

Neuroimaging in autism—from basic science to translational research

Christine Ecker and Declan Murphy

Abstract | Over the past decade, human neuroimaging studies have provided invaluable insights into the neural substrates that underlie autism spectrum disorder (ASD). Although observations from multiple neuroimaging approaches converge in suggesting that changes in brain structure, functioning and connectivity are associated with ASD, the neurobiology of this disorder is complex, and considerable aetiological and phenotypic heterogeneity exists among individuals on the autism spectrum. Characterization of the neurobiological alterations that underlie ASD and development of novel pharmacotherapies for ASD, therefore, requires multidisciplinary collaboration. Consequently, pressure is growing to combine neuroimaging data with information provided by other disciplines to translate research findings into clinically useful biomarkers. So far, however, neuroimaging studies in patients with ASD have mainly been conducted in isolation, and the low specificity of neuroimaging measures has hindered the development of biomarkers that could aid clinical trials and/or facilitate patient identification. Novel approaches to acquiring and analysing data on brain characteristics are currently being developed to overcome these inherent limitations, and to integrate neuroimaging into translational research. Here, we discuss promising new studies of cortical pathology in patients with ASD, and outline how the novel insights thereby obtained could inform diagnosis and treatment of ASD in the future.

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Introduction

The term autism spectrum disorder (ASD) encompasses a group of life-long neurodevelopmental conditions characterized by a triad of symptoms: impaired social communication, deficits in social reciprocity, and repetitive, stereotyped behaviour.¹ The aetiology and neurobiology of ASD are complex, resulting in considerable heterogeneity among affected individuals.² Currently, the diagnosis of ASD is made entirely on the basis of behaviour, leading to collections of behaviourally similar but biologically heterogeneous participants being included in (mostly unsuccessful) treatment trials. As a result, few effective treatments have been developed specifically for ASD, and use of medications developed for other conditions that have not been tested for efficacy in ASD is common practice. Hence, we need to develop tools that can help us diagnose and treat ASD at the earliest opportunity, and identify biomarkers that predict which therapies will be most effective.

Characterization of the underlying pathology of ASD is likely to require an integrative platform that enables researchers to combine findings across various scientific disciplines. For example, observations from human neuroimaging studies could be linked with findings obtained using experimental techniques (such as cellular assays, histology and animal models) that can characterize pathological processes at a high level of specificity

and resolution. So far, however, neuroimaging studies of brain structure and function in ASD have mostly been conducted in isolation, which has limited the translatability of their results. At the same time, neuroimaging remains one of the few techniques that can be used to investigate brain pathology in living humans and, therefore, offers a unique opportunity to provide information that could facilitate the diagnosis and treatment of ASD in the clinical setting. Consequently, pressure is now growing to develop neuroimaging into a translatable tool—that is, a tool that forms part of a chain of multidisciplinary inputs and outputs, provides results that can be translated into medical practice, and can be used as a measure of health outcomes. In the future, efforts to integrate neuroimaging into the translational research cycle (Figure 1), and to facilitate translation of findings from bench to bedside, will be crucial.

So far, development of translatable neuroimaging markers has been mainly hampered by the low specificity of the outcome measures that neuroimaging techniques generally provide. For example, neuroimaging studies in ASD and other neurodevelopmental conditions have mostly focused on global or large-scale structural brain abnormalities, such as measures of brain volume or white matter connectivity. Little is currently known about alterations in brain anatomy and function on the local, microscopic level. Although global descriptors of atypical brain anatomy provide important insights into the underlying neural substrates of ASD,

Sackler Institute for Translational Neurodevelopment, Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, King's College, P050, De Crespigny Park, London SE5 8AF, UK (C. Ecker, D. Murphy).

