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Autism 2

Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan

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Over the past decade, in-vivo MRI studies have provided many invaluable insights into the neural substrates underlying autism spectrum disorder (ASD), which is now known to be associated with neurodevelopmental variations in brain anatomy, functioning, and connectivity. These systems-level features of ASD pathology seem to develop differentially across the human lifespan so that the cortical abnormalities that occur in children with ASD differ from those noted at other stages of life. Thus, investigation of the brain in ASD poses particular methodological challenges, which must be addressed to enable the comparison of results across studies. Novel analytical approaches are also being developed to facilitate the translation of findings from the research to the clinical setting. In the future, the insights provided by human neuroimaging studies could contribute to biomarker development for ASD and other neurodevelopmental disorders, and to new approaches to diagnosis and treatment.

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Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder that develops in early childhood. It is diagnosed on the basis of a combination of behavioural observations and clinical interviews that assess deficits in social communication, social reciprocity, and repetitive and stereotyped behaviours and interests.[1] These core symptoms of ASD typically manifest from around age 2 years and are accompanied by developmental differences in brain anatomy, functioning, and connectivity that affect behaviour across the lifespan. The causes of ASD are complex and include genetic and environmental risk factors.[2] For instance, on the genetic level alone, more than 100 genetic and genomic loci have been implicated in autism.[3] However, the ways in which these risk factors lead to the biological differences underpinning ASD or whether they could provide crucial clues to help in the development of new treatments is unknown.

In this Series paper, we review insights into atypical brain development across the human lifespan in ASD, and respective neurodevelopmental differences in brain anatomy, functioning, and connectivity within the neural systems that underlie specific autistic symptoms. Various neuroimaging techniques can been used to investigate the brain in vivo (eg, PET and magnetoencephalography), but most studies of the brain in ASD have used MRI, which provides the basis for our review. We discuss the potential for translation of in-vivo neuroimaging findings from the research to the clinical setting. In particular, we focus on the clinical utility of multivariate pattern classification approaches, which are becoming increasingly popular for the analysis of complex biological data to make clinically relevant predictions (eg, diagnostic category or clinical outcome). Despite the promise of such approaches, studies investigating the biomarker potential of neuroimaging measures for ASD have, so far, been confined to the research setting, and how well these approaches might work in the real-world clinical setting remains unknown.

Atypical brain development
Early brain development

Findings from several structural MRI studies have shown that toddlers with ASD (age 2–4 years) have, on average, a larger brain volume than typically developing children (ie, those without a medical or psychiatric diagnosis).[4–6] However, this increased brain volume seems to disappear around the age of 6–8 years, when growth curves intersect;[5] after this, no substantial increases in total brain volume commonly occur.[7] These findings, and others, contributed to the notion that the neurodevelopmental trajectory of brain maturation is atypical in ASD and includes a period of early overgrowth followed by arrested growth and possibly a decrease in brain volume at older ages.[7] Moreover, the altered neurodevelopmental trajectory of the brain in ASD seems to vary across different brain regions, with the frontal and temporal lobes being affected more than the parietal and occipital lobes.[6] This finding suggests that the temporal and regional sequence of typical early brain development (ie, from back to front)[8] is perturbed in ASD. However, others have also reported generalised enlargements throughout the cerebral cortex in children with ASD between age 18 months and 35 months,[9] which will not only affect the structure of isolated brain regions, but also lead to differences in brain anatomy and connectivity on a systems level.[10] Some of the systems-level abnormalities in an early-onset disorder such as ASD will probably differ from those found in disorders that are usually present later in life (eg, schizophrenia). However, the stage in brain development during which the first differences in brain anatomy and connectivity associated with ASD occur is poorly understood.

Findings from several longitudinal MRI studies of infants at high familial risk of developing ASD (eg, siblings of affected individuals) suggest that differences in the brain can already be seen in the first 2 years of life. For example, Schumann and colleagues[11] reported brain

enlargement in 2·5-year-olds with ASD, which was accompanied by an abnormally increased growth rate of the cortex. A general cerebral enlargement in ASD was also noted by Hazlett and colleagues[12] at this age; however, by contrast, this enlargement was not associated with an increased growth rate. Hence, there is compelling evidence that individuals with ASD have an early enlargement of the brain that is already visible at around age 2 years.

Whether this enlargement represents an ongoing accelerated growth of the cortex or is the end result of previous differences in brain growth remains unclear. Moreover, the pathological process that underpins differences in brain volume is unclear, although recent progress has been made. For instance, Hazlett and colleagues[12] did not identify an increase in cortical thickness and so argued that the increase in brain volume in ASD must be driven by an increase in brain surface area before the age of 2 years. The distinction between cortical thickness and surface area is important because both measurements are related to distinct aspects of cortical neuropathology and represent different features of the cortical architecture.

In terms of phylogeny, cortical thickness and surface area are now widely believed to be determined by different types of progenitor cells, which divide in the ventricular zone to produce glial cells and neurons. Cortical thickness has been related to intermediate progenitor cells,[13] which divide symmetrically at basal positions of the ventricular surface. These progenitor cells only produce neurons,[14,15] which then migrate along radial glial fibres to form ontogenetic columns arranged as radial units. According to the radial unit hypothesis,[16] cortical thickness is determined by the neuronal output from each radial unit amplified by intermediate progenitor cells and, therefore, is related to the number of neurons produced in each unit. By contrast, cortical surface area has been associated mainly with radial unit progenitor cells, which divide at the apical ventricular surface. The early proliferation of radial unit progenitor cells leads to an increase in the number of proliferation units, which in turn increases the number of ontogenetic columns, resulting in increased surface area.[13] The finding of early enlargement of the brain in ASD, caused by an accelerated expansion of cortical surface area but not cortical thickness,[12] is hence of importance because it points towards specific genetic and neurobiological mechanisms that might be impaired in ASD, and highlights the need for the development of novel neuroimaging measurements that offer a higher degree of specificity with regard to particular underlying mechanisms.

Figure 1 shows the chronological progression of MRI measurements from volumetric features[4,17–19] to surface-based geometric measurements[10,20–23] of brain anatomy and diffusion tensor imaging measurements of structural brain connectivity.[24] These findings support suggestions that the accelerated expansion of the cortical surface and a potential pathological change in the number or size of cortical neurons or minicolumns in ASD[25,26] leads to the brain being connected differently;[27] for example, as evident in early white matter differences in ASD.[28] Thus, atypical development of the cortical grey matter in ASD is likely to be linked to abnormal maturation of the cortical white matter, and multimodal neuroimaging studies examining the relation between the development of cortical grey matter and white matter will be a crucial next step in the identification of the mechanisms that drive perturbed brain maturation in ASD.

