Research Report

Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders

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ARTICLE INFO

Article history:  
Accepted 24 December 2009  
Available online 4 January 2010

Keywords:  
Functional MRI  
Resting-state fMRI  
Regional homogeneity  
Autism spectrum disorder

ABSTRACT

Measures assessing resting-state brain activity with blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) can reveal cognitive disorders at an early stage. Analysis of regional homogeneity (ReHo) measures the local synchronization of spontaneous fMRI signals and has been successfully utilized in detecting alterations in subjects with attention-deficit hyperactivity disorder (ADHD), depression, schizophrenia, Parkinson’s disease and Alzheimer’s dementia. Resting-state brain activity was investigated in 28 adolescents with autism spectrum disorders (ASD) and 27 typically developing controls being imaged with BOLD fMRI and analyzed with the ReHo method. The hypothesis was that ReHo of resting-state brain activity would be different between ASD subjects and controls in brain areas previously shown to display functional alterations in stimulus or task based fMRI studies. Compared with the controls, the subjects with ASD had significantly decreased ReHo in right superior temporal sulcus region, right inferior and middle frontal gyri, bilateral cerebellar crus I, right insula and right postcentral gyrus. Significantly increased ReHo was discovered in right thalamus, left inferior frontal and anterior subcallosal gyrus and bilateral cerebellar lobule VIII. We conclude that subjects with ASD have right dominant ReHo alterations of resting-state brain activity, i.e., areas known to exhibit abnormal stimulus or task related functionality. Our results demonstrate that there is potential in utilizing the ReHo method in fMRI analyses of ASD.

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Abbreviations: KCC, Kendall’s coefficient of concordance; ReHo, regional homogeneity

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1. Introduction

Autism is a developmental disorder that is characterized by impaired social interactions, communication deficits and a restricted, repetitive pattern of interests and activities. Autism is classified as a part of Autism Spectrum Disorders (ASD), also known as Pervasive Developmental Disorders, which are a broad spectrum of developmental disorders affecting children and adults. The range of these disorders varies from severely impaired individuals with autism to individuals who exhibit abnormalities in social interaction and restricted repetitive behavior, but normal intelligence, Asperger syndrome (AS) (American Psychiatric Association, 2000; NIMH, 2004; WHO, 1993).

Task-based BOLD fMRI studies of autism (e.g. Allen and Courchesne, 2003; Dapretto et al., 2006; Just et al., 2007; Kana et al., 2007; Kennedy et al., 2006; Müller et al., 2003; meta-analysis: Di Martino et al., 2009; reviews: Courchesne and Pierce, 2005; Hughes, 2007; Mizuno et al., 2006; Redcay, 2008) have revealed abnormalities in the patterns of brain BOLD activations in subjects with ASD. In the future, it may be possible to differentiate between various cognitive disorders by utilizing BOLD fMRI combined with different stimulus patterns. Nonetheless, there will invariably be situations where stimuli cannot be successfully applied due to the patient’s condition, a lack of appropriate MRI-compatible stimulus devices, or a lack of required extended study and patient training time. In this sense, fMRI during patient’s rest is a more applicable option, one that can be implemented in connection with normal diagnostic anatomical MRI study without complex preparations or rehearsal. The measurements assessing resting-state brain activity with fMRI BOLD scans can reveal cognitive disorders at an early stage (Greicius et al., 2004; Liu et al., 2008). Resting-state data obtained through the scans can be analyzed with a variety of methods, e.g., functional connectivity (Cherkassky et al., 2006; Kennedy and Courchesne, 2008a), independent component (ICA) (Kiviniemi et al., 2003) and regional homogeneity (ReHo) (Zang et al., 2004) analyses. In the future, a combined spectrum of established methods of analysis for resting-state brain fMRI data might even result in definitive diagnostic conclusions.

There have been very few resting-state fMRI studies conducted on autism. Cherkassky et al. (2006), who used fixation block data between tasks for functional connectivity analyses, detected similarities in the volume and organization in resting-state networks in autism and control groups, but functional underconnectivity was observed in the anterior–posterior connections. Kennedy and Courchesne (2008a) used resting functional connectivity MRI to reveal that in autism, the task-negative default mode network (e.g., Buckner et al., 2008)