Correspondence to:
C. Ecker
christine.ecker@kcl.ac.uk

Competing interests

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Key points

- The focus of neuroimaging in mental health research is increasingly on translational approaches
- A multidisciplinary approach is required for clinical translation of neuroimaging findings in autism spectrum disorder (ASD)
- Improving the specificity of neuroimaging markers will substantially enhance the translational potential of this modality
- Novel neuroimaging markers that accurately reflect specific pathological processes in ASD are required to link with data from other scientific approaches, such as genetic or molecular studies
- Neuroimaging findings could provide biomarkers that facilitate diagnosis and prediction of response to treatment, and enable stratification of individuals with ASD

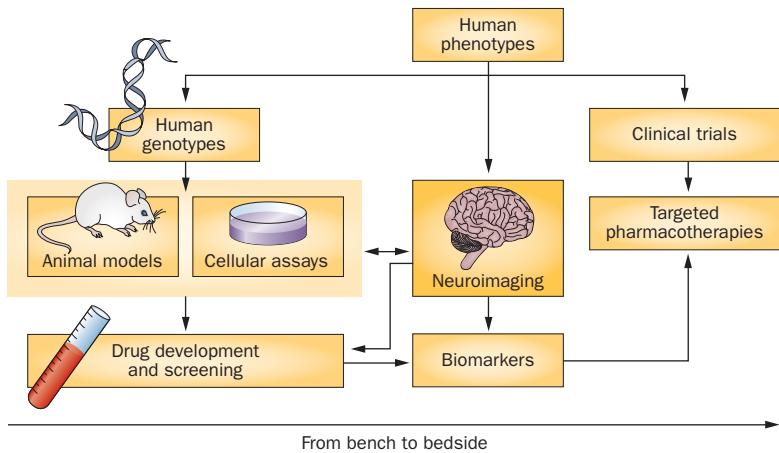


Figure 1 | The role of human neuroimaging in the translational research cycle of ASD. This cycle integrates phenotypic and genotypic characterization of individuals with ASD, as well as data from animal and *in vitro* models, to enable the translation of multidisciplinary research from bench to bedside. In the future, human neuroimaging studies will offer considerable translational research value with regard to elucidating the aetiology and neurobiology of ASD, by providing *in vivo* biomarkers that facilitate stratification of individuals for clinical trials, development and screening of novel pharmacological targets, and development and validation of animal and *in vitro* models. Abbreviation: ASD, autism spectrum disorder.

they are less useful for generating hypotheses. Novel and innovative imaging measures that enable conclusions beyond the native resolution of the acquired data to be drawn are currently being developed to investigate specific aspects of the neuropathology underlying ASD. These approaches are expected to generate hypotheses that can be meaningfully tested *in vitro* or in animal models.

This Review describes state-of-the-art neuroimaging approaches and their relevance to ASD, and highlights attempts that have been made to integrate neuroimaging into the translational research cycle. For example, we focus on advances in structural neuroimaging techniques to illustrate how such integration might be achieved, and how neuroimaging biomarkers could be meaningfully applied in the clinical setting.

Increasing neuroimaging specificity

The interpretation of neuroimaging findings is naturally constrained by their spatial and temporal resolution, which conventionally lies in the range of millimetres and milliseconds, respectively. This level

of resolution at which neuropathology in individuals with ASD can be examined greatly restricts the ability of neuroimaging to illuminate specific aspects of neuropathology. Furthermore, differences in the level of data generated by human neuroimaging, genetic studies and experimental *in vivo* and *in vitro* techniques (such as histology, cellular and molecular assays, animal models, electrophysiology and stem cell transplantation) have so far prevented meaningful integration and translation of research findings across disciplines. Establishing more-specific human neuroimaging markers will increase the potential for insights to be obtained into the neural underpinnings of ASD that can be subsequently applied in translational studies. This issue has been addressed in several structural neuroimaging studies over the past 2–3 years.

