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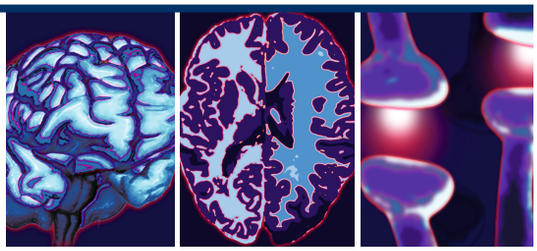

Recent advances in neuroimaging in autism

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Practice points

- Autism spectrum disorders (ASDs) are a family of neurodevelopmental syndromes with a population prevalence of approximately 0.5–1.5%.
- The neurobiology of ASDs are unclear. Early neuroimaging studies reported abnormal findings in a minority of cases, but varied widely.
- Newer imaging techniques have revealed more about the disorder. Functional MRI has shown abnormalities in brain activation during task performance and at rest.
- Magnetic resonance spectroscopy has become an increasingly popular tool for investigating neurochemistry *in vivo*.
- Multivariate pattern classification analysis has shown promise in detecting subtle anatomical and functional differences between ASD and control brains.
- Studies have begun to examine neurobiological differences between the various clinical and genetic subtypes of ASDs.
- In the near future, advances in positron emission tomography should help investigate the molecular basis of ASDs, including the GABA and glutamate systems.

SUMMARY Autism is a neurodevelopmental syndrome characterized by impairments in three domains of behavior: social interaction, social communication and repetitive behaviors. Early neuroimaging investigations of autism examined gross brain structure and metabolism. These studies revealed some abnormalities, for example, in overall brain size, but these were typically of small magnitude, or only seen in a minority of cases. However, the introduction of new neuroimaging and statistical techniques has led to many recent advances in this field. Functional MRI and magnetic resonance spectroscopy have shed light on the function and neurochemistry of the brain in autism. Advanced approaches to data analysis have recently shown great promise in picking up complex structural and functional

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differences. Conceptual shifts in the understanding of autism have begun to be reflected in the neuroimaging literature, and upcoming advances in positron emission tomography should help elucidate the molecular basis of autism. This update is intended to provide a concise and accessible overview of the latest work in this area and to outline promising avenues for the future.

Autism spectrum disorders

Autism spectrum disorders (ASDs) are a family of neurodevelopmental syndromes with a population prevalence of approximately 0.5–1.5% [1,2]. ASDs are more common in males than in females, with a gender ratio of approximately 4:1 [1,2]. ASD is a modern term which includes the older concept of 'autism' or 'childhood autism', but also covers cases which, while sharing many of the symptoms of autism, do not meet the strict criteria for this disorder [3].

ASDs are diagnosed on the basis of a triad of impairments in three domains of behavior: social interaction and relationships, language and communication, and repetitive, restricted interests and behaviors [4]. These symptoms are present from early life (before 36 months of age). ASDs are clinically heterogeneous, with the severity of the various symptoms, and the impairment they cause, varying widely [3].

Some people with an ASD also have an intellectual disability (low IQ), but at least 25% of people with 'classic' autism, and a higher proportion of those with milder ASDs, show normal or superior intellectual function [5]. ASD featuring both a normal range IQ and a history of normal language acquisition is called Asperger's syndrome [6]. However, it is debated whether Asperger's is truly a syndrome distinct from autism [7] and proposed new DSM-5 diagnostic criteria would remove it as a separate diagnosis as well as introducing other changes to ASD diagnosis [7].

ASDs are known to be highly heritable, with estimates of heritability ranging from approximately 0.5 to 0.9 [8]. However, environmental risk factors, including perinatal and obstetric complications, also play a role [9]. First-degree relatives of affected individuals are at increased risk of an ASD, and also of milder social and communication impairments dubbed the 'broader autism phenotype' [10].

Early neuroimaging investigations of ASDs

Early studies of the brain in autism focused on brain structure (using computer tomography or MRI scanning) and regional blood perfusion or metabolism using positron emission tomography (PET).

These studies provided a mixed picture. Clear-cut abnormalities were observed in some individual cases of ASD, however, there was great variability in these reports, with a range of different focal and generalized pathologies being reported in various studies.

The majority of individuals with an ASD showed no qualitative abnormalities detectable with these methods, although some quantitative differences were found, for example, decreased volume of the corpus callosum and cerebellum, and increased volume of the caudate nucleus in ASD, on average, compared with controls, although with small or medium effect sizes (for a review and meta-analysis, see [11]).

