Whole-brain voxel-based morphometry study of children and adolescents with Down syndrome

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Summary

In order to investigate alterations in brain morphology and a possible temporal pattern of neuroanatomical abnormalities in the gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) of young patients with Down syndrome (DS), high-resolution magnetic resonance imaging (MRI) voxel-based morphometry (VBM) was performed on 21 children and adolescents with this chromosomal aberration and 27 age-matched participants as controls.
In comparison with control subjects, children and adolescents with DS showed not only an overall smaller whole-brain volume, but also volume reductions of the GM in the cerebellum, frontal lobes, frontal region of the limbic lobe, parahippocampal gyri and hippocampi and of the WM in the cerebellum, frontal and parietal lobes, sub-lobar regions and brainstem. By contrast, volume preservation was observed in the GM of the parietal lobes, temporal lobe and sub-lobar regions and in the WM of the temporal lobe and temporal regions of the limbic lobe. A lower volume of CSF was also detected in the frontal lobes.
This study is the first to use the high-resolution MRI VBM method to describe a whole-brain pattern of abnormalities in young DS patients falling within such a narrow age range and it provides new information on the neuroanatomically specific regional changes that occur during development in these patients.

KEY WORDS: Down syndrome, magnetic resonance imaging, voxel-based morphometry

Introduction

Down syndrome (DS), or trisomy 21, is the most common genetic, non-heritable cause of developmental disability; it occurs in about one out of 800 live births worldwide (Nadel, 2003). Individuals with DS present delayed development of motor and mental functions, such as language and memory; this delay is usually associated with low muscle tone and poor postural control, balance and gait, and accompanied by multiple congenital malformations of the cardiovascular, endocrine, gastrointestinal and skeletal systems.
Postmortem studies on DS adults have reported several neuropathological brain abnormalities, including a reduced gross brain weight, a lower number and depth of cerebral sulci, and ventriculomegaly and hypoplasia of several structures, including the brainstem, cerebellum, and frontal and temporal lobes, which feature a narrow superior temporal gyrus. By contrast, subcortical structures are relatively well preserved (Zellweger, 1977; Kemper, 1991). Postmortem studies on children with DS, on the other hand, have shown delayed maturation of the central nervous system and cortical dysgenesis, which suggests that the above brain abnormalities could be related, at least in part, to prenatal arrest of neurogenesis and synaptogenesis (Wisniewski, 1990).
With a view to defining the neuroanatomical pattern underlying DS, a large number of *in vivo* magnetic resonance imaging (MRI) studies have been carried out (Schapiro et al., 1989; Pearlson et al., 1990; 1998; Weis, 1991; Jernigan et al.,1993; Kesslak et al.,1994; Raz et al., 1995; Aylward et al., 1997a, 1997b, 1999; Pinter et al., 2001a, 2001b; Kates, et al., 2002; White et al., 2003). However, these have generally been based on classic region-of-interest (ROI) volumetry, which, although highly sensitive, is only able to detect volumetric changes in predetermined brain regions manually or semi-automatically delimited on MR images. As this is a time-consuming and poorly reproducible process, whose findings generally relate to only a small number of brain regions (Hasboun et al., 1996; Pruessner et al., 2000; Chan et al., 2001), voxel-based morphometry (VBM) has more recently been employed to study the DS brain (White et al., 2003).
Voxel-based morphometry is a fully automated technique, involving voxel-by-voxel analysis of the whole brain in spatially standardized MR images. This technique has the potential to detect global structural abnormalities (Wright et al., 1995; Ashburner and Friston, 2000) and has been extensively cross-validated with

ROI and functional analyses (Richardson et al., 1997; May et al., 1999; Good et al., 2001; Tisserand et al., 2002). In fact, White et al. (2003) recently performed a high-resolution MRI VBM study on a sample of 19 non-demented DS adults, yielding findings consistent with previous neuroimaging and postmortem evidence (Shapiro et al., 1989; Pearlson et al., 1990; Wisniewski, 1990; Kemper, 1991; Weis, 1991; Kesslak et al., 1994; Raz et al., 1995; Haier et al., 1995; Nadel, 1999; Zellweger, 1997; Aylward et al., 1997a, 1997b, 1999; Pearlson et al., 1998; Pinter et al., 2001a, 2001b) and providing additional insight into the three-dimensional topography of the morphology throughout the DS brain. VBM was also used to look for a relationship between cerebral volumes and walking patterns in nine DS children, in order to investigate the origin of the motor problems seen in DS (Rigoldi et al., 2009). The results of this study showed a strong relationship between cerebellar vermis volume reduction and quality of gait.

Voxel-based morphometry and ROI-based methods could be combined to provide a useful analytical strategy: the results of an initial VBM analysis could be used to define the cerebral regions which would then be examined in depth using an ROI-based method (Testa et al., 2006).

Nonetheless, neither of these approaches has been used extensively to examine brain morphology in young people with DS (Jernigan et al., 1993; Pinter et al., 2001a, 2001b; Kates et al., 2002), and the studies that have been performed have relied mainly on ROI-based volumetry to investigate volumetric differences in only a small number of brain regions, thereby failing to provide a comprehensive assessment of the brain's structural neuroanatomical characteristics. Furthermore, comparison of the results of these studies is hampered by the very different age ranges of the patients examined, while the fact that they also included young adults makes it difficult to establish whether brain abnormalities observed in adults with DS are primarily due to early developmental differences or to neurodegenerative changes.