Previous neuroimaging investigations into the neurobiology of ASD mainly focused on three aspects of global cortical pathology—namely, atypical brain anatomy, connectivity and function. These aspects of ASD neuropathology do not develop in isolation, but interact with each other during development (and, subsequently, with environmental factors). This interaction gives rise to differences within large-scale neural systems that mediate diagnostically relevant autistic symptoms and traits—that is, behavioural and neuropsychological deficits—that are characteristic of ASD in the mature brain.³ The components of these neural systems are well documented, and primarily include regions that form part of the frontothalamic–striatal system, frontotemporal circuitry, and frontocerebellar network.⁴ Moreover, differences in the volumes of these brain regions are associated with the severity of particular domains of autistic symptoms; for example, differences in frontotemporal regions and amygdala have been associated with abnormalities in socioemotional processing,⁵ whereas volumetric differences in the frontostriatal system have been linked with repetitive and stereotypical behaviour.⁶ Most of these traditional investigations into atypical brain structure in ASD were based on volumetric analyses at the regional,⁷ lobular⁸ and whole-brain⁹ levels. These studies were important first steps towards defining the neuroanatomy of ASD. Traditional volumetric studies, however, are not well suited to elucidation of which particular aspects of the cortical neuroanatomy are implicated in ASD, and/or to guiding future aetiological investigations.

ASD comprises a group of conditions with a large degree of aetiological and phenotypic heterogeneity.² Perhaps we should not be surprised, therefore, that the diagnosis of ASD continues to be made on the basis of symptoms rather than aetiology, nor that individuals with suspected ASD are conventionally assessed via behavioural observations and/or clinical interviews. Although the behavioural diagnosis of ASD has clear advantages in the clinical setting, it is less beneficial for the development of new treatments and interventions. For example, clinical trial cohorts typically exhibit a high degree of clinical and/or phenotypic heterogeneity, and potentially include individuals belonging to different

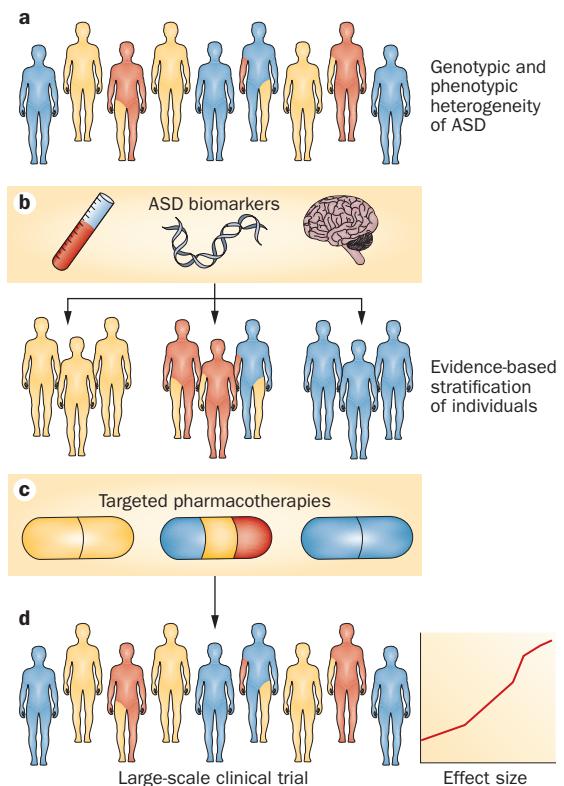


Figure 2 | Personalized diagnosis and treatment of ASD. **a** | Clinical trials using a ‘one size fits all’ approach to the treatment of ASD typically show small to moderate effect sizes, owing to considerable genetic and phenotypic heterogeneity of the condition. **b** | The development of reliable ASD biomarkers is, therefore, crucial for phenotypic stratification of individuals in clinical trials. **c** | Treatments and interventions could be tailored to the patient’s specific ASD phenotype. **d** | Stratification could increase the effect sizes of interventions in large-scale clinical trials. Abbreviation: ASD, autism spectrum disorder.

biological subgroups within ASD, who are unlikely to be treatable using a ‘one size fits all’ approach.¹⁰ Strong effects of a given treatment within a biologically homogeneous subgroup of patients with ASD might be masked by a small effect of the treatment across the whole cohort, which might partially explain why clinical trials in patients with ASD so far have shown small to moderate effects.¹¹ Neuroimaging techniques might enable stratification of patients into homogeneous subgroups of individuals who are more likely to respond to a given treatment (Figure 2). In addition, the development of novel imaging measures that can discriminate between individuals with and without ASD could contribute to the development of biomarkers for the condition, and facilitate their application in the clinical setting.

