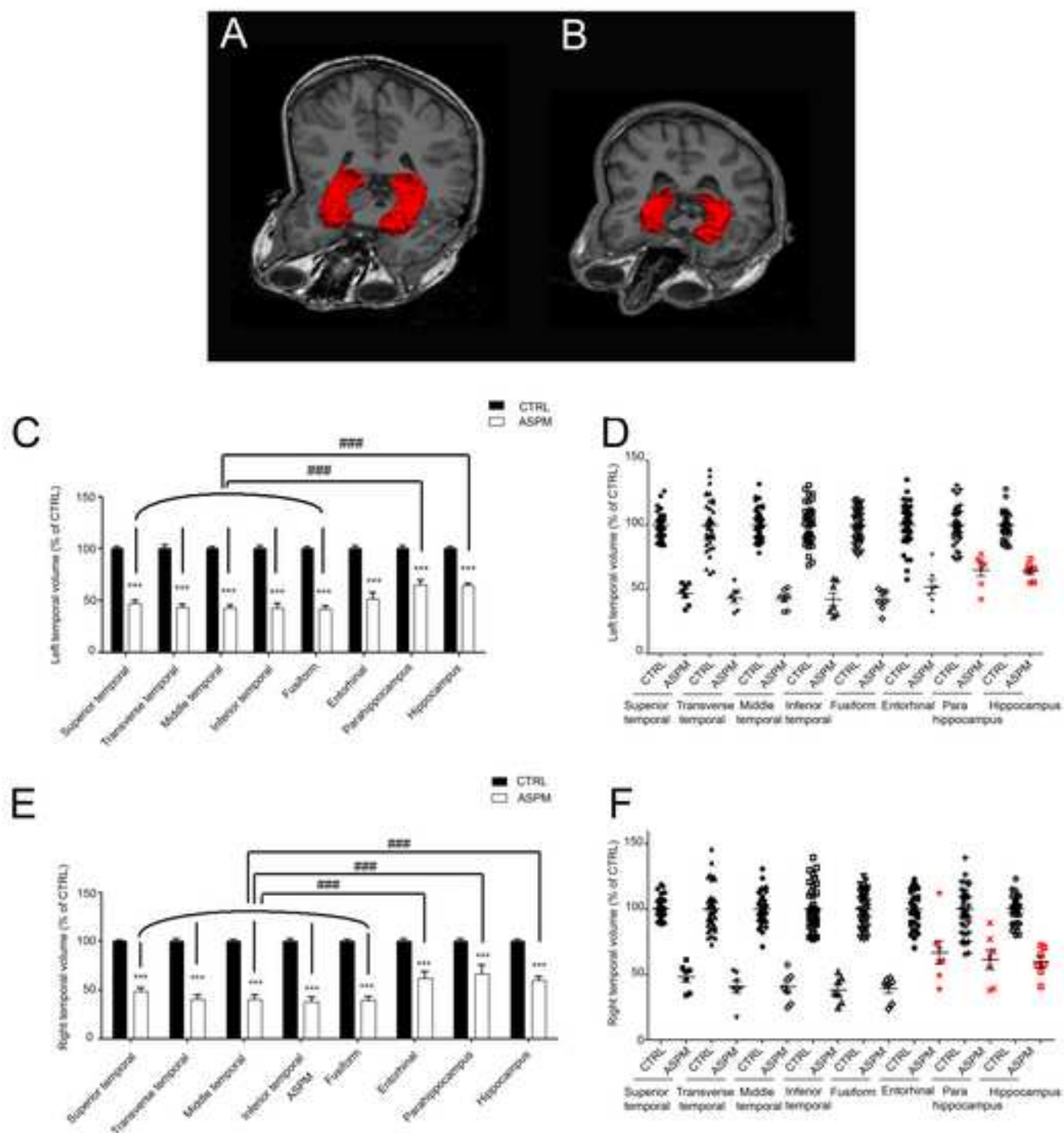


Figure 3

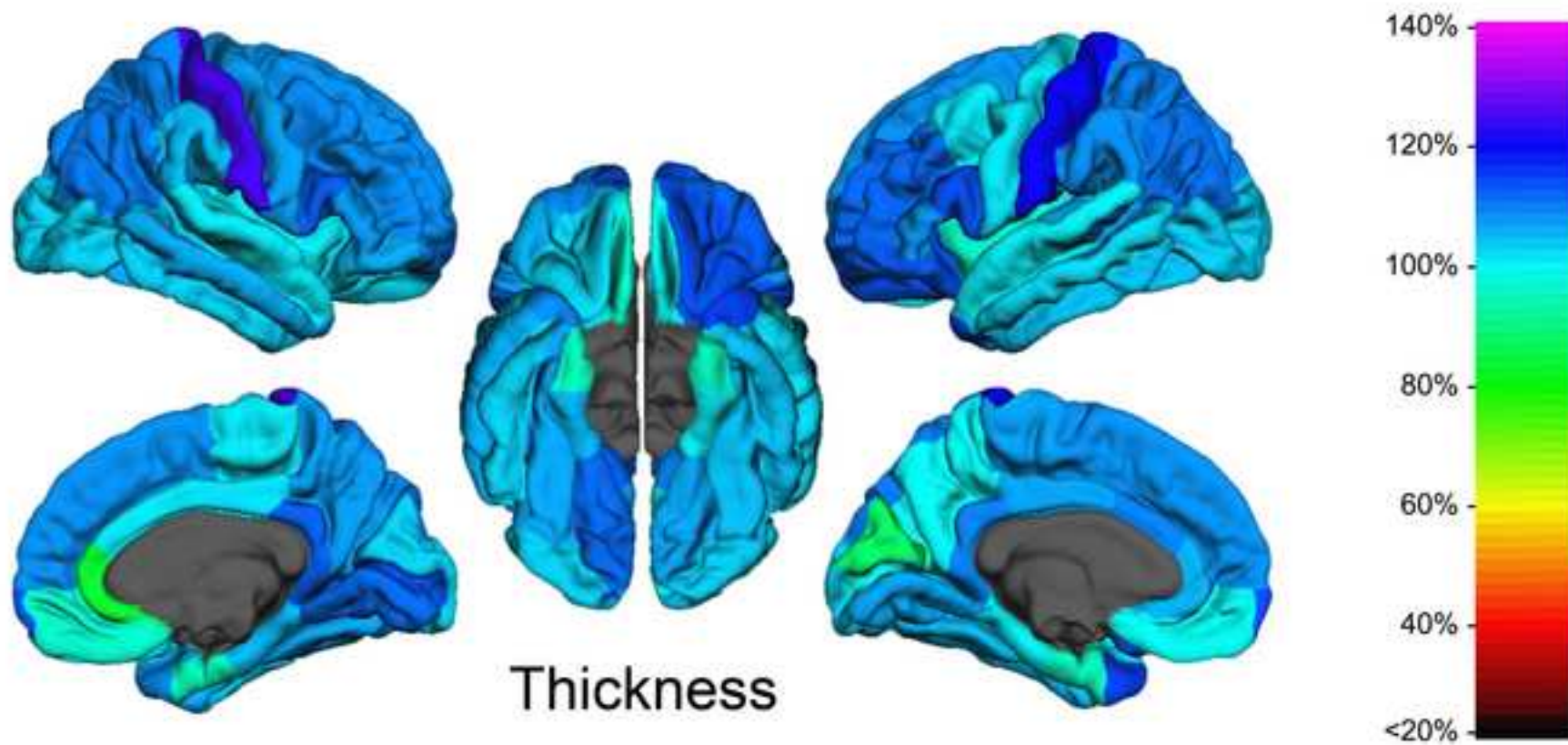


Figure 4

Figure 5
[Click here to download high resolution image](#)