Nevertheless, all these studies reported similar results, namely a volumetric reduction of the DS brain as a whole and a decrease in cerebral volumes (Jernigan et al., 1993; Pinter et al., 2001a; 2001b; Kates et al., 2002), as well as a clear reduction in both total gray matter (GM) (Jernigan et al., 1993; Pinter et al., 2001b; Kates et al., 2002) and white matter (WM) volumes (Pinter et al., 2001a; Kates et al., 2002). In particular, reductions were found in i) the total cerebellar volume (Pinter et al., 2001b; Jernigan et al., 1993), with a reduction in both total GM and WM volumes (Pinter et al., 2001b), and ii) the volume of the occipital lobe (Pinter et al., 2001b), with normal GM and WM volumes (Kates et al., 2002).

However, dissimilar and/or contrasting results were reported for other specific brain regions. Indeed, while Pinter et al. (2001b) and Kates et al. (2002) found a normal total volume of the frontal lobes, Jernigan et al. (1993) showed reduced volume in the anterior cortex; however, these studies cannot easily be compared due to the considerable size of the cortical region analyzed and the inclusion of the anterior portions of the temporal lobes in the latter case. Other discrepancies can be found in results of volumetric studies of the parietal and temporal lobes. For example, Kates et al. (2002) found a reduction in the total volume of the parietal and temporal lobes, whereas Pinter et al. (2001b) showed an increase in their total volume.

A more recent study, using a VBM technique, identified several cerebral abnormalities in a homogeneous group of twelve adolescents with DS, as compared to normal healthy controls (Menghini, et al., 2011). The adolescents with DS showed a significant regional reduction in GM density in the left cerebellum (posterior), the right inferior temporal gyrus/medial temporal lobe (fusiform gyrus and hippocampus), and the left medial temporal lobe (fusiform gyrus). Conversely, they showed a significant increase in GM density in the left cerebellum (anterior), the right medial temporal lobe (fusiform gyrus), the left and right basal ganglia (putamen, caudate nucleus), the left and right insula, the left and right superior frontal gyri, the right superior and middle temporal gyri, and the left and right inferior frontal gyri (Menghini et al., 2011).

In order to furnish further information on the structural neuroanatomy of children and adolescents with DS and to perform a whole-brain assessment of the developmental features of the nervous system in this condition, we performed a high-resolution MRI study in 21 children and adolescents with DS, using the VBM technique and the experimental design previously reported by White et al. (2003). Our aim was to gather whole-brain volumetric data in DS patients throughout development, in a manner that would allow comparison with similar studies performed in adult subjects. In particular, we were looking for information on the temporal pattern at the onset of early cerebral volumetric changes, which might help to define the expression patterns of chromosome 21 and to elucidate how these may herald the degenerative events typically seen at a more advanced age.

Materials and methods

Participants

The study group consisted of 21 children and adolescents with DS (11 males and 10 females; mean age±SD =10.5±3.3 yrs; range: 7-16 yrs). These participants were recruited from a larger sample of individuals with DS referred to the Child Development Department of the San Raffaele Pisana Scientific Institute between September 2003 and June 2004. The diagnosis of DS was confirmed in all cases by karyotype examination that showed trisomy of chromosome 21.

All children and adolescents with DS included in this study were right-handed, and were screened for neurological impairments and any history of learning disability or developmental delay. Participants were excluded if they presented untreated medical conditions that might have affected cognition (e.g., hypothyroidism), or had a history of severe head trauma requiring medical attention. MR image acquisition was performed on these participants when a possible atlo-occipital joint malformation was suspected. All DS participants underwent neuropsychological evaluation with the Revised Wechsler Intelligence Scale for Children (WISC-R; Wechsler, 1974), recording their intelligence quotient (IQ) scores (mean verbal IQ±SD: 49.8±5.7; mean performance IQ±SD: 52.5±9.6; mean full scale IQ±SD: 47.1±8.3).

Two of the DS participants required sedation during the MR image acquisition.
As controls, a group of 27 age-matched (p>0.8) right-handed, developmentally normal children and adolescents (14 males and 13 s; mean age±SD: 10.7±2.9 yrs; range: 7-16 yrs), recruited through local young people's associations, were assessed. Control participants underwent neuropsychological evaluation (WISC-R) to establish their IQ. Exclusion criteria included IQ <70 and any known neurological disability or contraindications to MR image acquisition.
All participants and their parents were informed about the study protocol, and written consent was obtained. The study had institutional review board approval.

Magnetic resonance image acquisition

High-resolution contiguous axial T1-weighted MR image acquisition was performed at the Radiology Department of the San Raffaele Pisana Institute, using a 1.5 Tesla (clinical) Philips Intera scanner (Philips Medical Systems, N.A., Bothell, WA). All MR images were acquired using the same parameters (3D MR acquisition; repetition time TR=18.0 ms; echo time TE=9.2 ms; flip angle =30°; matrix 256x256; field-of-view=24 cm; voxel size 1.0x1.0x1.0 mm^3), saved in 2-D DICOM format, and finally converted into 3-D ANALYZE 7.5 format (Biomedical Resource, Mayo Foundation, Rochester, MN) by means of the MRIcro software tool (www.psychology.nottingham.ac.uk/staff/cr1/mricro.html).
Image quality and the absence of structural abnormalities and motion artifacts were verified by two examiners (P.O., F.C.), who were unaware of each other's findings. In cases of inter-observer disagreement, a final consensus was achieved after discussion. The quality of image segmentation and the accuracy of the spatial normalization step were separately controlled and verified for each participant and sequence.