Novel measures of brain connectivity

ASD has been suggested to result from atypical brain connectivity.^{12,13} Evidence of altered brain connectivity in ASD has been obtained from many neuroimaging studies, which have so far mostly focused on between-group differences in cortical white matter that reflect extrinsic corticocortical connections. For example,

individuals with ASD have widespread reductions in the volume of white matter during childhood, adolescence and adulthood compared with age-matched controls, as measured using voxel-based morphometry.^{3,14} Atypical structural connectivity in patients with ASD has also been noted in numerous studies using diffusion tensor imaging (DTI). Interestingly, altered fibre tract connectivity is observed in limbic and language pathways, frontostriatal circuitry and the corpus callosum, and these changes are likely to mediate autistic symptoms and traits.^{15–17} These reports were important first steps towards characterizing large-scale alterations in brain connectivity in ASD, and most studies agree with the general notion that global hypoconnectivity of the brain is present in ASD, which has also been confirmed using functional connectivity MRI.^{18,19}

Results of genetic studies (discussed in depth below), imply that the atypical brain connectivity in ASD may not be restricted to white matter, but could also affect direct neuronal connections within cortical grey matter. Although neuroimaging measures of cortical white matter connectivity are well established, neuroimaging of grey matter (that is, intrinsic) brain connectivity is inherently difficult. Intrinsic corticocortical connections are well described in histological studies, and generally refer to connections formed by axon collaterals that are confined to the cortical grey matter, and run parallel to the cortical surface.^{20,21} Intrinsic connections are, therefore, not explicitly quantifiable by conventional measures of brain volume or cortical thickness, which largely reflect the vertical architecture of the cortex.

A novel framework has been developed for estimating grey matter connectivity in humans. Using the framework, researchers examined differences in local and global intrinsic wiring costs of the brain (that is, the minimum length of horizontal connections required to link brain regions within the cortical sheet) in individuals with ASD and control participants.²² Wiring costs *per se* do not represent the actual length of axonal connections—which are not directly accessible by MRI in humans—but instead are estimated using so-called geodesic distances,²³ a measure that represents the shortest possible path linking two points along the cortical surface. Theoretically, shorter geodesic distances are associated with lower wiring costs, which in turn indicate the intrinsic wiring potential of a brain region: reduced wiring costs can facilitate formation of intrinsic corticocortical circuits. Our research group showed that the brain’s intrinsic connectivity in individuals with ASD significantly differs from that in controls, and that intrinsic wiring costs were significantly reduced in ASD, predominantly in frontotemporal regions.²³ Furthermore, the decrease in wiring costs correlated with the severity of autistic symptoms, particularly the tendency to engage in repetitive behaviour. Taken together, these findings suggest that abnormal brain connectivity in patients with ASD is not restricted to white matter connections, but might also affect the intrinsic neural architecture and connectivity within the cortical grey matter, and could influence specific autistic symptoms.²²