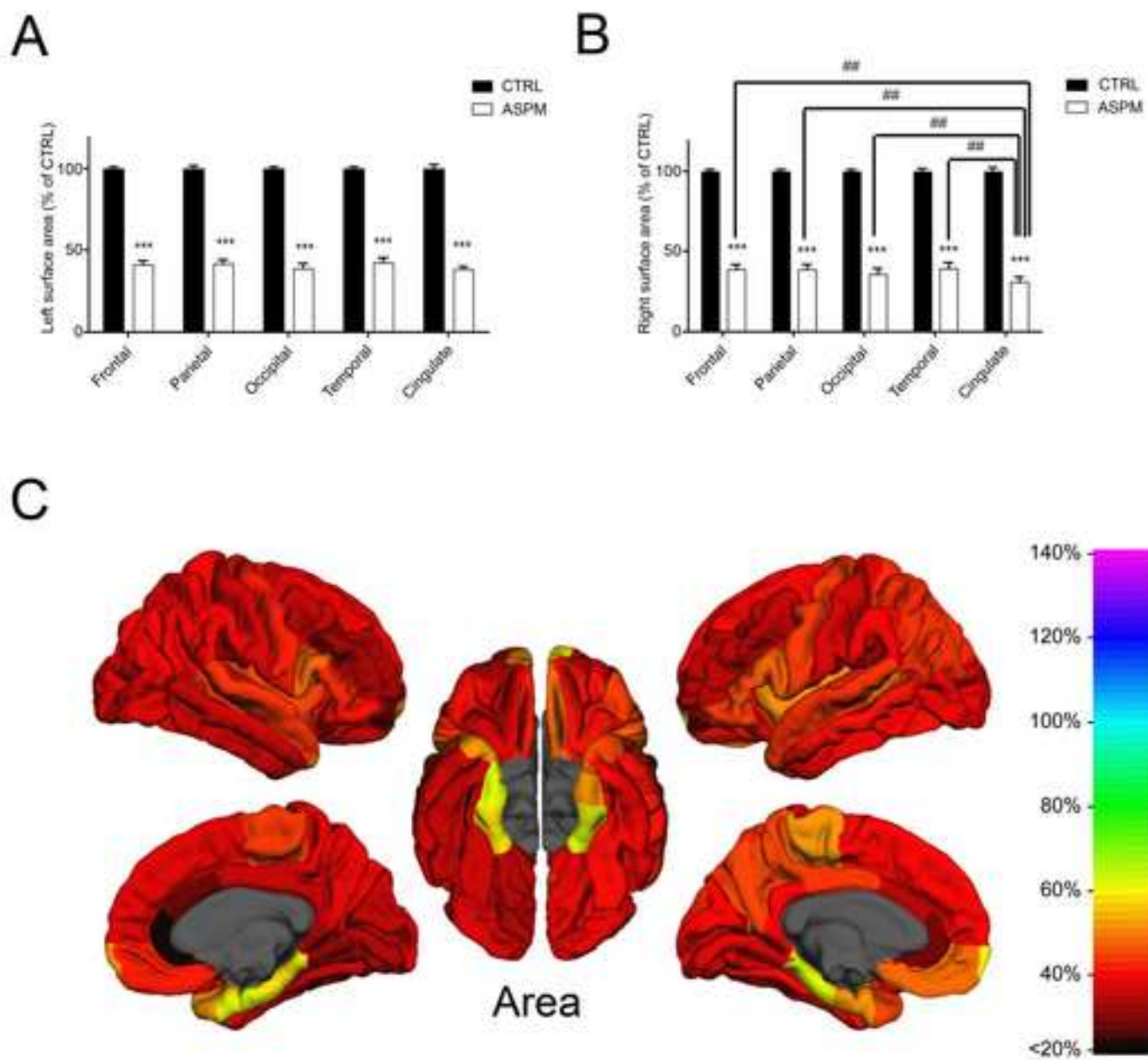


Figure 6
[Click here to download high resolution image](#)