Data analysis

Image processing and analysis were performed on a personal computer based on an Intel Pentium 4 processor 520 supporting HT technology (2.8 GHz; 1 MB L2 cache; 800 MHz FSB; 1 GB DDR; 64 MB VRAM; O.S. Windows Xp), using an SPM2 analysis software package (Department of Cognitive Neurology, University College London, UK; http://www.fil.ion.ac.uk) running under MATLAB 7.0 (R14; Mathworks Inc., Natick, MA, USA).

Optimized VBM analysis

All MR images were processed according to the optimized and modulated protocol for VBM (Ashburner and Friston, 2000; Good et al., 2001; White et al., 2003) to automatically compute local absolute differences in the volumes of GM, WM and CSF, respectively. Optimized modulated VBM was performed according to the following image data processing pipeline: i) creation of customized GM, WM, and CSF templates to take into account the young age of the participants and the particular neuroanatomy of the DS group; ii) spatial normalization and segmentation of the original images by means of the three customized templates, to improve the results of the GM, WM and CSF partitions, respectively [variability due to registration errors during the normalization procedure was assumed to be smaller for subcortical structures than for cortical areas (Grachev et al., 1999)]; iii) correction for volume changes, i.e. modulation of the improved partitions by means of the Jacobian determinants derived from the spatial normalization step in order to preserve the total local volume of respective tissues; iv) calculation of the total intracranial volume (TIV), used in the subsequent statistical analysis, to take into account global differences in head size, integrating the voxel values across the modulated GM, WM, and CSF partitions of each participant; and v) smoothing the modulated partitions with a 12-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel to increase the validity of the parametric statistical tests (Worsley, et al., 1995) and to compensate for the inaccurate nature of spatial normalization. The GM partition was further smoothed with a 6-mm isotropic kernel to evaluate an expected decrease in the volume of small GM regions (i.e. the hippocampus) in the DS group (Maguire et al., 2000; Kubicki et al., 2002; White et al., 2003). Additional details of the processing methods are published in Good et al. (2001).
The optimized modulated VBM sequence was applied using a specific MATLAB script (dbm.neuro.uni-jena.de/vbm). The creation of the customized GM, WM and CSF templates was performed in two steps. First, all MR images were normalized with a 12-parameter affine transformation to the standard SPM template. This template is described in the MNI stereotaxic space (Montreal Neurological Institute; Montreal Canada), which is very similar to the Talairach space (Talairach and Tournoux, 1988). Second, the normalized images of each participant were averaged and smoothed with an 8-mm FWM isotropic Gaussian kernel, and the resulting averaged images were used as the new, customized templates. These averaged templates were also employed in the second normalization step, in which the original images were normalized to the new templates using a nonlinear spatial transformation and corrected for gray non-uniformities. The new normalized images were segmented in GM, WM and CSF partitions, and the non-brain voxels were removed using a series of morphological operators (erosions and dilations) (Good et al., 2001). The remaining voxels were modulated through multiplication by the Jacobian determinants resulting from the spatial normalization step (Good et al., 2001). Finally, the segmented modulated WM and CSF images were smoothed with a 12-mm FWHM isotropic Gaussian kernel, while the GM images were separately smoothed with two 12-mm and 6-mm FWHM isotropic Gaussian kernels used, respectively, in the global and hippocampal region analyses.

Statistical analysis

The MRI data met the normality (Shapiro-Wilk test) and heteroscedasticity (Bartlett's χ^2 test) criteria necessary for parametric statistical analysis. First, a standard unpaired t-test was used to compare TIVs between the DS and control groups. Imaging data were then analyzed to determine regional brain volume differences between the two groups.
Regional differences in GM, WM and CSF volumes across the whole brain and between groups were as-

sessed using the theory of Gaussian fields (Worsley et al., 1995; Friston et al., 1995); the assessments were carried out using the general linear model as implemented in SPM2. Statistical inferences were made voxel-by-voxel using an analysis of covariance design (ANCOVA), wherein the TIV, age and sex of each participant were regarded as nuisance covariates (White et al., 2003). Non-sphericity correction was used. Voxels with an intensity of less than 0.1 were excluded from the statistical comparison.

The set of voxel values resulting from the single comparisons gave a statistical parametric map of the t statistic (SPM{*t*}). The SPM{*t*} maps, comprising the results of statistical tests on each voxel, were transformed into the unit normal distribution (*Z*) and then: i) thresholded at $p<0.001$, uncorrected for multiple comparisons, to generate the figure and tables, and ii) corrected for multiple comparisons at $p<0.05$, using the false discovery rate (FDR) approach, to allow discussion of meaningful results (White et al., 2003).