For example, early case reports identified cerebellar hypoplasia and ventricular enlargement in ASD cases [12], but other investigators reported no MRI abnormalities in most cases, and normal cerebellar development [13]. In Asperger's syndrome, individual case reports of left temporal [14], left frontal and bilateral opercular cortical abnormalities [15] were reported, with little consistency. The authors of one large 1990 MRI study cautioned [16]: "MR findings did not present a single pattern … Autism is a heterogeneous disease entity containing different clinical subgroups, which do not manifest similar radiologic pictures."

Early PET studies likewise produced mixed findings. An early report revealed widespread increases in glucose metabolism across the brain in some autistic adults [17], but another study found no differences [18]. Others reported reduced metabolism in particular brain structures [19].

These studies imply that, while some cases of ASD are associated with qualitative neurological abnormalities, there is no clear localization to particular regions of the brain, with various cortical, subcortical and cerebellar regions all having been implicated in different cases. In addition, although quantitative abnormalities in the mean volume of various areas have been found, these are of modest magnitude, with substantial overlap between ASD and control groups. Hence, in order to understand the neurobiology of autism, an approach considering the whole brain, rather than individual regions of interest, is required.

Conventional neuroimaging is, however, still used clinically in terms of detecting any gross neuropathological abnormalities that may present as ASD symptoms. Neuroimaging thus plays a role akin to genetic screening for single gene mutations known to cause syndromes characterized by ASD symptoms, such as Fragile X (*FMR1*) [20] and Rett's (*MECP2*) [21]. While most cases of ASD are not associated with such mutations, they are sufficiently common and clinically important that they are routinely screened for as part of many diagnostic services.

New neuroimaging techniques

While technical limitations meant that early studies were limited to investigation of the structure and metabolism of individual brain regions, recent advances have allowed a richer approach to the neurobiology of ASDs **(Table 1)**.

■ MR spectroscopy

MRI scanners rely on the phenomenon of nuclear magnetic resonance (NMR). NMR is widely used in chemistry to investigate the chemical composition of samples and to determine the molecular structure of novel compounds.

Conventional MRI uses NMR to produce images of the body, however, MRI scanners can also be used to perform *in vivo* NMR. This is known as MR spectroscopy (MRS). The most popular approach is proton MRS, ^{1}H-MRS. Protons resonate at particular frequencies depending upon the molecules in which they form a part. The amplitude of the signal at a particular frequency reflects the concentration of the corresponding molecule, in this example, the concentration within a particular area of the brain (voxel).

In vivo MRS therefore provides a powerful way to quantify a range of neural metabolites, including the neurotransmitter glutamate and its metabolic product glutamine. Others include *N*-acetylaspartate (NAA), a marker of neuronal density and mitochondrial function, and choline-containing compounds, a measure of membrane synthesis and turnover (for a review, see [22]).

MRS offers the opportunity to test hypotheses about the neurochemistry of ASDs. For example, it has been suggested, on the basis of genetics and animal model evidence, that ASDs are associated with an imbalance in glutamate neurotransmission [23,24]. However, before the advent of MRS, it was impossible to measure glutamate levels in the living human brain.

There have now been five published ^{1}H-MRS studies reporting on glutamate in ASDs, three of which were in children. One reported a widespread decrease in cortical combined glutamate/glutamine signal (Glx) as well as NAA [25], one reported a nonsignificant reduction in Glx in the left thalamic voxel [26], and the other found no difference in glutamate levels between groups [27].

In adults, Page *et al.* reported that adults with ASD had a significantly higher concentration of Glx in the right amygdala–hippocampal complex [28], while another study found reduced Glx in the right anterior cingulate cortex in adults with ASDs [29].

^{1}H-MRS has also been used to test the hypothesis [30] that some cases of ASDs are

Table 1. Some recently developed neuroimaging techniques, with examples of the insights they have provided into autism spectrum disorders†.

