Voxel-based morphometry in adolescents with autism spectrum disorder

Anita Bryńska¹, Tomasz Wolak², Patrycja Naumczyk², Tomasz Srebnicki¹, Tomasz Wolańczyk¹

¹ Department of Child Psychiatry, Medical University of Warsaw
² Institute of Physiology and Pathology of Hearing

Summary

Aim. The aim of this article is to evaluate changes in the grey matter volume using the VBM method in a group of adolescents with ASD who met the criteria for Asperger syndrome.

Material and methods. Voxel-based morphometry (VBM) was performed in 37 male adolescents, aged 12 to 19 (M = 14.3 ± 2.0), with autism spectrum disorder who met the DSM–IV–TR criteria for Asperger syndrome and 15 neurotypical adolescents matched by age. Significance was set at \( p < 0.05 \) with FWE (family-wise error) correction and \( p < 0.007 \) without FWE correction.

Results. The decrease in the volume of grey matter was observed in ASD group including the pre- and postcentral gyrus, the superior and middle frontal gyrus, the inferior and superior parietal lobule, the precuneus, the anterior and posterior cingulate cortex, the fusiform gyrus, the parahippocampal gyrus, the lingual gyrus, the middle occipital region, the cuneus and the angular gyrus, the regions of calcarine sulcus, and the cerebellum. The majority of changes were localized bilaterally.

Conclusions. The decrease in the volume of grey matter observed in ASD group, which can be functionally related to the characteristics of deficits observed in autism spectrum disorder, highlights the role of abnormal organization of numerous CNS structures in the genesis of symptoms observed in cognitive and behavioral domains.

Key words: autism spectrum disorder, voxel-based morphometry, CNS structure

Introduction

The etiopathogenesis of autism spectrum disorders (ASD), which are characterized by social communication deficits, limited and repetitive patterns of behavior, and a chronic course [1], still remains to be thoroughly explained. Abnormalities in the volume of grey and white matter and CNS structure abnormalities have been reported
by neuroimaging and histopathological studies across many years [2, 3]. Morphometric and volumetric studies based on voxel-based morphometry (VBM) use automatized techniques which provide information about abnormalities in the grey and white matter in the whole brain without making assumptions about localization [4] and without preselection of regions of interest (ROI).

Though many VBM-based studies targeted identification of abnormal CNS structures in the ASD population, a common view on characteristic locations still remains to be determined. The results of studies are inconsistent and sometimes even contradictory. The repeatability of results was related to the volume of grey matter in several regions of the brain which are involved in social, linguistic and executive functions, including prefrontal, temporoparietal and limbic regions, corpus striatum and the cerebellum [3, 5]. With regards to the direction of differences, the results are inconsistent – an increase in the volume of the grey matter or thickness of the cortex was observed in some studies [6-8], while others report a decrease in those parameters [9-11] or no differences in subjects with ASD [12, 13].

The Activation Likelihood Estimation (ALE) methods were used in meta-analyses in order to unscramble the results, yet without definite conclusions, as emphasized by the authors. According to Cauda et al. [2], the available data are indicative of significant increases of the grey matter in the ASD population in the cerebellum bilaterally, the middle temporal gyrus, the right anterior cingulate cortex, the head of caudate nucleus, the insular cortex, the fusiform gyrus, the precuneus, the posterior cingulate cortex and the left lingual gyrus. On the other hand, the decrease in the volume of grey matter is observed repetitively in the cerebellar tonsil bilaterally, the inferior parietal lobule, the right amygdaloid nucleus, the insular cortex, the middle temporal gyrus, the tail of caudate nucleus, the precuneus and the left precentral gyrus. Nickl-Jockschat et al. [3] marked several regions for which the results of VBM studies were convergent: 1. the extensive lateral occipital regions (the connection of the lateral occipital sulcus and the inferior temporal sulcus), 2. the region around the central sulcus (the left superior part), 3. the region which encompasses the middle regions of the right temporal lobe, 4. the basal ganglia (the head of the right caudate nucleus and the putamen of the left caudate nucleus), 5. the region of the insular cortex and the right parietal operculum. The detailed analyses of the changes of the grey matter volume in the ASD group showed a decrease in the left and right caudate nucleus putamen, the vermis (bilaterally), the left hippocampus/amygdaloid nucleus, the left parietal operculum, the left superior region around the central sulcus, the right middle temporal gyrus and the left precentral gyrus. The increase in the volume of grey matter involved the right and left temporo-occipital region, the right precuneus, the right and left hemisphere of the cerebellum, regions of the right lingual gyrus and the right inferior occipital gyrus. The question about the relationship between the thickness and volume of the cortex and the intensity of ASD symptoms remains to be answered. Ecker et al. [14] concluded that the changes observed in the frontal and temporal regions correlated significantly with the intensity of symptoms and age of the participants, while DeRamus and Kana [15] observed an age-related decrease in the grey matter.
volume in the parietal and the inferior temporal regions as well as an increase in the volume in the frontal and the anterior temporal regions.