Findings from several recent studies have shown the importance of considering wider contextual issues when interpreting reports of early brain overgrowth in ASD. For example, in 2013, Raznahan and colleagues[29] suggested that the well-replicated finding of a significant increase in head circumference in ASD—which accompanies the increase in total brain volume—might in fact be a result of a bias in population norms rather than a replicable pattern of dysregulated growth.[29] Future investigations into population norms will also be important when considering the large phenotypic diversity of the brain in ASD, which can only be reliably interpreted in the context of the wide neuroanatomical diversity within the general population.[30] Evidence also suggests that early brain overgrowth in ASD is not restricted to the brain exclusively, but that early generalised patterns of physical (ie, somatic) overgrowth occur in ASD, particularly in boys.[31,32] Thus, dynamic changes in population norms and factors responsible for both neural and non-neural tissue development should be considered to provide a more comprehensive assessment of atypical brain development in ASD.

Neural systems underlying autistic symptoms and traits

The components of the neural systems that probably underlie ASD are well established and most likely incorporate the frontotemporal and frontoparietal regions, amygdala–hippocampal complex, cerebellum, basal ganglia, and anterior and posterior cingulate regions.[33] Moreover, these core regions have been suggested to mediate specific clinical symptoms. For example, abnormalities in (1) Broca's area and Wernicke's area have been associated with social communication and language deficits;[34] (2) frontotemporal regions and the amygdala have been related to abnormalities in socio-emotional processing;[35,36] and (3) the orbitofrontal cortex and the caudate nucleus (ie, frontostriatal system) might mediate repetitive and stereotyped behaviours.[37] Findings from a voxel-based morphometry study[38] suggest that the neuroanatomy of ASD is also modulated by sex, and might be related to gender differences in neurocognitive profiles.[39] Moreover, although abnormalities in these brain regions seem to be common in ASD, evidence suggests that the differences in these regions are not specific to the disorder. For example, neuroimaging evidence suggests that the

caudate nucleus is enlarged in ASD,[40] and that this enlargement is associated with the severity of repetitive and stereotyped behaviours.[41] The amygdala is also significantly enlarged in children with ASD compared with typically developing children.[42] However, enlargement of the basal ganglia has also been noted in individuals with obsessive–compulsive disorder without a comorbid diagnosis of ASD,[43] and increased total amygdala volume has been reported in children with general anxiety disorders.[44] Finally, frontal lobe abnormalities, which are a hallmark of ASD, have also been found in other neurodevelopmental disorders such as schizophrenia.[45] These findings suggest that phenotypic similarities in brain anatomy between ASD and other psychiatric disorders might be related to similarities in their respective clinical phenotypes. However, future research is needed to establish the extent and origins of such shared neuro-anatomical variation, and to establish whether there are common or distinct genetic and molecular mechanisms for similar symptoms across disorders.

Neurodevelopment across the human lifespan

Less is known about neurodevelopment during adolescence and adulthood in ASD. Although early brain development in ASD seems to be dominated by an accelerated increase in volume of the frontal and temporal lobes,[46] brain development after early adolescence seems to be dominated by an accelerated age-related decline in total brain volume[47] and its two constituents (ie, cortical thickness and surface area). For
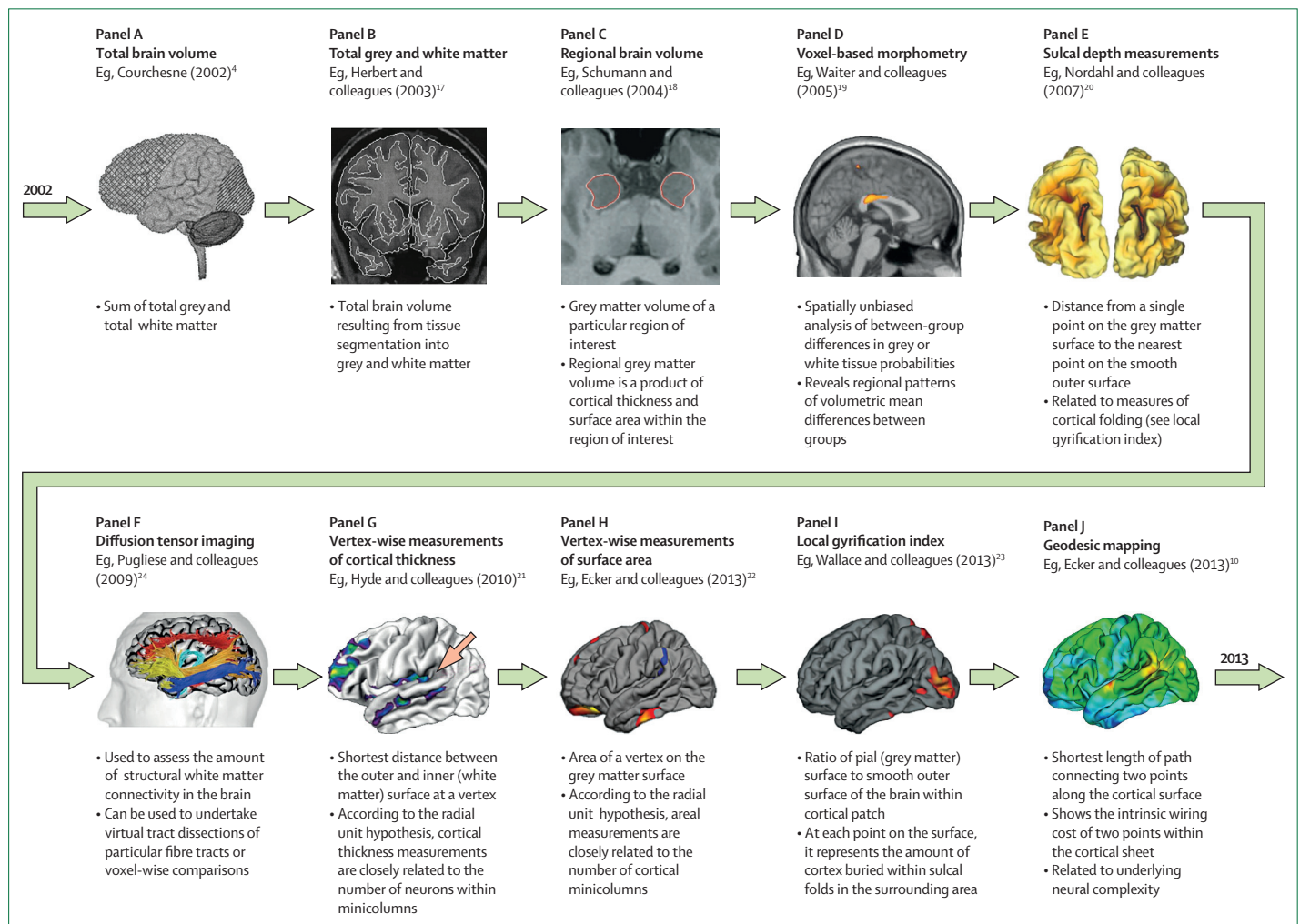