Fig. 1 – T-statistical difference map between ASD subjects and controls (P < 0.05, corrected). Warm and cold colors indicate ASD-related ReHo increases and decreases, respectively. The left side of the image corresponds to the right side of the brain in axial (a, b, d, e) and coronal (g, h, i) slices. The left side of the image corresponds to the anterior side of the brain in sagittal slices (c, f). Slice coordinates according to Talairach space are shown in the upper right corner of the slices, indicating Z-axis in axial, X-axis in sagittal and Y-axis in coronal slices.
involved in social and emotional processing was disrupted. In particular, specific abnormalities were found in the medial prefrontal cortex (MPFC) and left angular gyrus. However, the task-positive dorsal attention network involved in sustained attention and goal-directed cognition was found to be intact and similar to that of the control group. Later Kennedy and Courchesne (2008b) provided a more detailed view of the default mode network (DMN) abnormality in autism as their study results suggested that the dysfunction of the ventral MPFC/ventral anterior cingulate cortex (ACC) is task-independent and pervasive, but deficits in the dorsal MPFC and retrosplenial cortex/posterior cingulate cortex (PCC) are task-specific. While functional connectivity can reveal the synchronization of remote brain regions, ReHo measures the local synchronization of spontaneous fMRI signals by calculating similarity of dynamic fluctuations of voxels within a given cluster (Long et al., 2008; Zang et al., 2004; Zou et al., 2009). Zou et al. (2009) investigated the static and dynamic characteristics of cerebral blood flow (CBF) in the resting-state using an arterial spin labeling (ASL) perfusion imaging technique. Consistent with previous PET results, static CBF measured by ASL was significantly higher in the PCC, thalamus, insula/superior temporal gyrus (STG) and MPFC than the average CBF of the brain. These results suggested that higher ReHo in the DMN is significantly higher in the PCC, thalamus, insula/superior temporal gyrus (STG) and MPFC than the average CBF of the brain. These brain regions also had high temporal synchrony, as measured by ReHo. Their report suggests that higher ReHo in the DMN is not simply due to the high CBF baseline or SNR but rather by ReHo. Analysis of remote brain regions, ReHo measures the local synchrony, as measured by ReHo. Their report suggests that higher ReHo in the DMN is not simply due to the high CBF baseline or SNR but rather reflects the local synchrony (Zou et al., 2009). Long et al. (2008) used three methods to analyze resting-state fMRI data: ReHo, linear correlation and ICA. All three methods revealed the existence of the default mode (e.g., Buckner et al., 2008). Since the BOLD signal of fMRI reflects neural activity (Logothetis and Wandell, 2004), abnormal ReHo is probably related to changes in the temporal aspects of the spontaneous neural activity in the regional brain. It can be speculated that an abnormal ReHo may be a sign of disrupted local functionality. In fact, analysis of ReHo has been successfully utilized in detecting alterations in subjects with ADHD (Cao et al., 2006; Zhu et al., 2008), depression (Yuan et al., 2008; Yao et al., 2009), schizophrenia (Liu et al., 2006), Parkinson’s disease (Wu et al., 2009) and Alzheimer’s dementia (He et al., 2007).

The aim of this study was to explore the regional brain activity of the ASD subjects by using resting-state fMRI with the ReHo method and to provide a proof of concept about potential utility of ReHo in this context. We hypothesized that ReHo of resting-state brain activity would be different between ASD subjects and typically developing controls in brain areas known to display functional alterations in previous stimulus or task-based fMRI studies.

2. Results

In comparison with the typically developing controls, the subjects with ASD displayed significantly decreased ReHo in right superior temporal sulcus (STS) region (Figs. 1e, h), right inferior (IFG) and middle frontal gyri (MFG) (c, e), bilateral cerebellar crus I (a, b, c, i), right insula (c, e) and right postcentral gyrus (c, g). Significantly increased ReHo was discovered in right thalamus, mainly in its ventral posterolateral nucleus (VPN), but extending to lateral geniculum (c, g). All three methods revealed the existence of the default mode (e.g., Buckner et al., 2008). Since the BOLD signal of fMRI reflects neural activity (Logothetis and Wandell, 2004), abnormal ReHo is probably related to changes in the temporal aspects of the spontaneous neural activity in the regional brain. It can be speculated that an abnormal ReHo may be a sign of disrupted local functionality. In fact, analysis of ReHo has been successfully utilized in detecting alterations in subjects with ADHD (Cao et al., 2006; Zhu et al., 2008), depression (Yuan et al., 2008; Yao et al., 2009), schizophrenia (Liu et al., 2006), Parkinson’s disease (Wu et al., 2009) and Alzheimer’s dementia (He et al., 2007).

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### Table 1 - Brain areas of ReHo difference between two groups (ASD – controls).