In the analysis of the GM partitions smoothed with the 6-mm FWHM isotropic Gaussian kernel, a small volume correction (SVC; 40x40x40 mm^3) procedure was employed for the hippocampal region, rather than applying it to the whole brain, with a threshold set at $p<0.05$, corrected. A similar analysis has previously been utilized in other studies (Maguire et al., 2000; Kubicki et al., 2002; White et al., 2003), the latter (White et al., 2003) focusing on DS participants. Furthermore, since regional effects from SPM2 were localized in MNI stereotaxic space, they were transformed into Talairach space (Talairach and Tournoux, 1988) and anatomically labeled by means of a specific MATLAB script (MNI Space Utility – MSU http://www.ihb.spb.ru/~pet_lab/MSU/MSUMain.html) which computes a non-linear transformation of MNI to Talairach coordinates and describes SPM2 clusters in terms of Talairach Daemon anatomical regional labels (Talairach Daemon; www.talairach.org/applet.html).

These labels, electronically derived from axial sectional images in the Talairach and Tournoux Atlas (1998), are organized into five hierarchical levels: hemisphere, lobe, gyrus, tissue type, and cell type. The lobe level consists of the four main lobes (frontal, temporal, parietal, and occipital), a limbic lobe as a single lobular equivalent deep within the brain, and a sub-lobar region. The limbic lobe includes portions of frontal, parietal and temporal brain regions. According to this labeling scheme, the cingulate is within the limbic lobe and the insula within the sub-lobar region.

Results

A significant decrease ($p<0.01$) in TIV was observed in the DS as compared to the control group (DS: mean=1458.1±139.3 cm^3; control: mean=1638.9±282.0 cm^3). The results of the ANCOVA statistical analysis, performed with TIV, age and gender as nuisance variables, were corrected for multiple comparisons using the FDR approach ($p<0.05$) at cluster level, with a minimum cluster extension set at 50 contiguous voxels; however, these data could be biased by a less homogeneous smoothness compared with other functional imaging techniques (Ashburner and Friston, 2000; Kaasinen et al., 2005). Percentage values are also provided in order to report the relative proportion of the cluster in the brain regions (Kaasinen et al., 2005).

Gray matter

Table I and figure 1 (over) show the regional changes in GM volume in the DS subjects after the effects of TIV, age, and gender had been factored out. The DS group was characterized by a significant and symmetrical GM volume reduction in the cerebellum, mainly localized in the posterior lobe. A global rightward reduction in cerebrum GM volume was detected in the frontal lobes and at the level of the cingulate gyri in the frontal region of the limbic lobes. At the level of the frontal lobe, a rightward reduction in GM volume was found in the medial/inferior frontal gyri, while an almost symmetrical GM volume reduction was observed at the level of the precentral gyri. Finally, a unilateral GM reduction in the right middle frontal lobe was observed.

After SVC analysis, the left and especially the right parahippocampal gyri and hippocampi showed a significant reduction in GM volume. By contrast, significant preservation (increase) in GM volume was observed in the left middle temporal lobe, the right sub-lobar region (lentiform nucleus) and, almost symmetrically, in the parietal lobes.

White matter

Table II and figure 1 (over) show the results of the statistical analysis, performed with TIV, age and sex as nuisance variables. The DS group was characterized by a significant unilateral WM volume reduction in the left posterior/anterior lobes of the cerebellum, left brainstem and frontal and parietal lobes, and sub-lobar region. In the frontal lobes, the distribution of WM reduction was characterized by a symmetrical pattern overall, although there was a rightward volume reduction in the precentral gyrus and in the sub-gyral region and a leftward volume reduction in the superior/medial frontal gyri.

Asymmetrical distributions of WM reduction were revealed at the level of the sub-lobar region and parietal lobe, leftward in the former and rightward in the latter. Preservation (increase) of WM volume was detected in the left temporal lobe, the right sub-lobar region and, almost symmetrically, in the parietal lobes.

Cerebrospinal fluid

The DS group showed lower levels of CSF surrounding the left superior and left/right middle frontal lobes. No regions with significantly higher levels of CSF were detected (Table III and Fig. 1, over).

Discussion

Postmortem and *in vivo* MRI studies on adults with DS have shown a common pattern of volume reduction in the whole brain, cerebellum, brainstem, frontal, parietal and temporal lobes, cingulate gyrus and hippocampal structures, contrasting with volume increases in the parahippocampal gyrus and a normal basal ganglia volume (Zellweger, 1977; Schapiro et al., 1989; Pearlson et al., 1990, 1998; Weis, 1991; Kemper, 1991; Kesslak et

Table I - Locations of regional decreases in Down syndrome gray matter volume.