In light of the results described above which confirm the complexity of autism spectrum disorder as a neurodevelopmental problem, some authors suggested to bridge the structural abnormalities of the CNS observed in ASD with the “social brain” hypothesis. Based on the results of many studies, an idea was suggested that the morphostructural changes affect mainly the regions of the amygdala nuclei (associated with the processing of emotions), superior temporal sulcus and fusiform gyrus (responsible for perception and recognition of faces), orbitofrontal cortex and superior frontal gyrus (responsible for the theory of mind (ToM) functions) [16, 17]. Regrettably, the very “social brain” hypothesis is only based upon theoretical assumptions about the regions of the brain whose involvement in ASD deficits is only hypothetical, and not on actual locations of the CNS.

The novel approach to the analysis of morphometric studies was proposed by Grecucci et al. [18]. Source-based morphometry (SBM) enabled them to discover the extensive network encompassing the regions of the brain where the functional and structural abnormalities reported in other studies were confirmed [3, 5, 14, 19, 20] (also known as autism-specific structural network, ASN). The ASN involved extensive regions of the temporal lobes, including the inferior, middle and superior temporal sulci, the fusiform gyrus, the parahippocampal gyrus, the superior and inferior frontal gyrus, the precentral gyrus and the cerebellum. A significant correlation was demonstrated between structural changes observed within the ASN and the intensity of behavioral symptoms in ASD.

Paucity of observations and inconclusiveness thereof warrant a need for the continuation of morphometric studies in ASD groups characterized by different levels of intensity of basic deficits.

The aim of the study is to evaluate changes in the grey matter volume using the VBM method in a group of adolescents with ASD who met the criteria for Asperger’s Syndrome.

**Material**

The evaluated group consisted of 63 male adolescents aged 12 to 19 years – 42 adolescents with ASD and 21 adolescents in the control group. Due to the presence of movement artefacts and low data quality, 52 subjects were included in the final analysis. The ASD sample consisted of 37 adolescents (ASD group) who met DSM-IV-TR [21] criteria for Asperger’s Syndrome – the diagnosis was confirmed by experienced clinicians (the assessment included an analysis of the Childhood Asperger’s Syndrome Test – CAST results [22], developmental history with particular emphasis on data pertaining to specific aspects of social, linguistic, cognitive and motor competences, qualitative interest analysis, psychiatric examination and behavioral observation). The control group included 15 neurotypically developing adolescents matched according to age (HC group). No significant differences were observed in terms of age between the ASD and HC group (14.3 years ± 2 vs. 14.4 years ± 2). The selection to study groups did not
take into account the criterion of homogeneity of IQ results. Intellectual functioning, as measured with the Wechsler Intelligence Scale for Children – Revised (WISC-R) or Wechsler Adult Intelligence Scale – Revised (WAIS-R) was normal, in both ASD (full scale IQ = 106 points ± 17, range 77-135 points; verbal IQ = 107 points ± 18, range 85-146 points; non-verbal IQ = 102 points ± 17, range 70-141 points) and control group (full scale IQ = 128 points ± 13, range 95-144 points; verbal IQ = 125 points ± 14, range 93-145 points; non-verbal IQ = 126 points ± 13, range 97-142 points).