<table>
<thead>
<tr>
<th>Areas</th>
<th>Hemisphere</th>
<th>Cluster size (volume, mm³)</th>
<th>Peak t-value</th>
<th>Peak t-value Talairach coordinates (x, y, z)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gray matter</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Decreased ReHo in ASD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MTG, STG, STS</td>
<td>R</td>
<td>1632</td>
<td>−4.81</td>
<td>57 –37 6</td>
</tr>
<tr>
<td>Cerebellum crus 1 (Decive)</td>
<td>R</td>
<td>1624</td>
<td>−4.34</td>
<td>29 –85 −22</td>
</tr>
<tr>
<td>MFG, IFG</td>
<td>R</td>
<td>984</td>
<td>−4.09</td>
<td>37 –43 6</td>
</tr>
<tr>
<td>Cerebellum crus 1 (Tuber)</td>
<td>L</td>
<td>672</td>
<td>−3.81</td>
<td>−45 −73 −26</td>
</tr>
<tr>
<td>Insula</td>
<td>R</td>
<td>472</td>
<td>−3.59</td>
<td>41 1 0</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>R</td>
<td>432</td>
<td>−3.89</td>
<td>45 −19 50</td>
</tr>
<tr>
<td><strong>Increased ReHo in ASD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus (ventral posterolateral nucleus)</td>
<td>R</td>
<td>552</td>
<td>4.58</td>
<td>19 −19 6</td>
</tr>
<tr>
<td>Cerebellum lobule VIII (ISLL)</td>
<td>L</td>
<td>512</td>
<td>3.91</td>
<td>−17 −61 −42</td>
</tr>
<tr>
<td>Cerebellum lobule VIII (ISLL)</td>
<td>R</td>
<td>464</td>
<td>4.95</td>
<td>9 −65 −36</td>
</tr>
<tr>
<td>IFG, SG</td>
<td>L</td>
<td>400</td>
<td>3.45</td>
<td>−25 31 −10</td>
</tr>
<tr>
<td><strong>White and gray matter</strong></td>
<td></td>
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<tr>
<td>Increased ReHo in ASD</td>
<td></td>
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</tr>
<tr>
<td>CC, OR, MOG, IOG, FG</td>
<td>L</td>
<td>1752</td>
<td>4.74</td>
<td>−31 −67 8</td>
</tr>
<tr>
<td>OR, MTG</td>
<td>R</td>
<td>1278</td>
<td>5.05</td>
<td>37 −61 10</td>
</tr>
<tr>
<td>CC</td>
<td>R</td>
<td>1240</td>
<td>4.27</td>
<td>25 −49 20</td>
</tr>
<tr>
<td>OR, SOG</td>
<td>L</td>
<td>984</td>
<td>4.67</td>
<td>−15 −83 16</td>
</tr>
<tr>
<td>CC, MCC</td>
<td>L</td>
<td>728</td>
<td>3.63</td>
<td>−13 13 26</td>
</tr>
</tbody>
</table>

CC=Corpus Callosum, FG=Fusiform Gyrus, IFG=Inferior Frontal Gyrus, IOG=Inferior Occipital Gyrus, ISLL=Inferior Semi-Lunar Lobule, MCC=Middle Cingulate Cortex, MFG=Middle Frontal Gyrus, MOG=Middle Occipital Gyrus, MTG=Middle Temporal Gyrus, OR=Optic Radiation, SG=Subcallosal Gyrus, SOG=Superior Occipital Gyrus, STG=Superior Temporal Gyrus, STS=Superior Temporal Sulcus.
and bilateral cerebellar lobule VIII (Figs. 1a, f, i). Significantly increased ReHo in clusters including both gray and white matter were also detected. Left cerebral hemisphere clusters included inferior, middle and superior occipital gyri and fusiform gyrus with optic radiation, but also middle cingulate cortex with corpus callosum (Figs. 1d, e, f, i). In the right hemisphere, increased ReHo in optic radiation with middle temporal gyrus was found (Figs. 1e, i). Simply by adding up the gray matter ReHo clusters (Table 1), right side changes total 6160 voxels and left side 1584. When white and gray matter areas are included, the voxels for right side added up to 9128 and left to 5048.