Modality	Technique	Example findings
Chemical	Proton MR spectroscopy ([^{1}H]MRS)	No evidence of elevated lactate in the brain of 45 children with ASDs, arguing against hypothesis of a mitochondrial defect in autism [31]
Functional	Functional MRI	Reduced activity in so-called 'mirror neuron' systems, possibly underlying social cognition impairments [36]
	Functional connectivity MRI (fcMRI)	Increased randomness (decreased coherence) of resting-state spontaneous neuronal activity [51]
Structural	Diffusion tensor imaging (DTI)	Reduced integrity of frontostriatal white matter tracts [47]
	Longitudinal structural morphometry	Children with ASDs have enlargement of cerebral cortex during early life (before 2 years of age), but normal growth rate after this [57]
	Multivariate pattern classification	Able to classify individuals with ASD at a sensitivity and specificity of up to 90 and 80%, respectively, using a support vector machine on cortical surface morphology [40]

†This is not intended to be a comprehensive list of results, but an overview of methods.
ASD: Autism spectrum disorder.

associated with mitochondrial disease: Corrigan *et al.* reported no evidence of elevated lactate in the brain of 45 children with ASD, and argued that this was inconsistent with a mitochondrial deficit [31].

■ Functional MRI

Functional MRI (fMRI) is a technique that allows the detection of neural activity by measuring local activation-related changes in the level of oxygen in blood – the Blood Oxygenation Level Dependent response (BOLD response) [32].

The first published fMRI study of ASD appeared in 1999 [33], and revealed that people with an ASD show reduced neural responses to facial emotional expressions in brain areas known to be engaged by these stimuli in people without the disorder, such as the amygdala [33]. Recently, abnormal amygdala responses to facial emotion were also shown in unaffected siblings of autistic probands [34], consistent with the fact that ASD is associated with deficits in social cognition, such as in recognizing other's emotions.

More recently, fMRI studies have found reduced BOLD signal in visual area V2, which may be associated with the bias towards detail-orientation 'local' perception in ASDs [35] and reduced activity in so-called 'mirror neuron' systems [36], possibly associated with impairments in imitating and understanding the actions of others, although this is controversial [37].

However, while fMRI can provide important insights into the neurobiology of ASD symptoms, the interpretation of these results is rarely straightforward. For instance, the finding of reduced neural activity to images of faces displaying emotions could reflect the fact that people with ASD tend not to direct their gaze towards the eyes of such images, the areas which are richest in emotional information [38]. In other words, such differences in activation could be the indirect product of a behavioral trait, rather than direct evidence of a neurobiological abnormality.

■ Multivariate analyses

Multivariate analyses – also known as pattern classification, machine learning and automatic classification – to analyze brain structure and function have recently shown promise in characterizing complex structural and functional differences.

Conventional approaches to the analysis of neuroimaging data are univariate. Each region of the brain, or each voxel making up an image, is considered as a separate variable, and statistical analyses are performed on all regions separately. However, while useful, univariate approaches are unable to detect relationships between the structure and function of different brain regions. Multivariate analyses overcome this difficulty.

Several multivariate analyses of brain structure in ASDs have recently been published [39]. For example, Ecker *et al.* used support vector machine (SVM), a form of multivariate pattern classification, to predict the presence or absence of ASD in male adults. The results revealed that SVM applied to a set of five measures of cortical morphology (including cortical surface area) was able to classify individuals with ASD at a sensitivity and specificity of up to 90 and 80%, respectively. The classification was also shown to discriminate ASD from ADHD individuals [40].

Other studies from independent centers have recently confirmed the utility of multivariate approaches in ASD. For example, Hoeft *et al.* showed that a SVM applied to regional gray matter density was able to successfully distinguish individuals with Fragile X syndrome (a single gene disorder with prominent autistic symptoms as well as mental retardation [41]) from healthy controls and from individuals with idiopathic i.e., non-fragile-X ASD, with over 80% accuracy [42]. It was able to distinguish idiopathic ASD from controls at an accuracy of 75% somewhat less successfully than Ecker *et al.* [40]. Future studies using this novel technique should address the issue of replicability, by verifying the performance of their classification algorithm in independent data sets [39].

■ Connectivity (fMRI & diffusion tensor imaging)

It has long been theorized that ASD might be associated with abnormal connectivity between brain regions leading to impaired integration of information between brain systems (see, for example, [43].) Recent advances in neuroimaging technology have allowed this hypothesis to be tested directly.

In terms of the structure of the brain, diffusion tensor imaging (DTI) is an MRI technique which makes use of the fact that water molecules within white matter tracts tend to diffuse along the direction of the myelinated fibers more readily than in orthogonal directions. This fractional anisotropy (FA) of water diffusion can be quantified using MRI and this provides a tractographic

map of white matter pathways, allowing the integrity (FA) of individual regions to be measured and compared.