p	k	Z	Talairach coordinates x	y	z	Hemispheres	Lobes (%k;%L)	Regions (%k;%R)	BA (%k;%BA)
0.000	16521	5.54	12	19	41	L cerebrum	frontal lobe (19.1;1.2)	medial frontal gyrus (12.1;6.3)	6 (8.5;6.1)
								paracentral lobule (5.7;16.9)	31 (2.3;6.1)
									6 (8.5;6.1)
							limbic lobe (8.0;2.1)	cingulate gyrus (7.1;4.6)	31 (2.3;6.1)
						R cerebrum	frontal lobe (43.7;2.8)	medial frontal gyrus (32.6;16.2)	6 (13.2;9.4)
									32 (4.7;14.6)
								cingulate gyrus (14.7;8.6)	32 (4.7;14.6)
								paracentral lobule (3.6;9.5)	6 (13.2;9.4)
							limbic lobe (13.5;3.3)	cingulate gyrus (14.7;8.6)	32 (4.7;14.6)
0.000	63449	5.24	-29	-66	-51	L cerebellum	anterior lobe (3.8;8.7)	pyramis (4.1;34.3)	
								nodule (1.2;75.2)	
								fastigium (0.5;80.4)	
							posterior lobe (20.4;20.6)	inferior semi-lunar lobule (4.6;43.0)	
								pyramis (4.1;34.3)	
								cerebellar tonsil (3.7;16.5)	
								uvula (3.1;39.0)	
								declive (2.6;9.2)	
								fastigium (0.5;80.4)	
								declive of vermis (0.5;86.0)	
								uvula of vermis (0.2;100)	
								pyramis of vermis (0.2;100)	
								tuber of vermis (0.1;100)	
						R cerebellum	anterior lobe (4.7;10.7)	pyramis (3.8;31.9)	
								nodule (1.2;67.5)	
								fastigium (0.4;73.2)	
							posterior lobe (21.7;21.9)	declive (4.4;15.7)	
								cerebellar tonsil (4.4;19.3)	
								inferior semi-lunar lobule (4.3;39.6)	
								pyramis (3.8;31.9)	
								uvula (3.3;41.4)	
								declive of vermis (0.5;92.0)	
								uvula of vermis (0.3;100)	
								pyramis of vermis (0.2;100)	
								tuber of vermis (0.1;100)	
0.000	5933	4.08	56	9	35	R cerebrum	frontal lobe (98.8;2.2)	middle frontal gyrus (29.4;3.0)	9 (19.1;9.9)
									6 (17.6;4.5)
								inferior frontal gyrus (26.4;4.2)	9 (19.1;9.9)
									6 (17.6;4.5)
								precentral gyrus (33.2;6.1)	6 (17.6;4.5)
0.007	3436	3.97	-42	-11	22	L cerebrum	frontal lobe (80.7;1.1)	precentral gyrus (67.5;7.1)	6 (17.2;2.6)
									4 (4.1;3.0)
								inferior frontal gyrus (8.6;0.8)	9 (4.7;1.4)
								postcentral gyrus (4.9;0.7)	4 (4.1;3.0)
0.001	689*	5.22	17	-16	-18	R cerebrum	limbic lobe (49.3;0.6)	parahippocampal gyrus (42.2;1.8)	
								hippocampus (36.1;5.4)	
0.024	205*	4.67	-13	-12	-20	L cerebrum	limbic lobe (14.6;0.2)	parahippocampal gyrus (49.3;2.1)	
								hippocampus (25.3;3.8)	
Locations of regional increases in Down syndrome gray matter volume									
0.033	2387	4.54	39	-32	56	R cerebrum	parietal lobe (90.4;2.1)	postcentral gyrus (78.8;7.8)	3 (19.2;11.2)
									40 (21.5;4.0)
									2 (13.5;12.4)
								inferior parietal lobule (9.9;1.0)	40 (21.5;4.0)
0.025	2569	4.19	-31	-60	30	L cerebrum	parietal lobe (94.3;2.4)	precuneus (30.6;2.7)	7 (14.8;2.8)
								superior parietal lobule (29.6;11.3)	7 (14.8;2.8)
								inferior parietal lobule (9.6;1.0)	7 (14.8;2.8)
								angular gyrus (4.5;3.7)	39 (4.4;1.9)
							temporal lobe (5.6;0.1)	middle temporal gyrus (3.8;0.2)	39 (4.4;1.9)
0.023	2629	4.08	24	16	6	R cerebrum	sub-lobar (63.1;2.2)	lentiform nucleus (21.1;6.0)	
								putamen (21.1;8.2)	
								claustrum (6.5;10.1)	

Significant decreases and increases in DS gray matter volume. Cluster extension, which represents the number of contiguous voxels exceeding the threshold of ≥50, is shown by k-value. All clusters meet the significance level set at p<0.05 corrected. Brain regions are referred to the Talairach coordinates x,y,z, by means of the MNI Space Utility script. The hippocampi values are set at a threshold of p<0.05 corrected for the search volume (SVC*). BA=Brodmann area. Percentages indicate relative proportions of the cluster in the lobe/region/BA and of the lobe/region/BA covered by the cluster.