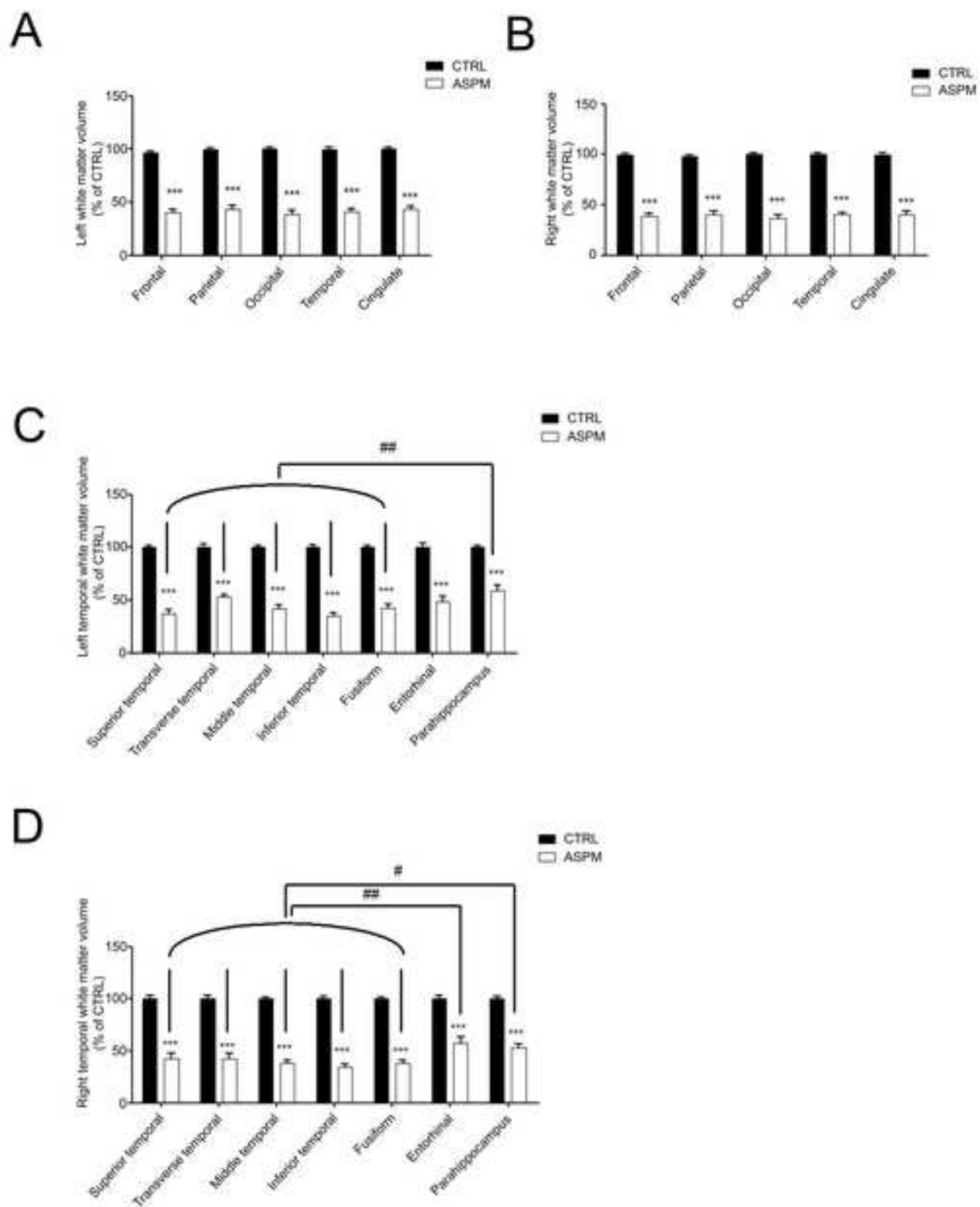


Figure 6

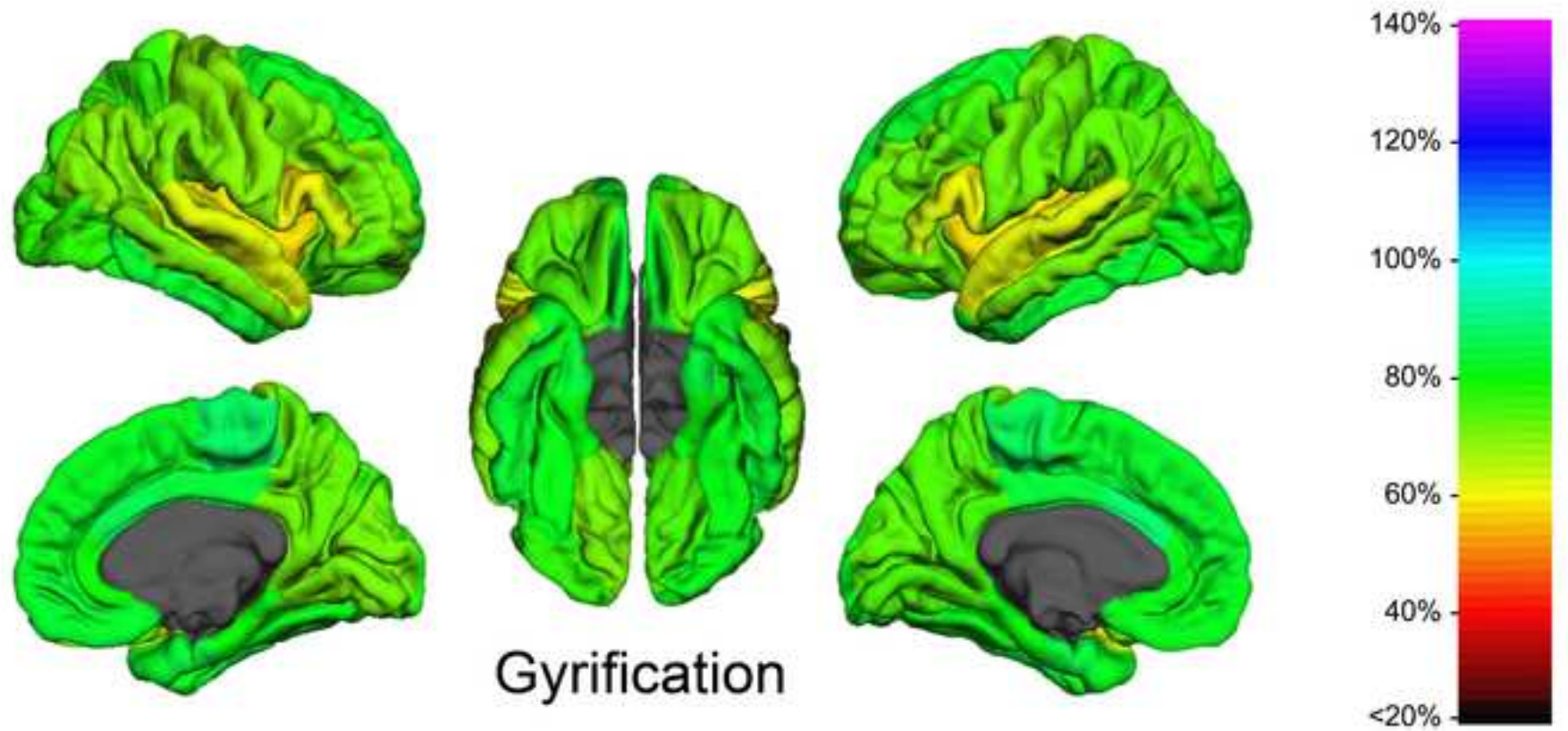


Figure 7

Figure 8
[Click here to download high resolution image](#)