Figure 1: **Progression of structural MRI measurements over time as applied to autism spectrum disorder**
Imaging measurements are becoming increasingly more specific with regard to particular underlying neural mechanisms, which will help to further disentangle different aspects of the cortical pathology of autism spectrum disorder in the future. Improvements in the specificity of imaging measurements will also increase their translatability across disciplines (eg, combining imaging and genetic studies). Panel A reproduced from Courchesne,[4] by permission of Nature Publishing Group. Panel B reproduced from Herbert and colleagues,[17] by permission of Oxford University Press. Panel C reproduced from Schumann and colleagues,[18] by permission of the Society for Neuroscience. Panel D reproduced from Waiter and colleagues,[19] by permission of Elsevier. Panel E reproduced from Nordahl and colleagues,[20] by permission of the Society for Neuroscience. Panel F reproduced from Pugliese and colleagues,[24] by permission of Elsevier. Panel G reproduced from Hyde and colleagues.[21] Panel H reproduced from Ecker and colleagues,[22] by permission of the American Medical Association. Panel I reproduced from Wallace and colleagues, by permission of Oxford University Press.[23] Panel J reproduced from Ecker and colleagues,[10] by permission of the National Academy of Sciences of the USA.

example, accelerated cortical thinning in various brain regions in ASD has been reported in several cross-sectional MRI studies,[48] and has been confirmed in a large longitudinal study of individuals aged 3–39 years.[49] Additionally, measurements of surface area decline more rapidly with age in individuals with ASD than in those without ASD both at the global (ie, total surface area)[50] and the local (ie, vertex wise)[51] levels. These findings also highlight the importance of accounting for the different neurodevelopmental stages when comparing individuals with ASD with typically developing controls because the rate of cortical development is not linear across the human lifespan, and precocious or delayed maturation in a group of individuals could lead to a substantial positive difference at one age and a substantial negative difference at another age.[48] For example, the amygdala is enlarged in toddlers and children with ASD, whereas no significant differences have been reported in adolescents with the disorder.[18,52] Thus, general effects of age and age-by-group interactions need to be accounted for in the statistical model when examining neuroanatomical variations across the human lifespan. However, overall, findings from neuroimaging studies suggest that atypical cortical development in ASD occurs in distinct stages that are dominated by an accelerated expansion of the cortical surface during childhood, and there is initial evidence that this expansion is followed by an acceleration in cortical thinning during adolescence and adulthood. If correct, these findings suggest that individuals with ASD might be at risk for accelerated cortical decline in later life. Hence, in the future, large normative datasets characterising the developmental timecourse of different morphometric features in different brain regions will be needed to fully characterise the cortical ontogeny of ASD. Moreover, in addition to investigating volumetric features of brain anatomy, examination of differences in geometric features (eg, cortical folding) in ASD will also be important.

Atypical cortical gyrification

If we accept that in ASD the brain undergoes a stage of accelerated expansion during childhood,[12] we can also expect the brain to differ in geometry from those without ASD. As the cortex expands, it eventually needs to fold to fit an increasing brain surface into the finite space of the skull. Consequently, the brain in ASD would be expected to show an increase in cortical folding.[12] The investigation of cortical folding would also allow us to better define the causative mechanisms associated with ASD. For example, axonal white matter fibres might exert a pulling force on the overlying neocortex, and so might modulate cortical folding extrinsically via mechanical tension.[53] Alternatively, cortical folding might be mediated by developmental changes intrinsic to the cortical sheet (ie, within the cortical grey matter). For instance, the formation of cortical gyri has been linked to an accelerated expansion of the outer cortical layers relative to the deeper layers[54] and

to the microstructural complexity of the associated grey matter determined by dendritic arborisation, synapto-genesis, and the alignment of neurons in space.[55] In other words, studies of cortical folding allow us to better define the cellular and microstructural processes underpinning differences in brain development, and in particular those of relevance to ASD.

Atypical cortical gyrification in the brain of individuals with ASD has been reported in several MRI studies, most of which involved manual assessments of the cortical surface. For example, a significantly higher incidence of cortical malformations have been reported in patients with ASD compared with healthy controls, including (1) polymicrogyria (too many small folds, thought to arise from atypical prenatal brain maturation); (2) schizencephaly (clefts lined within the cortical grey matter); and (3) macrogyria (increased size of gyri).[56] Evidence also suggests that some sulci (eg, the Sylvian fissure) seem to be further along the principal axes of the brain in children with ASD than in those without ASD,[57] and that there is significantly increased gyrification of the frontal lobe in children and adolescents with ASD.[58] These early neuroimaging studies are supported by investigations using automated techniques that provide measurements for gyrification in a spatially unbiased fashion. For example, an increasing number of studies have investigated brain morphology using a measurement known as the local gyrification index—a local variant of the classic two-dimensional gyrification index.[59] The local gyrification index at a given point on the cortical surface represents the amount of cortex buried within the sulcal folds and is computed as the ratio between the surface of a circular patch on the outer, smooth surface of the brain and the surface of the corresponding patch on the pial (ie, grey matter) surface (figure 2A).[60,62] In males with ASD compared with typically developing male controls aged 12–23 years, the local gyrification index is increased in bilateral posterior brain regions (figure 2B).[23] The local gyrification index is significantly reduced in the left supramarginal gyrus in males aged 8–40 years[63] and in the right inferior frontal and medial parieto-occipital cortices in children with ASD (figure 2C).[61]

Although these divergent findings can be partially explained by differences in sample size, participant demographics, and analytical techniques (eg, size of smoothing kernel), evidence also suggests that the particular pattern of cortical gyrification is variable across individuals—even in normative populations—and that both genetic and non-genetic factors contribute to patterns of cortical folding.[64] For instance, Kates and colleagues[65] directly examined the amount of concordance in gyrification index in monozygotic twin pairs in which one twin had a diagnosis of ASD and in typically developing unrelated controls. They reported a high level of discordance in cortical folding within ASD twin pairs across most lobular regions of the cortex, but

both children with ASD and their co-twins exhibited increased parietal lobe cortical folding compared with controls.[65] Thus, although patterns of cortical folding might be suggestive of an atypical neurodevelopmental trajectory driven by various genetic and molecular processes, environmental factors and experience-dependent mechanisms also probably modulate cortical morphometry, and perhaps especially cortical folding. This finding is in contrast to conventional measurements of brain anatomy (eg, total or regional brain volume), which are highly concordant between twins, and might thus be largely genetically determined.[66] Hence, future studies of the genetic and environmental factors that affect brain development in ASD will probably need to include measurements of cortical geometry in addition to those of volume. The examination of geometric features of the brain is of increasing importance in view of uncertainty about the heritability of ASD. For example, although in a traditional twin study the heritability of ASD (ie, proportion of liability attributable to genetic factors) was estimated at about 90%,[67] findings from a 2011 study[2] suggested that susceptibility to ASD has moderate genetic heritability (38%), with a large proportion of variance in liability explained by shared environmental factors (58%). Thus, to elucidate the contribution of genetic and non-genetic factors to brain development in ASD, various cortical features, including shape characteristics of the brain, should be examined to account for the large amount of phenotypic inter-individual variability typically noted in the brain in ASD.