Several DTI studies in ASD have shown reduced FA in adults with the condition, indicating reduced white matter pathway integrity, in several regions [44–46]. Some reports show pervasive abnormalities across the brain [44,46], others have shown selective changes in frontostriatal tracts [47], and in the frontal and parietal lobes [48]. However, results have been mixed with increases in FA seen in some areas including the cingulate gyrus, insula and cerebellar peduncle in some populations (see, for example, [49]).

DTI measures brain structural connectivity. A complementary approach, functional connectivity MRI (fcMRI), allows the investigation of dynamic connectivity in terms of brain activity. fcMRI is a form of fMRI, however, rather than focusing on task-related BOLD changes (activation and deactivation) in particular regions, fcMRI considers the correlation between neural activation across different brain regions over time.

This can be done either in the resting state, or during performance of a cognitive task, with distinct patterns of connectivity seen in different conditions [50]. Resting state fcMRI studies the intrinsic variability in brain activity over time.

Several studies have shown impaired functional connectivity in ASDs in the resting state. Lai *et al.* found increased randomness (decreased coherence) using fcMRI [51]. Longer-ranged connections seem to be more abnormal than very short range ones [52]. Interhemispheric connections, especially long ranged ones, have also been implicated [53].

A recent study building on these results used combined fcMRI and machine learning, achieving an accuracy of over 70% in the diagnosis of ASD based on resting state connectivity patterns [54]. The results showed a pattern of reduced functional connectivity in ASD, in both medium-range and long-range connections, but especially in terms of long-range, normally negatively correlated (anticorrelated) connections, which may represent inhibitory signaling.

This fMRI evidence converges with results using electrophysiological techniques showing impaired coherence between regions in ASD [55].

■ Brain maturation

Abnormal brain maturation has long been hypothesized to be associated with ASD. Macrocephaly was noted in five of the 11 of Kanner's original case series of childhood autism [56].

Recent neuroimaging studies have confirmed that increased brain volume is seen in some autistic children, but that this is true only of the earliest years of life [57–59]. Increased brain volume is not seen in later childhood or in adults [60]. This suggests that there is an abnormal brain 'growth spurt' in the immediate postnatal period, but that this is not sustained [57–59].

This underlines the importance of considering age as a factor in ASD neuroimaging research. It cannot be assumed that the same abnormalities will be seen across children, adolescents and adults with the condition. However, relatively few studies have adopted a longitudinal approach and examined the same ASD participants at different ages (reviewed in [61]).

Conceptual shifts in the understanding of autism

The past few years have seen a shift in the way that autism and related disorders are conceived, diagnosed and reported on. In the early years of autism research, autism was seen as something that a given individual either had or did not have (the categorical approach).

However, recently there has been a shift to a spectrum/dimensional approach. ASDs has become the preferred term in many quarters [3] – this is an umbrella term that includes the old categories of autism and Asperger's syndrome, but also other disorders in which autistic symptoms are less clear cut. The various manifestations of ASD have also been referred to as 'the autisms' [62].

Although most neuroimaging research continues to adopt a categorical approach, some recent studies have revealed differences between ASD subtypes. For example, Yu *et al.* performed a meta-analysis of structural MRI studies aiming to discover differences between the brains of cases with language delay ('high-functioning autism') compared to cases of ASD with normal language development ('Asperger's syndrome') [63]. They found that while both groups shared some features, such as reduced gray matter volume in the cerebellum compared to controls and increased left ventral temporal cortex, other areas of the brain differed between the groups. For example, autism was associated with increased volume of the caudate nucleus, part of the basal ganglia, while Asperger's was characterized by small amygdala.

These preliminary results underline the fact that studies which simply compare a sample of individuals with an ASD to a healthy control sample may fail to discover selective abnormalities in particular subpopulations. ASDs are known to be clinically heterogeneous. It will be important for future neuroimaging research to investigate the neural correlates of particular symptom domains.

The future? Imaging genetics in ASD

Although ASD is known to be heritable [8,64], until recently there were few replicated genetic correlates of ASD. However, advances in genomic technology have led to the emergence of a 'rare variant, common disease' model in which ASDs can result from a large number of diverse mutations, either genetic deletions or duplications.

Large rare deletions or duplications are called copy-number variants (CNVs) and several CNVs have been reliably associated with ASD including 7q11 duplications [65], 16p11.2 duplication [66], 17q12 deletion [67] and others.