3. Discussion

In this study, ReHo analysis was able to depict several brain areas, where local BOLD signal coherence was different in the subjects with ASD compared to the typically developing controls. Decreased or increased ReHo in ASD suggests that neural function in certain regions is less or more synchronized compared to typically developing controls. According to current theories and research on autism, no area of the brain can be unambiguously addressed as being more important than others. Nonetheless it is interesting to note, that the majority of the ReHo changes are right sided. Gray matter ReHo changes are indicative of decreased coherence or synchronization in local regional resting-state activity in the right frontal and temporal brain regions in the subjects with ASD. There can be many possible explanations for this uneven activity. For example, in the language domain earlier study results have indicated reduced hemispheric differentiation and atypical functional lateralization in the ASD (Kleinhans et al., 2008).

Compared to recent activation likelihood estimation meta-analysis of task-based functional neuroimaging studies of ASD (Di Martino et al., 2009), we were unable to detect significant ReHo abnormalities in ACC. However, estimated from the Talairach coordinates, common areas of functional brain abnormalities to both the task-based meta-analysis and this resting-state study include right STG, insula, thalamus, and bilateral cerebellar lobule VIII (Figs. 1a, f, i). Significantly increased ReHo in clusters including both gray and white matter were also detected. Left cerebral hemisphere clusters included inferior, middle and superior occipital gyri and fusiform gyrus with optic radiation, but also middle cingulate cortex with corpus callosum (Figs. 1d, e, f, i). In the right hemisphere, increased ReHo in optic radiation with middle temporal gyrus was found (Figs. 1e, i). Simply by adding up the gray matter ReHo clusters (Table 1), right side changes total 6160 voxels and left side 1584. When white and gray matter areas are included, the voxels for right side added up to 9128 and left to 5048.

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As noted, in this study many local ReHo changes display spatial resemblance to earlier autism studies and might be interlinked. For example, several current psychological theories of autism have implicated atypical sensory processing as a core feature of autism (Iarocci and McDonald, 2006). The decreased coherence in the resting-state BOLD activity detected in this study in both STS and insular regions may indicate that subjects with ASD experience problems integrating a multisensory input. As Redcay (2008) reviewed, a primary social cognitive function that recruits the STS is biological motion perception or the identification of a social form from a moving entity and attributing intentions or goals to that entity. In the language domain, the STS is involved in identifying linguistic units from a stream of auditory information and extracting the communicative significance out of these units. Furthermore, a large body of neuroanatomical, neurofunctional, behavioral and anec-

dotal evidence indicates that the temporal lobes and particularly the STS/STG are impaired in autism. Redcay (2008) argued that STS performs a common function for social and speech perception by parsing rapidly changing auditory and visual input and extracting meaning from this input and concluded that impairments in its function may underlie many of the social and language abnormalities seen in autism.

As mentioned earlier, the right insula in subjects with ASD showed decreased ReHo. The insular cortex is a complex structure containing areas that subserve visceral sensory, motor, vestibular, and somatosensory functions (Bamiou et al., 2003; Simmons et al., 2008). The right insula is a multimodally responsive area that responds to visual, tactile and auditory stimuli and has shown a significantly greater response to a novel versus a familiar stimulus. It is involved in the detection of temporal mismatch between simple stationary auditory and visual stimuli (Bamiou et al., 2003). The right insular activation has also been linked to perceived intolerance of uncertainty, in other words it is related to the degree to which uncertainty is processed as being aversive (Simmons et al., 2008). Results also indicate that the right claustrum/insula region is differentially activated in association with multisensory integration of conceptually related common objects (Naghavi et al., 2007). In addition, insula is a part of inhibition network with anterior and middle cingulate gyri, and subjects with ASD have shown lower levels of synchronization between inhibition network and the right middle and inferior frontal and right inferior parietal regions (Kana et al., 2007).

Cognitively demanding tasks that evoke activation in the brain’s central executive network (CEN) have been consistently shown to evoke decreased activation (deactivation) in the DMN. Sridharan et al. (2008) showed that particularly the right fronto-insular cortex (rIFIC) plays a critical and causal role in switching between the CEN and the DMN. They propose that a transient signal from rIFIC engages the brain’s attentional, working memory and higher-order control processes while disengaging other systems that are not task-relevant (Sridharan et al., 2008). Local decreased ReHo in the right insula and IFG may indicate disruptions to these processes, which participate in balancing one’s attention between external events (CEN) and internal reflections (DMN). Overall, considering its diverse connectivity and versatile functions, insula is one possible key area in the neurobiology of ASD.

The insula also has connections with several ventral thalamic nuclei, such as the VPN, which are the part of cerebello-thalamo-cortical pathways. The VPN itself relays somatosensory information to the postcentral area (Mizuno et al., 2006). We found increased ReHo in VPN of the subjects with ASD but decreased ReHo in postcentral gyrus.