Neural activation and functional connectivity

In view of the abnormal development of grey and white matter in autism, affected brain regions will inevitably be unable to generate connections that give rise to fully effective functional networks. Early functional MRI (fMRI) investigations focused exclusively on region-specific differences in the magnitude of activation. Because fMRI studies use different activation paradigms and task parameters, often in cohorts with different ages and levels of severity, results vary markedly. Results from face processing studies provide a stark example of the profound effects that minor task differences will produce; for example, early studies examining neural responses to emotional faces initially showed reduced activation in face-specific perceptual regions[68–70] in addition to limbic areas, particularly the amygdala.[71,72] However, further examination showed that activation differences between typically developing individuals and those with ASD were strongly affected by experimental factors such as gaze direction[73] and face familiarity.[74] Nonetheless, consistent differences in brain activation occur in regions that also show structural abnormalities, as highlighted in several recent reviews.[75–77] Findings from many studies have shown decreased activation in regions comprising the social brain network during tasks related to emotional processing or social cognition, including the amygdala, temporal–parietal junction, insula, and inferior frontal cortex;[71,78–84] in frontostriatal circuitry in response to cognitive control tasks and repetitive behaviours;[75,85–88] in language circuitry during communication tasks;[89–91] and in reward circuitry.[92–95] By contrast, abnormal increases in
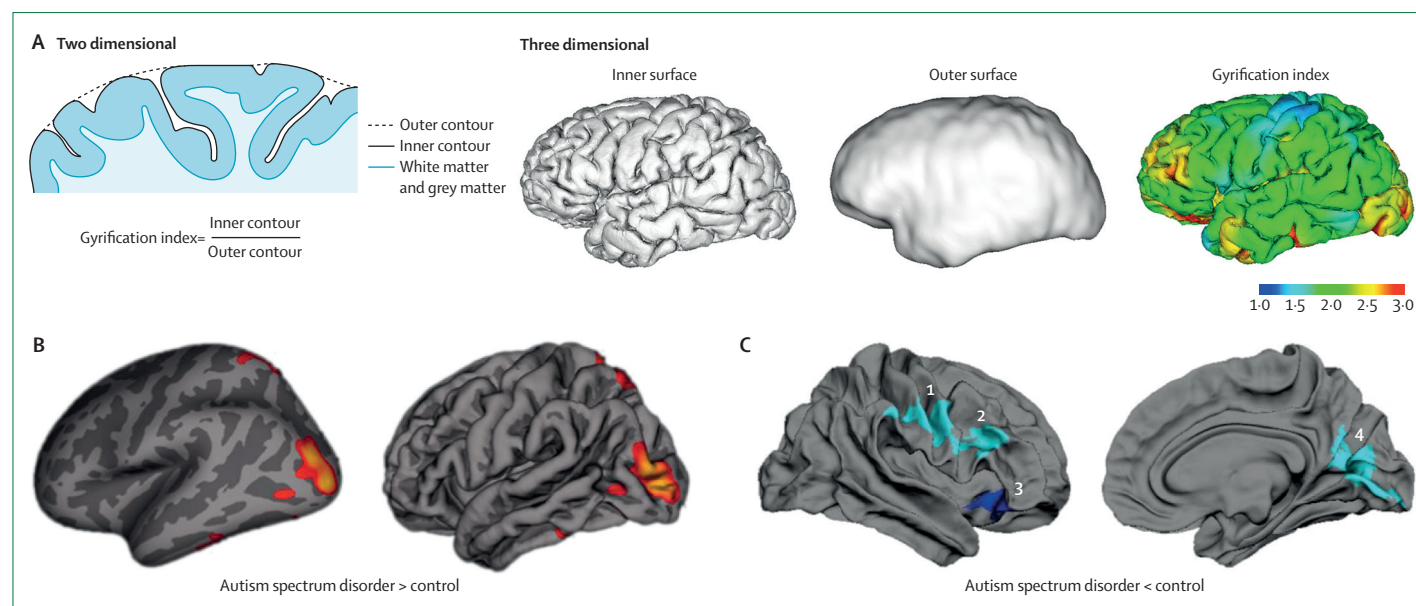
Figure 2: Cortical gyrification in autism spectrum disorder
(A) The local gyrification index allows quantification of gyrification at each vertex on the cortical surface. Reproduced from Mietchen and Gaser.[60] (B, C) In autism spectrum disorder (ASD), locally increased gyrification (B; reproduced from Wallace and colleagues,[23] by permission of Oxford University Press) and decreased gyrification (C; reproduced from Schaer and colleagues)[61] have been reported. The numbers in (C) correspond to four clusters of reduced local gyrification index in a group of children and adolescents with ASD compared with controls after correcting for multiple comparisons.

activation are found in response to irritants and direct gaze.[96,97] Taken together, these findings strongly support a link between regional structural brain abnormalities and functional sequelae, including both regional brain activation and phenotypic presentation.

In other studies, neural activity has been examined at the network level, focusing on brain connectivity either during task performance or in the resting state (panel; figures 3 and 4).[99–101] In most fMRI studies in people with autism, functional connectivity during task performance—measured by examining the correlation of fMRI activation changes between brain regions in typically developing participants versus those with ASD—is decreased. Such studies have typically included only high-functioning older participants. By contrast, resting-state fMRI examines spontaneous fluctuations in brain activity, measuring differences in the correlation between regions in well-defined functional networks. These studies have yielded mixed results, and there remains substantial controversy regarding the nature of connectivity impairment in autism, with researchers arguing in favour of under-connectivity,[102] over-connectivity,[103,104] or unique patterns of both under-connectivity and over-connectivity depending on the brain region.[105] Among the reasons for differences between studies are differences in the severity of ASD and, in fMRI activation studies, variable task choices, such as the choice of the smoothing filter or template. Choices in subtle aspects of image processing can also affect results.[106] Furthermore, findings from a 2013 review suggested that connectivity results are affected by the age of the population studied.[107] Thus, the nature and direction of connectivity differences in ASD might change across the lifespan.