Exclusion criteria for both groups involved: a diagnosed genetic disorder, CNS damage or seizures within the period of three years prior to the study, ferromagnetic metal implants, and – for the control group – a history of DSM-IV-TR Axis I psychiatric disorders. All participants signed an informed consent form.

For subjects younger than 18, a written consent was also signed by the caregivers. The study was approved by the Bioethics Committee at the Medical University of Warsaw.

Method

Functional testing was performed using a 3T Siemens Magnetom TRIO TIM magnetic resonance scanner (ver. VB17A) and 12-channel matrix head coil. For VBM analyses, a 3D T1-weighted MPR (multi-planar reconstruction) sequence was used with the following parameters: T1 MPR high-resolution sequence – 208 layers, sagittal images with isotropic resolution of 0.9 x 0.9 x 0.9 mm. All participants were scanned on the same scanner with the same sequence parameters. The data analyses were performed using SPM8 software (r4290) with MATLAB 7.9.0. Normalization as well as segmentation procedures were performed for all images using study-specific grey matter/white matter templates obtained as a result of DARTEL algorithm application. A border layer between grey and white matter was established with interpolation (defined by voxels, where one-third of the voxel was taken on the grey matter and two-thirds on the white matter). The variation of the grey matter thickness was predefined by the voxel intensity at a given location. The equalization of the homogeneity of the MR signal including the receiver coil geometry (noise correction) was performed when preparing images for analysis. After the normalization and segmentation procedures, a smooth filtration procedure was performed with a smoothed filter kernel the size of 6mm to modulate the signal. The final procedure was the two-sample T-test performed on the images and two contrasts were defined (ASD group vs. HC group and vice versa). Significance was set at $p < 0.05$ with FWE (family-wise error) correction and $p < 0.007$ without FWE correction.

Results

No significant differences were found between groups at $p < 0.05$ with FWE correction. On the other hand, the analysis at $p < 0.007$ without correction did not reveal regions with decreased grey matter volume in HC group compared to ASD group (ASD group vs. HC group), but it revealed 7 regions with decreased grey matter volume in
ASD group compared to HC group (HC group vs. ASD group). The differences were found in the following regions:

1. The left frontoparietal area, which primarily covered the fragments of the pre- and postcentral gyrus and the inferior parietal lobule and, to a lesser extent, the middle frontal gyrus and the superior parietal area. The following Brodmann’s areas are located in this region: BA 2, BA 3, BA 5, BA 4 and BA 40.

2. The right medial parieto-occipital region, which covered mainly the precuneus, and the superior parietal region (with superior parietal lobule), the fragments of the superior and middle occipital region, and, to a lesser extent, the areas of the cuneus and angular gyrus. The following Brodmann’s areas are located in this region: BA 7, BA 19 and BA 39.

3. The left occipital region which covered mainly the middle occipital gyrus. The following Brodmann’s areas are located in this region: BA 18 and BA 19.

4. The right middle-superior-medial frontal region, which covered mainly the cingulate gyrus, the middle frontal gyrus and the superior frontal gyrus. The following Brodmann’s areas are located in this region: BA 6, BA 24 and BA 32.

5. The post-central occipital region which covered mainly: the cuneus, the areas of the calcarine sulcus, the limbic lobes and the posterior cingulate cortex. The following Brodmann’s areas are located in this region: BA 17, BA 18, BA 23, BA 30 and BA 31.

6. The right parietal region, which covered mainly the fragments of the post-central gyrus and the inferior parietal lobule. The following Brodmann’s areas are located in this region: BA 2 and BA 40.

7. The occipitotemporal-cerebellar region, which covered extensive areas of both hemispheres of the cerebellum and the cerebellar vermis and included also: the fragments of the right and left fusiform gyrus, the right and left lingual gyrus, the right and left parahippocampal gyrus, the right calcarine sulcus, and the right and left inferior temporal region. The following Brodmann’s areas are located in this region: BA 18, BA 19, BA 20, BA 36 and BA 37.

The regions are listed in Table 1. The number of voxels assigned to individual regions (clusters) corresponded to the number of voxels where statistically significant differences were observed. The names of the structures listed in the table are in accordance with the international anatomical nomenclature and the names of areas are in line with Brodmann’s classification.