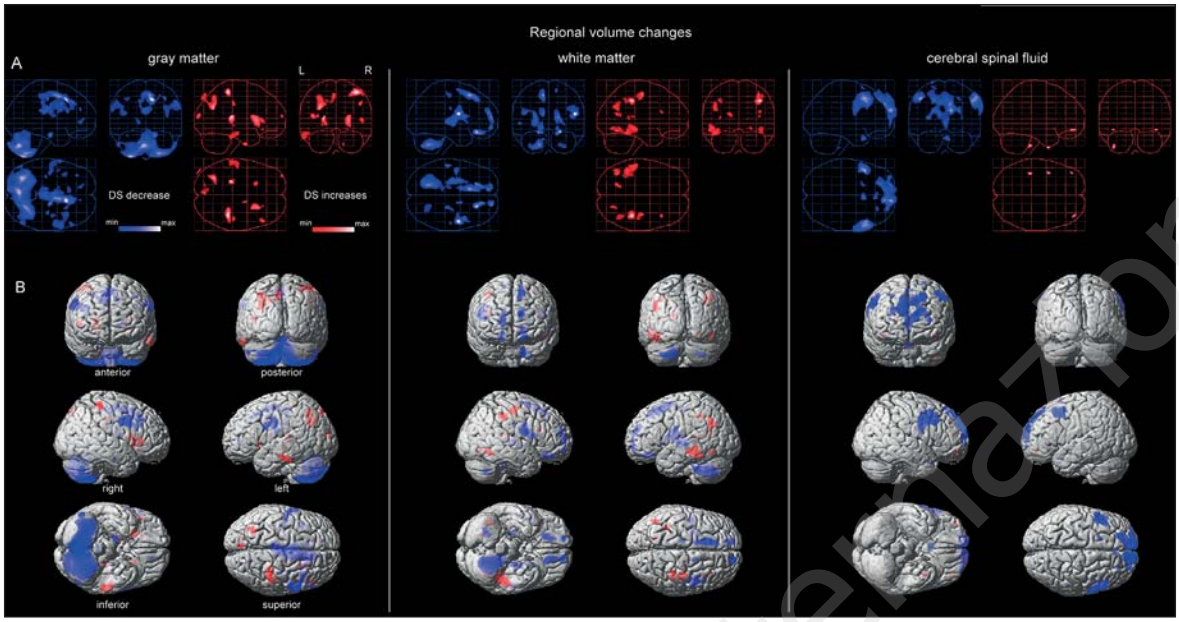

Figure 1 - Statistically significant decreases and increases in DS regional gray matter, white matter, and cerebral spinal fluid volumes shown as maximum intensity projections onto the standard "glass brain" (A) and onto the 3D surface renderings (six orthogonal views) of the standard T1 brain (B). The two-color bars in the figure show the Z scores corresponding to colors in the glass brain projections.

Table II - Locations of regional decreases in Down syndrome white matter volume.

p	k	Z	Talairach coordinates			Hemispheres	Lobes (%k;%L)	Regions (%k;%R)
			x	y	z			
0.000	4056	5.39	50	-2	17	R cerebrum	frontal lobe (86.3;1.3)	precentral gyrus (35.4;4.4)
								sub-gyral (30.0;1.2)
								inferior frontal gyrus (23.6;2.6)
							sub-lobar region (9.1;0.5)	insula (7.9;1.9)
							parietal lobe (4.6;0.2)	precentral gyrus (35.4;4.4)
								sub-gyral (30.0;1.2)
0.000	4270	5.00	-10	20	48	L cerebrum	frontal lobe (99.1;1.6)	superior frontal gyrus (60.6;5.6)
								medial frontal gyrus (32.9;4.4)
								sub-gyral (4.7;0.2)
0.000	9899	4.82	-17	-59	-31	L brainstem	pons (6.5;8.1)	
						L cerebellum	posterior lobe (71.1;11.3)	cerebellar tonsil (50.7;35.0)
								dentate (5.7;34.9)
								pyramis (11.3;14.6)
								inferior semi-lunar lobule (4.8;7.0)
								uvula (3.5;7.0)
							anterior lobe (22.0;7.9)	dentate (5.7;34.9)
0.017	1932	4.73	18	58	7	R cerebrum	frontal lobe (78.1;0.6)	superior frontal gyrus (63.9;2.6)
								medial frontal gyrus (36.1;2.1)
0.000	6738	4.37	-26	0	15	L cerebrum	sub-lobar region (68.2;6.1)	extra-nuclear (34.4;5.5)
							frontal lobe (12.1;0.3)	precentral gyrus (6.3;1.3)
								sub-gyral (6.1;0.4)
							parietal lobe (1.1;0.1)	sub-gyral (6.1;0.4)
Locations of regional increases in Down syndrome white matter volume								
0.001	3400	4.38	-52	-28	-6	L cerebrum	temporal lobe (95.4;2.8)	sub-gyral (35.9;1.2)
								middle temporal gyrus (35.1;3.0)
								fusiform gyrus (23.6;5.7)
								inferior temporal gyrus (2.1;0.7)
							limbic lobe (3.4;0.2)	parahippocampal gyrus (3.4;0.7)

Significant decreases and increases in DS white matter volume. k≥50; p<0.05 corrected. Refer to table I for a detailed explanation of the table layout.