Applications of graph theoretical approaches in ASD

Graph theoretical approaches can be used to identify new ways to describe the nature of both local and global network function, and can be applied to structurally or functionally defined networks; these approaches are also increasingly used to investigate connectivity impairments in ASD. These techniques describe properties of network dynamics that make up well-integrated biological systems and include metrics such as (1) modularity,

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Panel: **Network analysis of brain connectivity**

Resting-state functional MRI (fMRI) takes advantage of spontaneous low-frequency fluctuations in cerebral blood flow to measure correlations within and between brain regions.[98] Resting-state fMRI studies have identified networks of brain areas with similar levels of spontaneous activity at rest—such as sensorimotor, visual, and auditory systems; a language network, attention networks, and the default mode network; and a series of regions including the precuneus, medial frontal cortex, and hippocampus—that tend to show cerebral blood flow decreases during cognitive performance, but with relative increases at rest. Nodes in these networks can be identified in several ways—for example, by using a priori anatomical regions of interest or functional masks derived from previous studies of resting-state activity identified by independent components analysis. Graph theory analyses use all identified nodes and enter them into a correlation matrix. The data entering the correlation matrix can come from any modality, such as cortical thickness from structural MRI, fractional anisotropy from diffusion tensor imaging, temporal correlation coefficients from spontaneous fluctuations in resting-state fMRI, or activation in task-based fMRI. Graph analyses can be used to examine correlations within and between networks, and a wide range of metrics, some of which are interdependent, describe the properties of the networks derived from the correlation matrices. Figure 3 details a typical analysis pathway to derive a connectivity matrix from resting-state fMRI.[99] From this basic structure, several aspects of the resulting networks can be examined, including the extent to which specific regions form a correlated matrix that is separate from others (modularity); the number of connections needed to travel from one node to another (efficiency); and the extent to which specific nodes form hubs. Figure 4A shows the network structure of five

modules derived from a resting-state fMRI study.[100] The investigators optimised this analysis to identify functional modules by finding the best structure that maximally separated modules from one another while maximising correlations within nodes. The modules were consistent with combined somatosensory and auditory, attention, visual, default mode, and limbic, paralimbic, and subcortical networks. This pattern shows the hallmarks of a typical biological network: (1) there are distinct modules in which regions within the modules are highly connected to each other, and nodes within each module are locally efficient (there are direct paths from one node to another); and (2) the modules are distinctly segregated from one another, and there are few connections between the modules, so that the connections are clearly segregated. As a general rule, more paths would be needed for a node in one module to reach a node in another. A few regions seem to be hubs, connecting to several different modules. Only a few characteristics of a well-formed biological network enable specialisation of function, communication, and integration of information across networks that is efficient, non-random, and well organised. In the largest resting-state study in autism spectrum disorder (ASD) so far[101]—a multisite study combining images from 703 children and adolescents (360 with ASD and 403 with normal development)—widespread reductions in connectivity were reported among children with autism, spanning unimodal, heteromodal, primary somatosensory, and limbic and paralimbic cortices, whereas those with ASD had increased connectivity between a small set of nodes primarily in subcortical regions (figure 4B). This pattern of results supports a model of abnormal neurodevelopmental trajectories in ASD while providing a background for understanding various cognitive processing deficits.

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which shows the extent to which clusters of regions are associated with one another and are segregated from other modules; (2) local efficiency, which describes how efficiently local regions can communicate together; and (3) global efficiency, which refers to how densely regions across the brain are connected to one another.[108]

Network analysis during resting-state fMRI in ASD has yielded controversial results, mainly because some crucial metrics, including local versus global efficiency, are sensitive to motion artifacts.[109] Because children with ASD differ from typically developing children in their ability to self-regulate behaviour, increased motion in patients compared with controls could introduce a systematic bias, with apparent differences in functional connectivity resulting from motion artifacts. Similar confounds occur in diffusion tensor imaging studies.[110] In a recent study of a small sample of individuals with ASD, no global and only slight local differences in functional connectivity were noted when head motion was carefully eliminated by scrubbing or removing images affected by motion from the data.[111]

However, findings from most studies have continued to support the broad notion that, overall, individuals with ASD have poorer connectivity in regions spanning long distances in the brain than do typical controls, whereas connectivity seems to be increased in local circuits.[92,112–114] Furthermore, important properties of network connectivity seem to be altered in ASD; for example, the development of domain-specific function modules is reduced.[115,116] Although local connectivity within central hubs or rich clubs (ie, high-degree nodes that are more densely connected among themselves than nodes of a lower degree) seems to be increased,[117] the hub organisation is altered across the brain.[118] Taken together, findings from functional activation and functional connectivity studies suggest that, in children with autism, widespread patterns of developmental disconnection affect information processing at both the local and global levels; furthermore, the specific patterns of connectivity abnormalities relate to the severity of the autism phenotype.

Genetic risk factors for ASD and their association with measures of brain activation and connectivity

Findings from recent studies have linked genetic risk for autism to differences in functional brain activation and connectivity. Broadly, these imaging–genetics studies examined syndromes that carry a high risk for autism, such as fragile X, 15qdup, 22q11.2 deletion, and Angelman's syndrome, or investigated specific candidate risk genes more broadly in the population. Because patients with syndromic autism more commonly have marked cognitive dysfunction than do neurotypical controls, few fMRI studies have included these populations because of their reduced ability to actively participate and engage in tasks within the constrained fMRI environment. A few exceptions have been studies of fragile X[119–121] and 22q11.2 deletion syndrome.[122,123] Although results vary, in general, comparisons of syndromic autism versus controls yield similar results to those of ASD versus controls with regard to reduced connectivity, brain overgrowth, and abnormal functional activation, although a few consistent differences, particularly enlarged caudate volumes and caudate connectivity, seem to be specific to these syndromes.