In the ASD group, we found bilateral increased ReHo in cerebellar crus I but decreased in cerebellar lobule VIII. These cerebellar regions were among activated areas, when Dimitrova et al. (2003) evoked nociceptive leg withdrawal reflexes in healthy adults. Discrete right finger movements have been noted to activate the right lobule HVIII, whereas continuous movements activate bilateral crus I (Habas and Cabanis, 2008). A report from 2003 involving patients performing finger
movements demonstrated abnormal variability and scatter of functional maps, thus suggesting early-onset disturbances in the development of cerebello-thalamo-cortical pathways in autism (Müller et al., 2003). In addition certain nonmotor functional deficits, as seen in an attention task study by Allen and Courchesne (2003), may be related to cerebellar abnormalities in ASD. Indeed, evidence has been accumulating that the primate cerebellum is also involved in higher cognitive function. According to Ramnani (2006) the relatively well-developed models of how the cerebellum processes information from the motor cortex might be extended to explain how it could also process information from the prefrontal cortex. Prefrontal and motor cortices connect via pontine nuclei respectively to cerebellar lobules crus I and VIII (among others) and loop back via thalamus (Ramnani 2006).

In comparison with controls, the subjects with ASD had significantly decreased ReHo in right IFG and MFG. The frontal lobe hosts many essential higher functions, and it has been proposed that in autism, connectivity within this region is both excessive and disorganized, while connectivity between frontal cortex and other systems is poorly synchronized (Courchesne and Pierce, 2005). We did not find any significant ReHo changes in the pars opercularis part of IFG, which is considered to be an important part of the mirror neuron system, but there were decreases located more anteriorly in the parietal and triangularis. Furthermore, also insula is related to the system acting as an interface between the frontal component of the mirror neuron system and the limbic system (Dapretto et al., 2006).

We detected increased ReHo in the ASD group in an area including part of the left fusiform gyrus. One of the major clinical findings in the autism spectrum disorders is the lack of apparent recognition of faces. As reviewed by Hughes (2007), the fMRI studies of face recognition have shown weaker fusiform activity in the subjects with ASD, but also dysfunction of the modulating areas has been suspected.

One interesting aspect related to right temporal lobe ReHo differences has emerged from EEG research. In a retrospective review, Chez et al. (2006) showed that 60.7% of 889 ASD patients displayed abnormal EEG epileptiform activity in sleep with no difference based on clinical regression. The most frequent sites of epileptiform abnormalities were localized over the right temporal region, a location where we found decreased ReHo. It can be speculated that in some individuals this decreased local coherence in right temporal lobe spontaneous activity (as compared with the typically developing population) could possibly lower the threshold of an epileptiform activity or be a reflection of this kind of activity. Hughes (2007) stated that because discharges are usually regarded as focal and the seizures require that there is some spread from this focus, one problem in children with autism may be the deficiency of cortico-cortical fibers to account for this presumed lack of spread. Studies complementing fMRI with EEG could shed light on these speculations.

Kennedy and Courchesne (2008a) found specific abnormalities in the MPFC and left angular gyrus by using resting functional connectivity. Though the ReHo method is more sensitive to the activity within the task-negative network than the task-positive network (Long et al., 2008), we were unable to detect any significant differences in these areas between the ASD group and the controls. As noted earlier in this discussion, we were also unable to detect significant ReHo abnormalities in ACC. This suggests normal local coherence in these areas, which could also be interpreted as normal local connectivity. However, ReHo method is not able to identify such disruption of connectivity to more distant networks as found by Kennedy and Courchesne (2008a). This fact limits the comparison of our study to some current fMRI-evidence, suggesting that autism is an extensively distributed system-wide brain disorder, especially manifesting as underconnectivity (Cherkassky et al., 2006; Hughes, 2007; Kana et al., 2007). Further studies are needed to obtain a unified perspective including both local and distant networks.

While there is doubt about the validity of BOLD signal in white matter (Logothetis and Wandell, 2004), some previous studies have shown activation of corpus callosum (Mosier and Bereznaya, 2001; Tettamanti et al., 2002). Mezer et al (2009) were able to segment the white as well as the gray matter based on its temporal signal features. Similarly ReHo reflects the temporal homogeneity of the regional BOLD signal rather than its intensity. Deshpande et al. (2009) compared ReHo and another measure of local coherence called integrated local correlation (ILC) by examining their ability to discriminate between gray and white matter in the resting-state data. ILC was found to be more sensitive, and it would be interesting to compare these two techniques on this data.