Table 1. Clusters of lower grey matter volume (comparison: HC group > ASD group)

<table>
<thead>
<tr>
<th>Anatomical location</th>
<th>Side</th>
<th>Cluster size/ no. of voxels</th>
<th>Talairach coordinates for center of cluster x y z</th>
<th>Peak of significance T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precentral gyrus</td>
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<tr>
<td>Postcentral gyrus</td>
<td></td>
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<tr>
<td>Inferior parietal lobule BA 2 BA 3, BA 4, BA 5, BA 40</td>
<td>L</td>
<td>1377</td>
<td>-26 -33 55</td>
<td>5.18</td>
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</tbody>
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### Discussion

The results of our study indicate a decrease in the volume of grey matter in extensive regions of the brain cortex in adolescents with ASD and are largely similar to the findings of other studies [2, 3, 5, 6, 8, 14, 15, 20]. Importantly, we failed to observe regions with increased grey matter volume in the ASD group – the finding can be explained by a relatively small intensity of core symptoms observed in subjects with Asperger’s Syndrome for whom the increase in the volume of grey matter is regarded as a compensatory mechanism. The majority of localizations identified in our study can be functionally related to the characteristics of deficits observed in ASD, which is reflected by the results of functional studies.

The postcentral gyrus (BA 2, BA 3), the anterior cingulate cortex (BA 24, BA 32) and the inferior parietal lobule (BA 39, BA 40) correspond functionally to the primary and secondary somatosensory cortex and they are a part of the region involved in the mirror neuron system. The mirror neuron system is engaged in the recognition and

<table>
<thead>
<tr>
<th>Region</th>
<th>Loci</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>T</th>
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</thead>
<tbody>
<tr>
<td>Precuneus</td>
<td>R 1117</td>
<td>20</td>
<td>-61</td>
<td>42</td>
<td>3.62</td>
</tr>
<tr>
<td>Superior parietal lobule</td>
<td>R 153</td>
<td>-24</td>
<td>-90</td>
<td>0</td>
<td>3.26</td>
</tr>
<tr>
<td>Superior occipital gyrus</td>
<td>R 326</td>
<td>24</td>
<td>2</td>
<td>49</td>
<td>3.17</td>
</tr>
<tr>
<td>Middle occipital gyrus</td>
<td>L 415</td>
<td>3</td>
<td>-78</td>
<td>10</td>
<td>3.13</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>R 169</td>
<td>51</td>
<td>-33</td>
<td>39</td>
<td>3.12</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>L 9055</td>
<td>-35</td>
<td>-67</td>
<td>-27</td>
<td>3.65</td>
</tr>
</tbody>
</table>
understanding of intentions, behaviors and emotions of others. The results of functional studies [23] point to lower activations in the regions occupied by mirror neurons in individuals with ASD.

Other locations – the inferior temporal gyrus (BA 20), the parahippocampal gyrus (BA36), the fusiform gyrus (BA 37) and the anterior and posterior cingulate cortex (BA 24 and BA 32, BA 23 and BA 32) – are associated with the characteristic deficits of emotional recognition and processing of faces in ASD. These regions are involved in high-level visual processing and object recognition – faces in particular. The decrease in activations of the fusiform gyrus in ASD is the most frequently observed in functional studies [24].

The reduced volume of the grey matter in the superior parietal lobule (BA 5), the inferior parietal lobule (BA 39, BA 40), occipital regions (BA 18, BA 19) and the anterior cingulate cortex (BA 32) can be associated with executive functions deficits such as planning, cognitive flexibility and organization of actions. The results of several functional fMRI studies on executive functions [25, 26] revealed decreased activations in frontoparietal regions in ASD. Smaller activations were obtained in tasks involving response inhibition and working memory maintenance [27] in the areas including: the parietal (BA 7 and 40) and occipital regions (BA 18), as well as the anterior cingulate cortex (BA 32) associated with cognitive planning processes.