Table III - Locations of regional decreases in Down syndrome cerebral spinal fluid volume.

p	k	Z	Talairach coordinates			Hemispheres	Lobes	Regions
			x	y	z			
0.000	2928	4.81	-44	19	42	L cerebrum	frontal lobe	middle frontal gyrus
0.000	13764	4.72	11	64	19	L cerebrum R cerebrum	frontal lobe frontal lobe	superior frontal gyrus superior frontal gyrus
0.000	9502	4.66	50	20	38	R cerebrum	frontal lobe	middle frontal gyrus

Significant decreases in DS cerebral spinal fluid volume. k≥50; p<0.05 corrected. Refer to table I for a detailed explanation of the table layout.

al., 1994; Raz et al., 1995; Aylward et al., 1997a, 1997b, 1999; Krasuski, et al., 2002; White et al., 2003; Teipel et al., 2004). In young people with DS, on the other hand, previous *in vivo* MRI studies have consistently revealed a considerable decrease in the volume of the whole brain, cerebellum and hippocampal structures, and an increase in the volume of the subcortical regions (Pinter et al., 2001a, 2001b; Jernigan et al., 1993; Kates et al., 2002). By contrast, more heterogeneous results have been obtained in relation to the frontal, parietal and temporal lobes, thereby hindering global assessment of structural neuroanatomical aberrations of the brain.

The discrepancies in the results of these studies in young people with DS could be due to the different methodological approaches adopted; in particular, the samples comprised individuals of a wide age range, and also included young adults; the discrepancies might also depend on the small number of cerebral areas analyzed, the small number of participants, and the relatively low resolution of the MRI data.

Specifically, Jernigan et al. (1993) performed a low-resolution ROI study in six DS individuals, including young adults, with a wide age range (10 to 20 years; mean age 15.5±3.4 years). Pinter et al. (2001a, 2001b) performed two (manual/semi-automatic) volumetric studies on a broader sample of 16 children, adolescents and young adults with DS who had a mean age of 11.3±5.2 years and an age range of 5 to 23 years. Finally, Kates et al. (2002), also using a semi-automatic volumetric technique, investigated DS brain abnormalities in children only (n=12; age range 2.9 to 8.0 years, mean age 5.9±1.6 years).

Our findings indicate that, compared with the findings in developmentally normal participants, the brains of children and adolescents with DS show: i) a smaller whole-brain volume, ii) a GM volume reduction in the cerebellum, frontal lobes and frontal region of the limbic lobes (cyngulate gyri), and parahippocampal gyri and hippocampi; iii) GM preservation in the parietal lobes, left temporal lobe and right sub-lobar region, including the lentiform nucleus; iv) a decrease in WM volume in the left cerebellum, frontal lobes, parietal lobes, sub-lobar regions and left brainstem; v) WM volume preservation in the left temporal lobe and temporal regions of the left limbic lobe, including the parahippocampal gyrus; and, vi) a smaller volume of CSF surrounding the frontal lobes.

The results obtained, i.e. overall smaller whole-brain volumes in DS participants with respect to controls, including a strong bilateral symmetrical GM volume reduction and a left WM volume reduction in the cerebellum, concur with previous studies in young people and adults with DS, which showed that these changes are present at an early developmental stage (Aylward et al., 1997a; Jernigan et al., 1993; Posner and Dehaene, 1994; Pinter et al., 2001b; White et al., 2003; Ilg et al., 2007). The cerebellar hypoplasia reported in these subjects has been related to hypotonia, alteration in motor coordination, gait and speech disturbances (Ilg et al., 2007; Rigoldi et al., 2009). Recently, Menghini et al. (2011) found a significant regional decrease in GM density in the left cerebellum, the right inferior temporal gyrus/medial temporal lobe, and left medial temporal lobe. The authors found, in participants with DS, a positive correlation between regional GM density in the right and left cerebellum (posterior) and measures of linguistic abilities. With regard to long-term memory skills, a positive correlation was found between verbal memory measures (verbal long-term memory task) and GM density in the orbitofrontal cortex (Menghini et al., 2011).

At the level of the frontal lobes, our results showed a global volume decrease, which is in agreement with previous postmortem studies of DS individuals (Zellweger, 1977; Kemper, 1991). We also found an overall rightward GM volume reduction and an almost symmetrical pattern of WM volume decrease; Jernigan et al. (1993) found a GM volume decrease in the anterior cortex, including the frontal and anterior temporal lobe, while Pinter et al. (2001b) found smaller frontal lobe volumes before adjusting for overall brain volume. On the other hand, Kates et al. (2002) found normal total frontal lobe volumes in DS, with a normal total volume of both GM and WM in these lobes.

The frontal lobes have frequently been reported to be implicated in the cognitive deficits of DS, including executive dysfunction, inattention, and a tendency toward perseveration. This specific hypofrontality was clearly reported in all previous studies on adults with DS. Indeed, White et al. (2003), from whom we borrowed our methodological approach, reported a significant reduction in GM at the level of the left and right frontal lobes in DS adults. This last finding may suggest early damage to these neuroanatomical structures.

Furthermore, with regard to the cerebrum GM volume, we observed a bilateral rightward decrease in the frontal region of the limbic lobes at the level of the cingulate gyri; this resembles previous findings in adults with DS (Jernigan et al., 1993; Kesslak et al., 1994; Raz et al., 1995; White et al., 2003). It has been shown that the cingulate gyrus is involved in attention processes (Bench et al., 1993; Posner and Dehaene, 1994; Taylor, et al., 1994; Carter, 1999).

Moreover, we recorded WM preservation in the temporal region of the limbic lobe at the level of the left parahippocampal gyrus. This same result was reported by Jernigan et al. (1993) and in previous studies in adults with DS (Kesslak et al., 1994; Raz et al., 1995; Emerson, et al., 1995; White et al., 2003). The findings in adults with DS seem to show that the volume of the parahippocampal gyrus decreases with age. However, this decline seems to start from a developmentally spared or even increased parahippocampal volume (Teipel and Hampel, 2006).