As with other analyzing techniques, further studies are needed to clarify the relationship between ReHo method and structural MRI (e.g., T1 and diffusion tensor imaging) data. As far as we are aware, there is only one study (He et al., 2007) combining ReHo and structural MRI. It was shown that Alzheimer’s dementia (AD) patients had Mini Mental State Exam score, which correlated with ReHo decreases in the PCC/precuneus (PCu) when compared with the normal controls. When the regional PCC/PCu atrophy was controlled, these results still remained significant but with a decrease in the statistical power. Stanfield et al. (2007) performed a meta-analysis of existing structural magnetic resonance imaging studies of autism. Their conclusion was that autism is associated with generalized enlargements of the cerebral hemispheres, the cerebellum and the caudate nucleus, and with reductions in the size of the corpus callosum and possibly the midbrain and vermal lobules VI–VII and VIII–X. These alterations may be related to the cardinal features of the disorder.

### 4. Conclusion

It is concluded that subjects with ASD have right dominant ReHo alterations of resting-state brain activity in areas known to present altered functionality in stimulus or task-based fMRI studies. The decreased coherence or synchronization in local regional resting-state BOLD activity detected in subjects with ASD in both right STS and insular regions may reflect atypical sensory processing and problems in integrating multisensory input. Similarly, decreased ReHo in the right insula and IFG may be related to attention abnormalities, as rFIC has recently been shown to play a critical role in switching over between internally and externally oriented brain networks. The significant differences in right thalamus and bilateral cerebellum
may be related to abnormal or relatively overactive cerebellothalamo-cortical pathways or the role of cerebellum also in nonmotor functional deficits. Our results demonstrate that there is potential in utilizing the ReHo method in fMRI analyses of ASD.

5. Experimental procedures

Thirty participants with ASD were gathered from a community-based study conducted in 2000–2003 (see Mattila et al., 2007) and from a clinic-based study conducted in 2003 (see Kuusikko et al., 2009; Mattila et al., 2009). Thirty age and gender-matched controls were recruited from mainstream schools in Oulu (see Kuusikko et al., 2008; Jansson-Verkasalo et al., 2005). All participants and their parents gave written informed consent, and the study was approved by the Ethical Committee of the University Hospital of Oulu.

5.1. ASD diagnostics in the community-based study

All 8-year-old children born in 1992 and living within the Oulu University Hospital area in autumn 2000 were chosen for the target population (n = 5484). Of them, 4422 (81%) were rated by the Autism Spectrum Screening Questionnaire (ASSQ; Ehlers et al., 1999). Of 125 screened children, 110 (88%) accepted the invitation to participate in diagnostic examinations, including the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1995), the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), module 3, and the Wechsler Intelligence Scale for Children-Third Revision (WISC-III; Wechsler 1991). The ADI-R interviews of parent(s) and the ADOS observations of children were administered by a pediatrician (M.-L.M.), who was trained in the use of the ADI-R and ADOS for research purposes. The WISC-III assessments were performed by a clinical psychologist and a research psychologist (K.J.). Early development was checked from the patient records of Oulu University Hospital in the case of subjects for whom verification was considered to be essential after the ADI-R interviews. School-day observations were carried out in the case of 24 subjects for whom verification was regarded as necessary, 22 of them completely including observation of the child, structured (ASSQ) and non-structured teacher interview. After these examinations, 82 children remained for re-evaluations by reviewing all available data (ASSQs, ADI-R, ADOS tapes, school day observations, patient records); the ICD-10 research criteria were used to construct clinical consensus diagnosis of AS and autism (and “non-AS/non-autism”), based on all gathered information, between a pediatrician (M.-L.M.) having long-time clinical experience in ASDs and developmental disorders and a child psychiatrist having long-time clinical experience in ASDs and other psychiatric disorders.