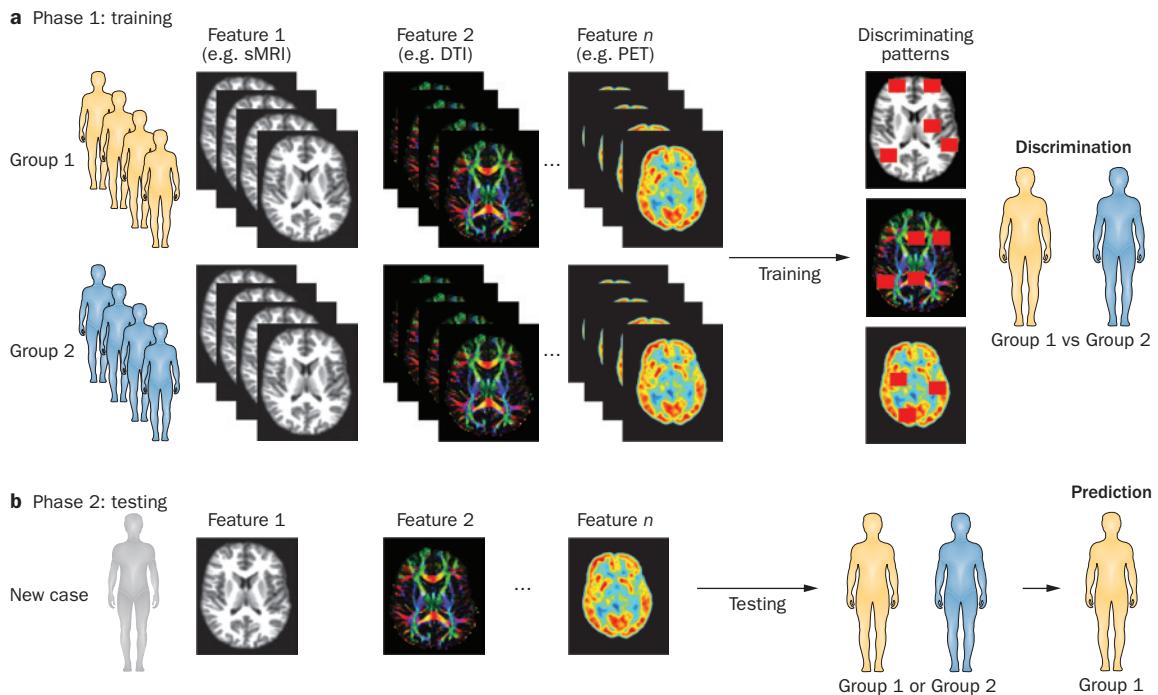


Figure 3 | Multivariate pattern classification can discriminate between multiple subgroups of ASD on the basis of neuroimaging data. **a** | Pattern classification models are initially trained (Phase 1) on well-characterized data obtained by structural MRI, DTI and/or PET, to derive potentially discriminative patterns of features. **b** | These patterns can then be used to determine whether patients in the validation cohort should be assigned to the ASD or control groups (Phase 2). The predictive value of pattern classification techniques makes this approach particularly suited for the development of MRI-based biomarkers that can be used for phenotypic stratification of patients with ASD. Abbreviations: ASD, autism spectrum disorder; sMRI, structural MRI; DTI, diffusion tensor imaging.

Machine learning in investigative settings

Traditional neuroimaging techniques were mainly designed to reveal brain pathology by testing for differences in average parameter values between two (or more) groups of participants, such as patients versus controls, providing little information about the existence of neuropathology in specific individuals. Contemporary techniques based on both neuroimaging data and other biological information now make it possible to distinguish between groups of individuals in an automated fashion, and can also be used to identify certain aspects of pathology in specific patients (Figure 3). These so-called multivariate pattern classification (MVPC) or machine-learning approaches²⁴ are, therefore, particularly suited for the phenotypic stratification of individuals with ASD, and are increasingly being tested and used in the clinical research setting. Although the total number of biologically defined ASD subgroups is currently unknown, research into the neurobiological background of ASD has highlighted several potential anatomical, functional, connectivity and neurochemical markers that could provide useful information for the phenotypic stratification of individuals on the autism spectrum.

With respect to neuroanatomical and functional markers, the results of imaging studies suggest that the total brain volume in patients with ASD is enlarged during early childhood (2–5 years of age) compared with age-matched individuals, affecting both grey and white

matter.^{9,25,26} Grey matter enlargement is most prominent in frontal and temporal cortices,²⁷ and seems to be driven by an increase in cortical surface area rather than cortical thickness.²⁶ No significant enlargement of the brain (versus healthy controls) is typically observed during subsequent childhood²⁸ and adulthood³ of these individuals, suggesting that ASD is accompanied by abnormal early brain development or maturation. The onset of such abnormal brain growth in ASD is currently being explored in several longitudinal neuroimaging studies examining infants <1 year of age (before the threshold of 2 years, after which making a reliable behavioural diagnosis is thought to be possible) who are considered to be at high risk of developing ASD (such as siblings of individuals with ASD). Researchers involved in one longitudinal study noted a significantly increased rate of brain growth during early childhood (2–5 years) in children diagnosed as having ASD,²⁵ whereas another group did not find increased brain growth rate during that period, and instead argued that the pathological perturbation must occur before the age of 2 years.²⁶