The precentral gyrus (BA 4 – primary motor cortex), the superior and middle frontal gyrus (BA 6 – premotor cortex), BA 24 (premotor region) and extensive regions of the cerebellum are associated with motor functions. Our results may contribute to the understanding of abnormalities in motor development and gesticulation in ASD. Activations in the same regions of the brain in motor tasks functional paradigms, including the primary sensorimotor cortex (BA 2, 3 and 4), the cerebellum and the supplementary motor area, were found both in ASD and neurotypicals. For the healthy individuals the activations were stronger in the frontal regions of the cerebellum, whereas for those with ASD they were stronger in the supplementary motor area [28]. It is worth noting that the available data suggest that the cerebellum also supports cognitive functions, including language and executive functions [29].

Locations for which the reduced grey matter volume was observed, including the superior frontal gyrus, the anterior cingulate cortex, the precuneus, the posterior cingulate cortex, the inferior parietal lobule and the parahippocampal gyrus, form parts of the default mode network (DMN) [30]. This network consists of the middle frontal regions (the middle prefrontal cortex, including the superior frontal gyrus and the anterior cingulate gyrus), the middle parietal regions (the precuneus and the posterior cingulate gyrus), the lateral parietal regions (the right and left angular gyrus, the right and left inferior parietal lobule) and the middle temporal regions (the parahippocampal gyrus). The strongest activations of the network are observed in the resting state (no-task condition) and during ToM tasks performance. These regions are significantly less activated in ASD subjects as a consequence of underdeveloped introspective and auto-reflexive thinking.

Particular attention should be paid to the decreased grey matter volume in the precuneus observed in our study. This region is located in the medial area of the parietal
lobe (BA 7 and partly BA 31) and is associated with many functions due to its extensive connections with the higher-order association cortex and subcortical structures. Nerve fibers of the precuneus can be found in many cortical areas (including the posterior cingulate cortex, the parietal cortex – the frontoparietal operculum, the superior and the inferior parietal lobule, the prefrontal cortex, the supplementary motor area, the anterior cingulate cortex, the temporal-occipital-parietal cortex), the thalamus (mainly the dorsal part which contains the thalamic nuclei which are connected with the higher-order association cortex; additionally fibers from the nuclei are connected reversely with the precuneus) as well as subcortical areas (the claustrum, the caudate nuclei, the corpus striatum, the zona incerta, the pretecal area, the superior colliculus). Activity of the precuneus is not associated with direct processing of external stimuli (due to the lack of connections with the somatosensory cortex), yet it has an influence on neural networks engaged in the processing of integrated and associated information. Interestingly, the human precuneus is much more developed compared with other species (as shown by a high precuneus-brain mass ratio), and it uses up to 35\% more glucose than other regions of the DMN in no-task condition [31]. Taking into account the results of functional studies and the extensivity of connections, the precuneus seems to play an important role in the coordination of higher-order cognitive functions. Observations suggest that the precuneus is involved in visuo-spatial imagination, retrieval of information from episodic memory, the processing of self-knowledge, self-experience as well as the awareness processes [31-33]. The precuneus, along with the prefrontal cortex and posterior cingulate cortex, is also activated during empathy-based task performance [34].

Taking into account that the primary visual cortex (the cuneus and the calcarine sulcus; BA 17) was activated during visual task performance by ASD subjects in other studies (e.g., detection of details) [35], we find our observations difficult to explain.

Limitations of the study

Firstly, the relatively small sample precluded more detailed analyses in the study groups, selected on the basis of age as well as identification of relationships between the presence of observed structural changes and age. Secondly, the diagnosis of ASD was not verified with ADOS-2 [36] and ADI-R [37] questionnaires – at the time of recruitment for the study, the center did not have the above-mentioned tools, and did not employ people trained in this field. Thirdly, the study groups were selected only on the basis of age and we did not take into account the significant differences in intellectual functioning between the ASD and HC group.

Conclusions

The decrease in the volume of grey matter observed in the ASD group, which can be functionally related to the characteristics of deficits observed in autism spectrum disorder, highlights the role of abnormal organization of numerous CNS structures in the genesis of symptoms observed in the cognitive and behavioral domain.
References


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Address: Anita Bryńska
Medical University of Warsaw
Department of Child Psychiatry
02-091 Warszawa, Żwirki i Wigury Street 63a
e-mail: abrynska@wum.edu.pl