5.2. ASD diagnostics in the clinic-based study

The target population was all registered outpatients with AS or AS traits or AS suspected (FSIQ > 80; WISC-III) at Oulu University Hospital by Spring 2003. The hospital records were evaluated during Autumn 2002, originally for the genetic part of the clinic-based study in high-functioning children with PDDs (Weiss et al., 2009). All outpatients had been diagnosed in the child psychiatric clinic or in the child neurological department supervised by a child psychiatrist or a child neurologist. Clinical diagnoses had then been assigned based on the ICD-10 criteria regarding only current behavior. Thus, a differential diagnosis between AS and autism had not been made. “AS traits” refers to the features of AS or autism. Participants had no other severe developmental disorders. The outpatients, who had already participated in the community-based study (Mattila et al., 2007), were removed from the diagnostic phase of the clinic-based study. Diagnostic examinations included the ADI-R and the ADOS, module 3, administered and videotaped by a research psychologist (K.J.), who was trained in the use of the ADI-R and ADOS for research purposes. Early development was verified from the patient records of Oulu University Hospital. After these investigations the clinical diagnoses of AS or autism were re-assigned by the psychologist (K.J.), consulting the pediatrician (M.-L.M.), in the case of subjects for whom a second opinion was considered to be essential. The ICD-10 research criteria were used in detail to derive a clinical diagnosis of AS or autism based on information obtained with the ADI-R and ADOS, and from patient records, now also taking into account development in the first 3 years of life.

In 2005, the Schedule for Affective Disorders and Schizophrenia for School Aged Children (K-SADS-PL; Kaufman et al., 1997), following the DSM-IV-TR criteria (American Psychiatric Association, 2000), was used for diagnostic interviews to detect other forms of psychopathology in the participants with ASD (T.H. and H.H.). When selecting participants for the present neuroimaging study, which took place in 2007, we excluded those with severe Tourette’s disorder or severe hyperactivity, due to the method and the rather long duration of the scanning procedure.

Finally, 30 subjects with ASD (FSIQ > 75) participated in the neuroimaging study using the fMRI. According to the ICD-10 research criteria (WHO, 1993), 19 met the diagnostic criteria for AS and 11 fulfilled the diagnostic criteria for autism.

The control adolescents, recruited in 2006–2007 from mainstream schools of Oulu, Finland, were first screened with the parent-rated ASSQ to exclude any individuals with ASD symptoms (Kuusikko et al., 2008; Jansson-Verkasalo et al., 2005). Secondly, controls and their parents were interviewed with the K-SADS-PL (S.K-G., T.H. and H.H.) to exclude those with current psychiatric disorders. IQ was not measured but considered to be in the normal range, because only children with normal intelligence attend mainstream education in Finland.

5.3. fMRI procedure

The imaging was performed using a 1.5-T General Electric Signa HDX 8-channel parallel imaging-coil ASSET system with 2.0 acceleration factor. Hearing was protected using ear plugs and motion was minimized using soft pads fitted over the ears. T1-weighted 3D FSPGR BRAVO-sequence images (FOV 24 cm × 24 cm, 256 × 256 matrix, whole brain coverage, flip angle 20°, BW 15.63 kHz, TR 12.4 ms, TE 5.2 ms) were taken in order to obtain anatomical images for co-registration of the fMRI data to the standard space coordinates. The functional
scanning was performed using EPI GRE sequence (FOV 25.6 cm × 25.6 cm, 64 × 64 matrix, flip angle 90°, TR 1800 ms, TE 40 ms) with whole brain coverage using 28 oblique axial 4 mm slices with 0.4 mm space between slices.

The resting-state scanning started the protocol and lasted for 7 min 36 s producing 253 brain volumes. First four volumes were excluded from analysis due to T1 equilibrium effects. During the scanning procedure the subjects were asked to lie still, stay relaxed and awake and stare at the cross on the screen. One subject with autism refused to undergo imaging in the MRI scanner room and thus a total of 29 subjects with ASD and 30 control adolescents were imaged. FMRI dataset of one subject with autism was lost. One control had teeth braces, which could not be removed and due to the resulting imaging artifacts, scanning was aborted. Two controls were discarded due to suprathreshold ASSQ score > 7. Consequently 28 ASD (19 AS, 9 autism; age 14.58 ± 1.62 years, 20 males, 8 females, three left-handed) and 27 control (age 14.49 ± 1.51 years, 18 males, 9 females, two left-handed) subjects were analyzed. Handedness was determined by self-report.

5.4. Pre-processing of imaging data

Head motion in fMRI data was corrected using multi-resolution rigid body co-registration of volumes as implemented in FSL 3.3 MCFLIRT software (Jenkinson et al., 2002). Default settings used were: middle volume as reference, three-stage search (8 mm rough + 4 mm initialized with 8 mm results + 4 mm fine grain initialized with previous 4 mm step results) with final trilinear interpolation of voxel