After SVC analysis, a rightward decrease in GM volume was observed in the hippocampi. This same result was found in young people with DS by Pinter et al. (2001a) and Jernigan et al. (1993), and in previous studies in adults (Raz et al.,1995; Aylward et al., 1997a, 1999; Pearlson et al., 1998; Pinter et al., 2001b; White et al., 2003). As the hippocampus is one of the main structures responsible for learning, its loss of functionality is assumed to interfere with different neural systems, including the subcortical structures (Devan and White, 1999). It could therefore be implicated in the learning deficits that emerge in DS individuals only some months or even years after birth. The unambiguous results concerning the hippocampal volume decrease found in both children/adolescents and adults with DS suggest that the decline in GM hippocampal volumes begins in childhood and continues throughout adulthood as a direct expression of the trisomic genetic disorder.

In the parietal lobes, we found almost symmetrical GM volume preservation, mainly localized in the right postcentral gyrus and in the left precuneus and superior lobule. This is consistent with previous studies in young adults with DS (Jernigan et al., 1993; Pinter et al., 2001b; Kates et al., 2002). In fact, previous studies in adults with DS have shown that the decrease in parietal lobe volume goes hand in hand with aging (Teipel et al. 2004). Indeed, White et al. (2003) found no significant volumetric alterations in this structure, suggesting that damage to the parietal lobe occurs much later in life.

In line with Kates et al. (2002), who reported findings in children with DS, we found a relative decrease in WM volume in the parietal lobes, structures involved in language and visuospatial abilities (Silverstein et al., 1982; Wang and Bellugi, 1994; Jarrold et al., 1999). The deficits in language and visuospatial processing seen in DS may therefore be partly due to excessive amounts of cells, leading to inappropriate function of the parietal lobes (Pinter et al., 2001b).

At the level of the temporal region, we found GM volume preservation (left lobe). Conversely, White et al. (2003) found a loss of GM in the temporal lobe, a result mirroring the results of previous studies in adults (Kesslak et al., 1994; Raz et al., 1995), as well as postmortem evidence (Wisniewski, 1990; Posner and Dehaene, 1994), which seems to support the hypothesis of late damage to this structure.

As previously stated, the reported results regarding the WM of the temporal region show more disagreement. Indeed, while Kates et al. (2002) found a WM reduction in the temporal lobes, Pinter et al. (2001a) found an increase in WM volume in the left temporal lobe, which might reflect a selective abnormality in WM maturation.

These latter findings show that the complexity of the temporal lobe does not allow a straightforward temporal pattern of lifelong structural changes occurring in this cerebral region, fundamentally involved in mnemonic and language abilities, to be discerned. In fact, although the results we obtained regarding GM preservation in the right sub-lobar region are in agreement with the studies by Jernigan et al. (1993) and Pinter et al. (2001b), they do not agree with the results recorded in adults with DS. Furthermore, Aylward et al. (1997a) reported reduced putamen volumes, whereas Raz et al. (1995) and White et al. (2003) failed to find any such difference. Nonetheless, the information obtained thus far seems to suggest possible and complex damage to these structures arising not in childhood, but later on in life.

On the other hand, consistent with postmortem and volumetric studies in DS adults, our findings clearly show a WM volume decrease in the left brainstem at the level of the pons (Zellweger, 1977; Schapiro et al., 1989; Lai and Williams; 1989; Wisniewski, 1990; Pearlson et al., 1990; Kemper, 1991; Weis, 1991; Kesslak et al., 1994; Haier et al., 1995; Raz et al., 1995; Aylward et al., 1997a, 1997b; Nadel, 1999; Lawlor et al., 2001; Ikeda and Arai, 2002). This morphological alteration is supported by neurophysiological data, i.e. auditory-evoked brainstem potentials in DS (Ferri et al., 1995). The authors found a significant reduction in V-wave latency, suggesting a correlation with different degrees of mental retardation.

Finally, we found a lower volume of CSF surrounding the frontal lobes, whereas we found none of the significant dilations of the lateral ventricles present in adults with DS (Pearlson et al., 1998; White et al., 2003). However, the decrease in the CSF surrounding the frontal lobes may be related to the overall reduction in head size of children and adolescents with DS, compared to euploid controls (Farkas et al., 1991). Adults with DS show a similar, although not statistically significant, CSF volume decrease (White et al., 2003), which may be ascribed to age-related changes and craniofacial anomalies.

In conclusion, this research provides the first whole-brain description of abnormalities in children and adolescents with DS based on data gathered using a high resolution MRI VBM method. Our data supply additional information on the temporal patterns of neuroanatomically specific regional changes in DS, and support the reliability of VBM in the investigation of brain abnormalities. Our results also show that gross regional pattern changes in the cerebellar, frontal, limbic and hippocampal regions are fairly stable throughout childhood, adolescence and adulthood. However, temporal, parietal and sub-lobar regions seem to be more sensitive to aging. In addition, the results of this VBM study also provide valuable information on the cerebral structures that require further analysis by the traditional ROI-based volumetry method.

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