Unlike studies of syndromic autism, the common polymorphism approach is used to examine the effects of autism risk genes independent of diagnosis, and thus has fewer confounds with variables such as impaired cognition. In several studies, associations have been reported between autism risk genes and brain connectivity.
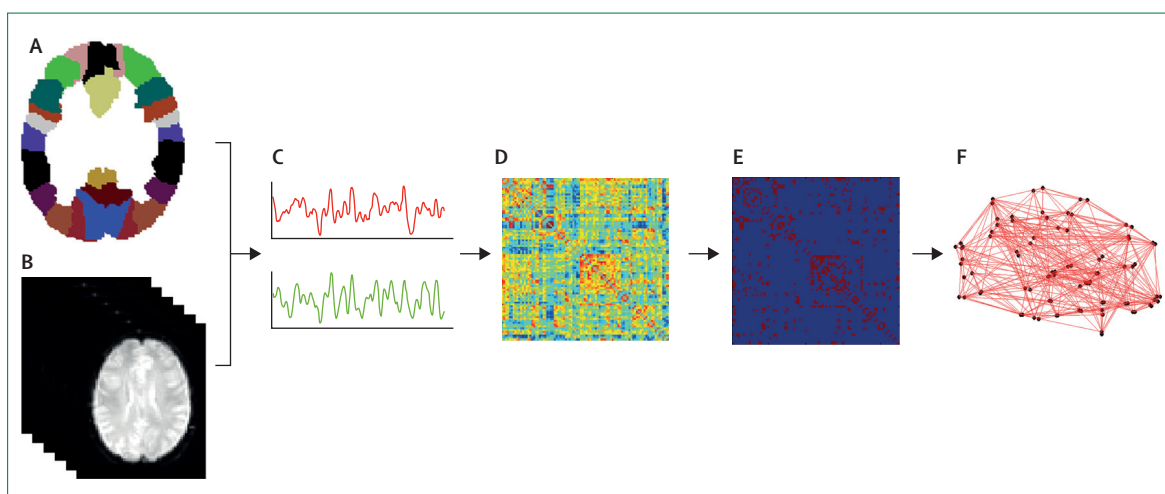

Figure 3: **Steps involved in deriving network structure from resting-state functional MRI data**
The network nodes are defined as anatomical regions of interest (A and B); they are applied to the time series of a resting-state functional MRI study collected for about 8 min. The derived time series from each node (C) is entered into a connectivity matrix (D), in which the strengths of the correlations are depicted as a colour code. This matrix is thresholded by applying a statistical threshold or cutoff (E) and the correlation strength between each thresholded pair is depicted graphically (F). Thus, nodes in the matrix represent regions of interest; edges are the relations between nodes. Reproduced from Wang and colleagues.[99]

For instance, the gene *CNTNAP2,* which confers risk for selective language impairment and for the language phenotype in autism,[124] is associated with abnormal structural and functional connectivity,[125,126] and specifically a pattern of increased short-range and decreased long-range connectivity.[92] Variations in oxytocin receptor genes, which confer a risk for autism, have been associated with differences in both amygdala volume[127] and functional

connectivity of the hypothalamus.[128] Similarly, the *MET* promoter variant—another autism risk gene—is related to both increased functional activation and decreased functional connectivity in neural networks associated with the processing of facial affect.[115] Taken together, studies that have examined the relation between genes associated with autism and brain structure and function support a model of abnormal developmental connectivity that leads to both reduced functional activation and decreased development of functional connections, particularly in long-range pathways; these differences occur in individuals who carry autism risk genes but who do not have autism.

Recent use of network analysis of gene expression profiles in tissue from patients with autism taken at post mortem[129] suggest the potential to better link genes and brain development in autism. Because network analysis is independent of modality, these techniques offer the potential to combine functional and structural imaging results, providing a comprehensive and potentially integrative model linking anatomical abnormalities to functional and phenotypic outcomes.

MRI-driven biomarker development

Typical brain development occurs as a dynamic yet ordered sequence of temporally distinct regional events,[8] which, if perturbed, would not only affect the development of isolated brain regions, but also lead to differences in brain anatomy and connectivity at the systems level.[130,131] Thus, multivariate approaches that offer high exploratory power by taking advantage of the correlated structure (ie, covariations) in a large and potentially complex (ie, multimodal) set of variables are particularly well suited to the investigation of the complex cortical pathology of ASD. Moreover, some multivariate techniques also enable researchers to make clinically relevant predictions. In the context of MRI, these techniques have been described as brain-decoding methods,[132] and they belong to a wider group of techniques known collectively as machine learning or multivariate pattern classification. The basic
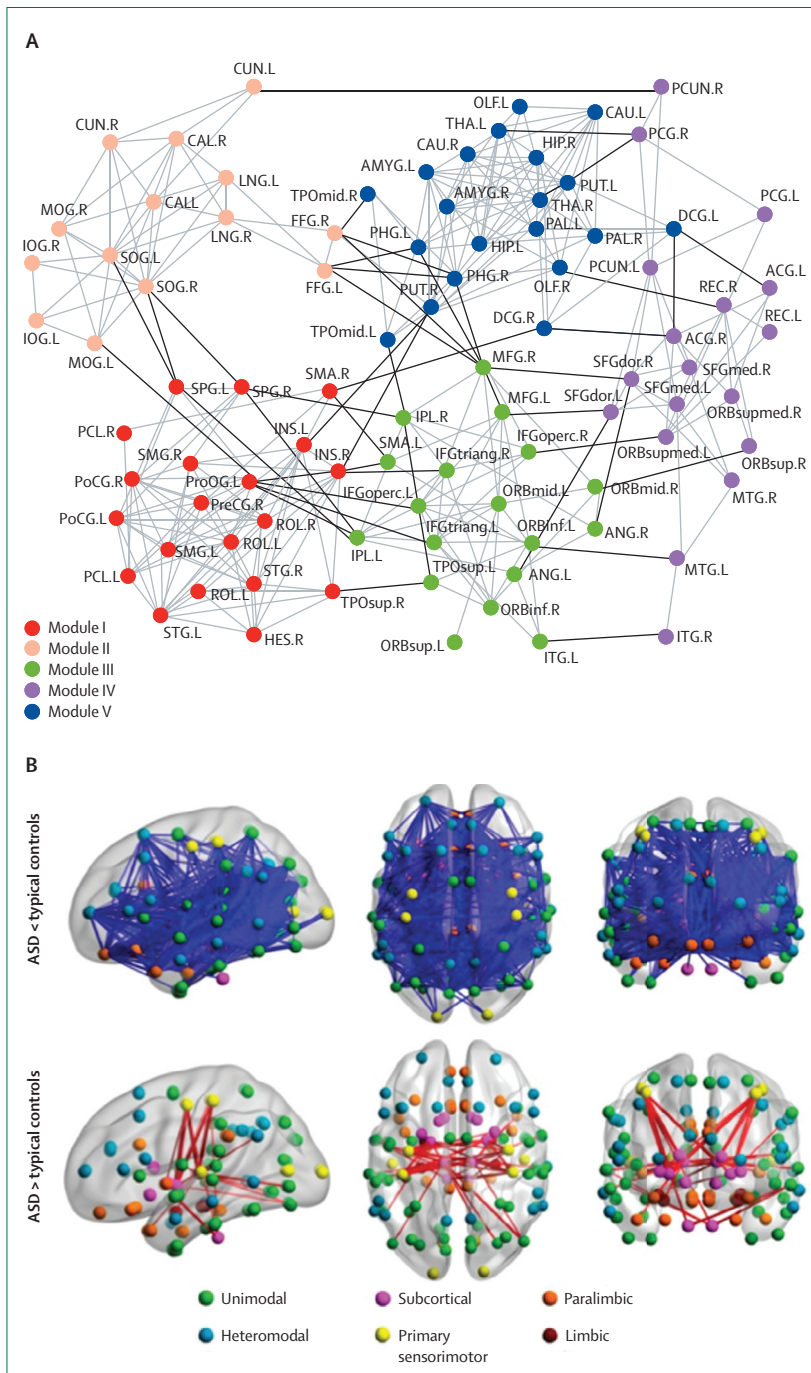