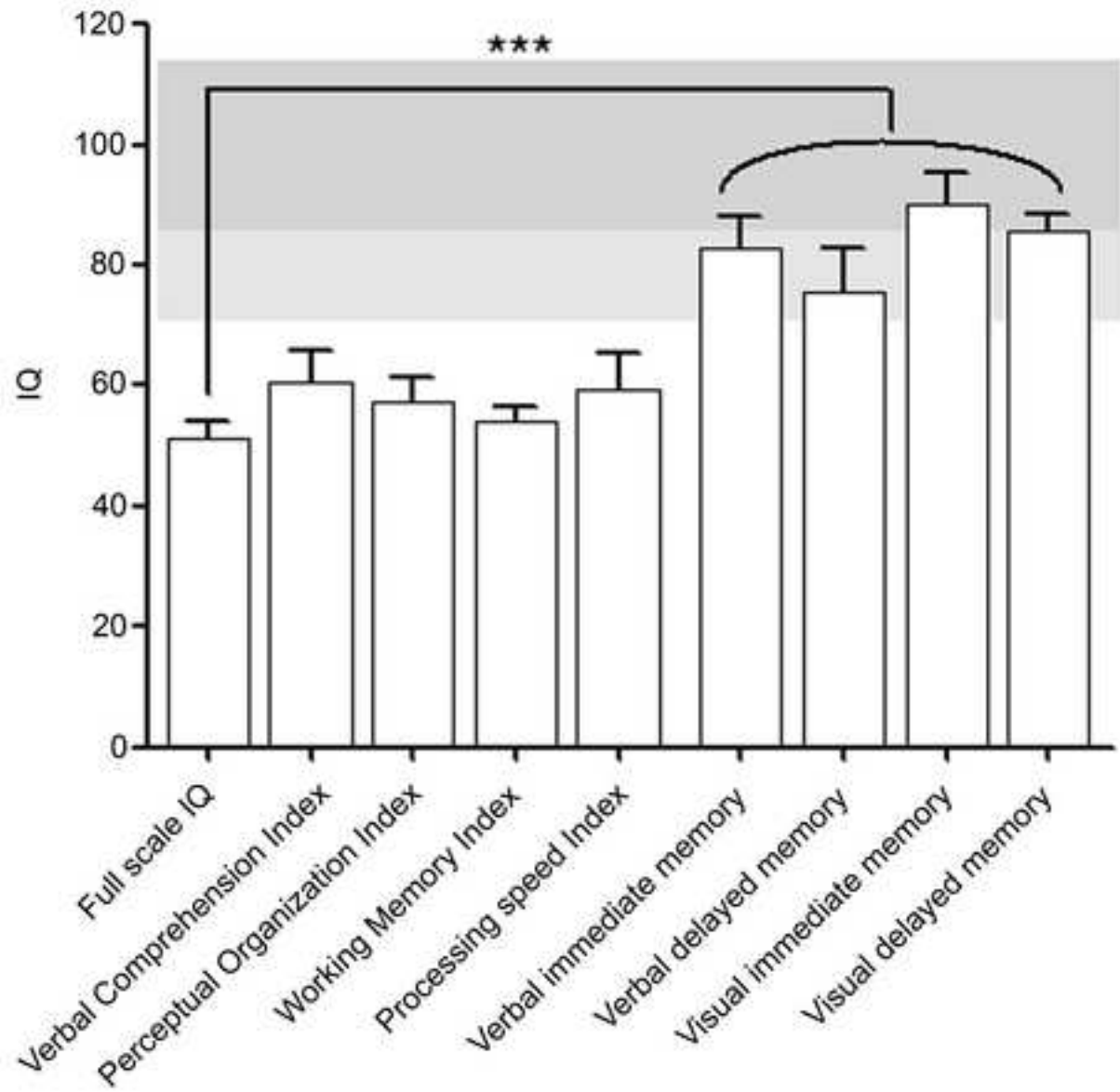


Figure 8