Emerging evidence therefore suggests that ASD, a clinically heterogeneous family of syndromes, is also genetically highly heterogeneous [68]. Genetics might provide a means to disentangle the neurobiological heterogeneity of ASD.

Very few studies have yet attempted to correlate imaging measures of brain structure and function with particular genetic variants in ASD, perhaps because the low prevalence of any particular rare mutation in the ASD population makes recruitment problematic. However, this can be expected to be a fruitful line of inquiry in the future. It is likely that particular mutations are associated with particular patterns of neurobiological abnormality, as has already proven to be the case with some genes associated with certain severe developmental syndromes [69]. For instance, a recent post-mortem neuropathology study examined abnormalities in ASD associated with the 15q11–13 microduplication syndrome, finding a higher incidence of hippocampal pathology but fewer cortical dysplasias in such cases compared to idiopathic ASD [70].

Future studies ought to use neuroimaging to examine structural, functional and neurochemical changes *in vivo* in 15q11–13 and other syndromes associated with ASD. Characterizing such genotype–phenotype relationships would shed light on the roles which these genes play in brain development and could contribute to reducing the heterogeneity in neurobiological studies of ASD.

The future? Technical advances

Early PET studies in autism were limited to measuring regional glucose metabolism or blood perfusion (see 'Early neuroimaging investigations of ASDs' section). However, advances in receptor-specific PET ligands may soon allow the measurement of particular neurotransmitters, re-uptake proteins and receptors, an approach which has proven productive in measuring, for example, dopaminergic abnormalities in schizophrenia [71].

A number of novel radioligands have recently emerged. ^{11}C Ro154513 is selective for the alpha5 subtype of GABA(A) receptors, which have been implicated in ASDs by studies using other methods [72]. ^{11}C Ro154513 PET has been successfully used to show reduced alpha5 expression in alcoholism [73] and recently, we found preliminary evidence of reduced alpha5 expression in adults with ASD in a small pilot study [74]. Other novel radioligands will allow specific quantification of the glutamate mGluR5 receptor [75]. There is strong evidence from various fields implicating these receptors, and other GABA and glutamate proteins, in ASDs [76,77], so this can be expected to be a very interesting line of inquiry.

In terms of MRI scanning, existing research MRI has almost always been conducted on scanners operating with 1.5 Tesla or 3 Tesla magnetic fields. However, an increasing number of centers are acquiring the capability to perform research MRI scanning at 7 Tesla. The stronger field has been shown to provide higher image resolution in both structural [78] and functional MRI [79], and better metabolite determination using MRS, so, despite the various technical challenges associated with ultrahigh field MRI [80], this can be hoped to provide further insights.

Clinical applications?

There has been much interest in the possible clinical applications of the new neuroimaging technologies, especially with regards to the use of multivariate machine learning approaches [40] for diagnostic purposes. As well as extensive coverage in the popular media, this issue has been discussed in several recent papers [81–83].

The discovery of a reliable and valid 'biomarker' for ASD – such as that provided by multivariate analysis of MRI – would have many benefits, since with increasing awareness of ASDs, more and more potential cases are being referred to diagnostic services in many countries [84,85] on the basis of behavioral symptoms.

This is in many ways a positive change, since ASDs are widely believed to have been seriously underdiagnosed in the past, and in many areas still are [86]. But as Murphy *et al.* recently pointed out, with regards to the Behavioral Genetics Clinic at the Maudsley Hospital in London (UK) [83], over half of referrals were judged not to have an ASD (53%) and in 60% of these cases, there was no evidence of any psychiatric or developmental disorder. This represents a drain on resources.

Biomarkers, including neuroimaging measures, could therefore improve the efficiency of services. It seems unlikely that they will be able replace an expert behavioral evaluation in the case of a complex disorder such as ASD. However, Murphy *et al.* argued that given the high costs of a full behavioral evaluation, MRI biomarkers could be cost efficient even though they only reduced the demand for behavioral diagnosis by 6.5%. Furthermore, biomarkers, especially neurochemical ones, as revealed using MRS or PET, could provide pointers for drug development.

Conclusion & future perspective

This paper has reviewed the recent development of neuroimaging in ASDs. Recent developments and future directions were discussed. While neuroimaging remains largely of research interest at present, it is used clinically as a means of detecting gross neuroanatomical abnormalities that can present with symptoms of ASD. However, recent advances in neuroimaging may soon provide valuable clinical tools for the diagnosis and assessment of ASD.

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