Figure 4: **Modular architecture of functional networks and functional connectivity in autism spectrum disorder**
(A) Modular architecture of functional networks in the human brain. This analysis was optimised to partition the network data to maximise modularity by identifying clusters of regions that best separated from other modules and were well integrated within modules. Grey lines represent within-module connections and black lines are connections between modules. Module I contains regions consistent with the somatosensory and auditory networks. Module II contains predominantly visual regions. Module III best resembles an attention network. Module IV resembles the default mode network. Module V contains limbic, paralimbic, and subcortical systems. Reproduced from He and colleagues.[100] (B) Group differences in intrinsic functional connectivity between individuals with autism spectrum disorder (ASD) and typical controls. The upper panel shows the intrinsic functional connections (blue lines) that were significantly weaker in ASD compared with typical controls. The lower panel shows the intrinsic functional connections that were significantly stronger in ASD compared with typical controls (red lines). Results were corrected for multiple comparisons using a false discovery rate at p<0·05. Reproduced from Di Martino and colleagues,[101] by permission of Nature Publishing Group.

idea of machine learning is to train a computer algorithm to identify a complex pattern of data that can then be applied to new individuals to make a prediction. Training usually occurs in a well-characterised sample by finding a boundary, or hyperplane, that best discriminates between different classes (eg, patients and controls). Once the classifier is trained, it can then be used to predict group membership of a new test example (eg, a new individual with unknown group membership; figure 5). A key feature of pattern classification is its potential to detect global, complex, and potentially multimodal patterns of abnormalities that cannot be efficiently identified with univariate methods. This aspect makes machine-learning approaches particularly suited to the search for autism biomarkers for case identification, patient stratification, or for the prediction of clinical outcomes.

Diagnostic methods for ASD and their limitations

At present, diagnosis of ASD is based on behavioural findings, clinical interviews, or both, and does not include the use of biomarkers. Although behavioural diagnosis of ASD is advantageous in the clinical setting because it can accommodate all variations of the autism spectrum regardless of their cause, it is insufficient to separate potentially different biological subgroups or strata of patients that would be expected to respond well to a particular treatment. Evidence suggests that there is a large amount of genetic, phenotypic, and clinical heterogeneity among individuals with ASD. For example, preliminary evidence suggests that about 30% of people with ASD might have hyperserotonaemia[133] and thus are likely to respond well to drugs that modulate the serotonergic system, whereas about 70% do not, which could also explain the low treatment response to serotonergic drugs in large-scale clinical trials. Thus, ASD probably cannot be treated with a one-size-fits-all approach; treatment needs to be more personalised and individually tailored.[134]

Also, diagnosis of ASD on the basis of behavioural findings can be problematic, particularly in adult populations. At present, ASD is diagnosed with the help of two assessment instruments: the Autism Diagnostic Interview–Revised (ADI-R)[135] and the Autism Diagnostic Observation Schedule (ADOS).[136] The ADI-R is a semi-
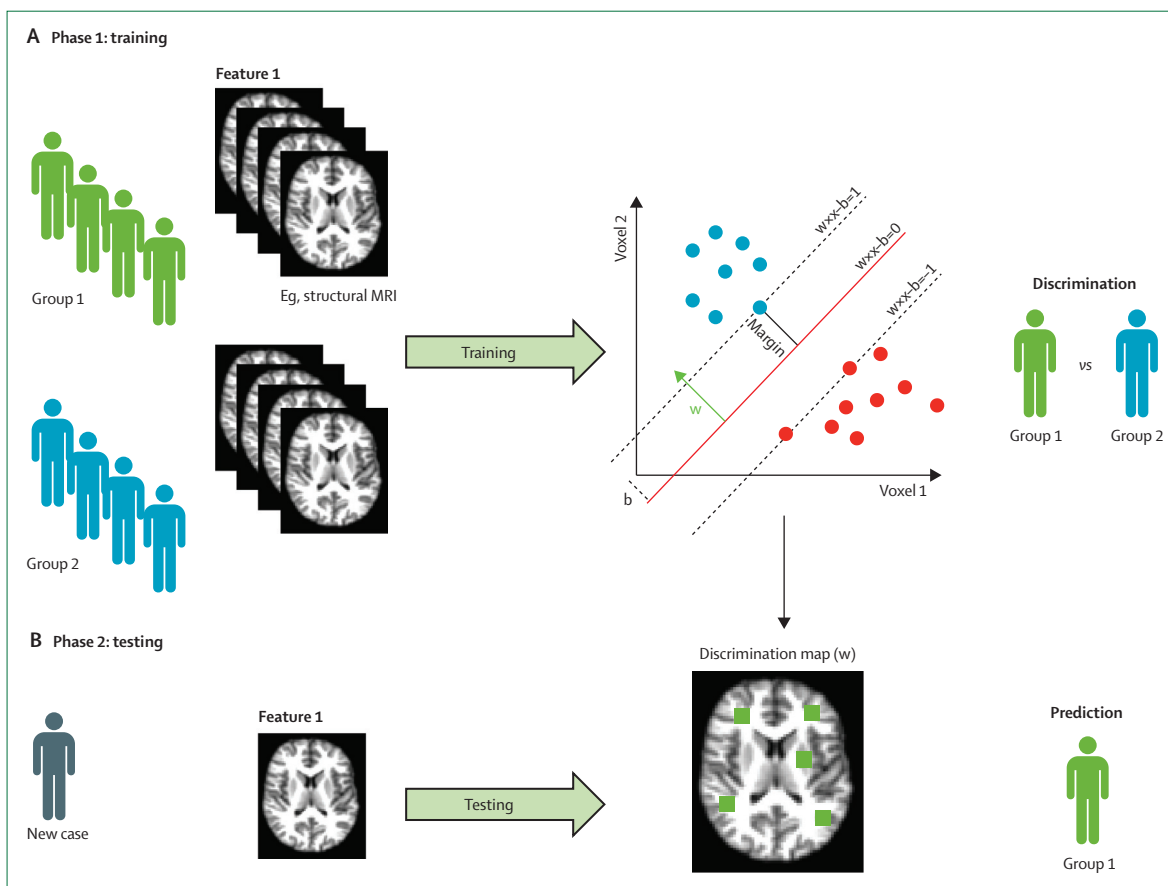