Fig. 2 – Results of regional homogeneity (ReHo) shown as Kendall’s coefficient of concordance (KCC) map across all subjects with autism spectrum disorder (ASD) in the resting state (one-sample t-test; P < 0.01, corrected for minimum volume of 456 mm³). The left side of the image corresponds to the right side of the brain. Z-coordinates according to Talairach space are shown in the upper left corner of the slices.
values and normalized spatial correlation as the optimization cost function. Brain extraction was carried out for motion corrected BOLD volumes with optimization of deforming smooth surface model as implemented in FSL 3.3 BET software (Smith, 2002) using threshold parameters $f = 0.5$ and $g = 0$ and for 3D FSPGR volumes using parameters $f = 0.25$ and $g = 0$. The BOLD data was temporally band-pass filtered ($0.01 < f < 0.08$ Hz) with AFNI (Cox, 1996) to reduce physiological noise (Lowe et al., 1998; Greicius et al., 2003) and to remove any linear trend.

Multi-resolution affine co-registration as implemented in FSL 4.0 FLIRT software (Jenkinson et al., 2002) was used to co-register mean non-smoothed fMRI volumes to 3D FSPGR volumes of corresponding subjects and 3D FSPGR volumes to Montreal Neurological Institute (MNI) standard structural space template (MNI152_T1_2 mm_brain template included in FSL). Trilinear interpolation was used, the correlation ratio was calculated as the optimization cost function and with regard to rotation parameters, the search was conducted in the full $[-\pi, \pi]$ range. Resulting transformations and trilinear interpolation were used to spatially standardize filtered BOLD volumes to 2 mm MNI standard space.

5.5. ReHo analyses
A within-subject analysis was first performed using the ReHo approach. Kendall’s coefficient of concordance (KCC) (Kendall and Gibbons, 1990) was calculated to represent the similarity

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Fig. 3 – Results of regional homogeneity (ReHo) shown as Kendall’s coefficient of concordance (KCC) map across all typically developing controls in the resting state (one-sample t-test; $P < 0.01$, corrected for minimum volume of 456 mm$^3$). The left side of the image corresponds to the right side of the brain. Z-coordinates according to Talairach space are shown in the upper left corner of the slices.
of the time series of each 27 nearest neighboring voxels and the KCC was given to the center voxel;

\[ W = \frac{\sum (R_i)^2 - n(\bar{R})^2}{K^2(n^3 - n)} \]

where \( W \) (ranged from 0 to 1) is the KCC among given voxels; \( R_i \) is the sum rank of the ith time point; where \( \bar{R}=\langle (n+1)K/2 \rangle \) is the mean of the \( R_i \)'s; \( K \) is the number of time series within a measured cluster (here \( K=27 \), one given voxel plus the number of its neighbors); \( n \) is the number of ranks (here \( n = 249 \)) (Zang et al., 2004; Yuan et al., 2008).

The individual ReHo map was obtained by calculating the KCC in a voxel-by-voxel way using the Resting-State fMRI Data Analysis Toolkit (REST, by Song Xiao-Wei et al., http://www.restfmri.net). Each individual ReHo map was divided by this subject's global mean KCC value within the brain mask. This is a similar standardization procedure as used in PET studies (Raichle et al., 2001). Standardized maps were smoothed with a Gaussian kernel (FWHM = 4 mm) for better anatomical comparability of ReHo values on group level.

In order to obtain a visual impression of ReHo, one-sample t-tests (against 1, i.e., the global mean KCC value after standardization procedure) were performed within each group (Figs. 2 and 3). The differences between ASD subjects and normal controls were examined with two-sample t-tests (AFNI 3dttest) between the two groups to create a group difference map. For the purpose of reporting results and multiple comparison correction, these statistical maps were then transformed to Talairach coordinates (Talairach and Tournoux, 1988) by using AFNI hand-landmarking. AFNI Monte Carlo simulation program AlphaSim (cluster connection radius 3 mm, individual voxel threshold probability 0.01, 1000 iterations) was used to obtain a corrected significance level of \( P < 0.05 \) for a minimum volume of 50 voxels (400 mm\(^3\)) in the group difference map (Fig. 1) (Cox, 1996). This enabled the identification of significant changes in the ReHo of ASD patients compared to normal controls. AFNI 3dclust was used to obtain clusters' sizes, locations and their respective t-values. Anatomical atlases included in AFNI were used to help locate anatomical areas corresponding to clusters (Eickhoff et al., 2007). Statistical maps were superimposed on the high resolution anatomical template available in MRicro (http://www.sph.sc.edu/comd/rorden/mricalo.html).

Acknowledgments

We thank the participants and their parents who graciously gave of their time to participate in this study. We also thank docent Eira Jansson-Verkasalo for her contribution to recruiting control subjects to this study.

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