***Figure 5:* Multivariate pattern classification**
(A) In the training phase, the model is initially trained on a well-characterised sample of biological data to derive discriminative patterns of features that maximally separate the groups. (B) In the testing phase, these patterns can then be applied to predict group membership of a new dataset. The separating hyperplanes are described by a learning weight vector, w, which shows the contribution of each voxel to the overall discrimination, and an offset, b.

standardised interview typically done with parents of individuals with ASD and assesses the severity of autistic symptoms at the age of 4–5 years, which qualifies for a diagnosis of childhood autism. The ADOS is a semi-structured assessment designed for use with the individual to be diagnosed and assesses the severity of present autistic symptoms. Thus, the use of either the ADI-R or the ADOS can be problematic for diagnosis of ASD in adult populations because (1) ADI-R assessments rely on the availability and reliability of an informant to give retrospective accounts of past autistic symptoms, which, in some cases, occurred many years ago; and (2) symptoms assessed in adult samples are often masked by coping strategies developed across the lifespan, and might have been alleviated by treatments and interventions. Thus, the availability of biomarkers that could assist behavioural diagnosis would be invaluable, particularly in cases in which behavioural accounts are insufficient to make a reliable diagnosis of ASD.

Applications of multivariate pattern classification

An increasing number of studies are using multivariate pattern classification techniques in the research setting to separate individuals with ASD from typically developing controls (ie, for case identification), and proof-of-concept studies were originally done in adult samples. For example, Ecker and colleagues[137] explored the diagnostic value of whole-brain structural MRI scans measuring regional grey and white matter volume for ASD using a common variant of machine learning, the support vector machine (SVM). In this sample, the SVM correctly classified individuals with ASD and controls into their respective diagnostic category on the basis of their neuroanatomy with about 80% accuracy. In addition to the binary classification, the SVM provided a test margin for each participant, showing the level of confidence with which a new individual could be classified.[137] The test margins were also positively associated with the severity of autistic symptoms, thus suggesting that the SVM could be used to capture ASD along a continuum that is also apparent in its neuroanatomical imprint. These original observations, which provided initial proof of concept, are supported by findings from several other neuroimaging studies with similar levels of classification accuracy in younger age groups,[138] females with ASD,[139] and autism-related disorders,[140] and with various anatomical and functional measurements.[141] Therefore, in cases in which diagnostic data for behavioural assessments are insufficient for a diagnosis of ASD, biomarkers might provide additional valuable information that could aid the expert clinical assessment of ASD.

Recent advances in analytical techniques now also make possible the prediction of quantitative outcomes, rather than simple binary categories (eg, patients *vs* controls, or responders *vs* non-responders). For instance, in 2013, Sato and colleagues[142] used support vector regression to predict the severity of autistic symptoms as measured with the ADOS using inter-regional correlations of cortical thickness measurements in a sample of individuals with ASD. The implementation of such quantitative (ie, dimensional) approaches in machine learning is crucial for the development of ASD biomarkers, which should act as a quantitative measure of a biological mechanism rather than simply testing for the existence or absence of a pathological phenotype. Moreover, ASD is a complex disorder with many causes and comorbid conditions, and a large amount of variability in the type and severity of systems expressed by different individuals. Thus, ASD is unlikely to be linked to a single biomarker (eg, an individual gene or brain region). Instead, ASD biomarkers are probably complex and multivariate, incorporating data from different biological processes as well as different measures for similar aspects of neurobiology. However, although the efficiencies of different data types have been compared with each other (eg, imaging *vs* genetics),[143] no-one has yet managed to meaningfully combine data across disciplines to assess their predictive value for ASD.

Although pattern-classification approaches hold promise for various clinical applications in ASD, several crucial issues must be addressed before these methods can be used in clinical practice, and to justify the increased costs of clinical MRI relative to the behavioural diagnosis. Most importantly, the extent to which automated classifiers can be used to generalise to the real-world clinical setting, in which clinicians are confronted with many psychiatric populations in addition to individuals with ASD and typically developing controls, remains to be established. In the future, identification of the clinical validity of these models using testing data from independent samples (ie, independent of the training set) that were acquired in the real-world clinical setting will be important. Furthermore, establishment of their clinical specificity will be crucial—ie, although the established methods might be successful in distinguishing individuals with ASD from typically developing controls, they might not be able to distinguish ASD from the various related comorbid conditions (eg, attention deficit hyperactivity disorder [ADHD], obsessive–compulsive disorder, or emotional disorders).[144] Preliminary evidence suggests that the clinical specificity of a classifier increases with its overall accuracy. For instance, Ecker and colleagues[145] showed that a classifier that is highly accurate for ASD did not allocate individuals with ADHD to the diagnostic category for cases but to the category of typically developing controls, whereas a classifier that did no better than random for individuals with ASD also randomly allocated individuals with ADHD to the two available categories (ie, ASD and control groups). This finding suggests that a classifier with high accuracy for ASD is also expected to be highly specific for the disorder. Finally, several technical limitations will have to be overcome before making automated approaches

more widely available. For various reasons, including hardware-related effects such as radiofrequency coil sensitivity and electronic amplifier gains, conventional structural MRI scans are not compatible across sites. Hence, a classifier that is highly accurate for ASD at one scanning site might not necessarily be highly accurate for classifying ASD at another site. This issue is also a problem for functional neuroimaging data acquired at several sites or on different scanner platforms.[146,147] Thus, the standardisation of multivariate classification approaches across sites or platforms at the level of data acquisition or image analysis, or both, will substantially contribute to the generalisability and availability of MRI-assisted classifiers for ASD in the future.

Conclusions

Over the past two decades, neuroimaging studies have provided many important insights into the pathological changes that occur in the brain in ASD in vivo. Most importantly, they have shown that ASD is accompanied by an atypical trajectory of brain maturation, which gives rise to differences in neuroanatomy, functioning, and connectivity within the wider neural systems that probably mediate autistic symptoms and traits. However, the development of the brain in ASD is complex and is mediated by many genetic and environmental factors, and their interactions. Hence, in the future, establishment of the specific environmental risk factors that contribute to ASD susceptibility in addition to genetic variations will be crucial. Moreover, investigation of the neurobiological and clinical phenotype of ASD compared with various neurodevelopmental disorders will be important to delineate the shared versus distinct molecular mechanisms that might be used to identify novel treatment targets, or to develop stratification instruments that can define more biologically meaningful subgroups within clinically and biologically heterogeneous disorders. Although substantial progress has been made in the development of animal models and cellular assays, neuroimaging approaches remain one of

the few techniques that allow us to directly examine the brain in vivo, and will probably facilitate the development of a more personalised, individually tailored approach to the treatment of ASD.

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