In addition to the above conflicting results, a further caveat is that although early brain overgrowth is observed in a large percentage (estimated to be as high as 90%²⁹) of children with ASD, it does not affect all individuals with the condition. These studies reveal considerable phenotypic variation among individuals with ASD, which is apparent even when examining large-scale measures of cortical pathology, such as total brain volume. Along

the same lines, several studies investigating head circumference in children with ASD report that macrocephaly (defined as a head circumference above the 97th percentile) affects only about 20% of all children with ASD.³⁰ Interindividual differences in total brain volume could provide valuable information not only for distinguishing individuals with ASD from controls, but also for stratifying individuals with ASD into biologically homogeneous subgroups.

Given the phenotypic complexity of ASD, we might reasonably expect that complex, multivariate approaches would provide higher discriminative power than single brain measures in ASD. For example, MVPC can distinguish between individuals with and without ASD (including those with other neurodevelopmental disorders) on the basis of spatially distributed patterns of differences in the cortical grey matter and white matter, and/or other brain morphological features.^{31–34} Measures of brain function and connectivity might also provide useful diagnostic information in ASD.^{35–39} In the future, MVPC could be used to inform and facilitate the conventional diagnosis of ASD, and might also be valuable in the stratification of patients for clinical trials.

MVPC also holds promise for establishing biomarkers that could be used for early detection and intervention in ASD. For example, an increasing number of neuroimaging studies are investigating brain development in infants (6–24 months) who are at high risk of developing ASD. The results of these studies suggest that differences in brain anatomy and connectivity associated with ASD can be observed in infants as young as 6 months of age.^{25,26} Furthermore, one study has reported evidence of atypical developmental patterns of brain chemistry in children as young as 3–4 years of age, with reductions in concentrations of N-acetylaspartate, choline and creatine, which were not observed in older age groups (9–10 years of age).⁴⁰ As such, neuroanatomical and neurochemical biomarkers, such as differences in certain neurotransmitter concentration might provide useful information for early detection of ASD (before a reliable clinical diagnosis can be obtained), and enable early treatment and intervention.

Although MVPC seems promising, application of this technique in routine clinical practice remains a vision for the future. Several crucial issues need to be addressed—most importantly, the clinical specificity of automated, biologically driven approaches has to be established. For example, although MVPC might be successful at distinguishing individuals with ASD from healthy controls in the research setting, how well MRI-based classification models will function in heterogeneous, real-world populations of individuals on the autism spectrum, and whether these models are capable of distinguishing ASD from several associated comorbid conditions (such as social anxiety disorders and attention deficit hyperactivity disorders) remains to be determined.

Another crucial issue is lack of rigorous testing of the assumption that the healthy control group used for training the model is negative for ASD. Gold standard diagnostic tools such as the Autism Diagnostic Observation

Schedule and Autism Diagnostic Interview-Revised are generally employed to characterize patients but not controls. Furthermore, autism biomarkers need to not only be able to deal with the clinical heterogeneity of ASD, but also take into account neurodevelopmental aspects of ASD that vary over time. Thus, large longitudinal studies are essential to provide the data necessary to train a classification model that can deal with the complexity as well as the trajectory of ASD,⁴¹ and to establish its specificity in real-world clinical settings. Finally, all existing classification algorithms are highly specific to the particular sample of patients used for training the model (such as male, right-handed, high-functioning individuals with ASD). Although use of a training cohort with similar characteristics assures optimal specificity with regard to that particular subgroup of individuals with ASD, the performance of the resulting model will be suboptimal in other subgroups on the autism spectrum.

Disentangling components of cortical volume

Measures of cortical volume are, by definition, a product of two separate neuroanatomical features: cortical thickness and surface area. Volumetric measures, therefore, do not represent a single aspect of the neural architecture, but rather can be underpinned by separable variations in cortical thickness and surface area, which in turn might have distinct genetic determinants, phylogeny and developmental trajectories.

So far, studies have mainly investigated either cortical volume or cortical thickness in isolation, and measures of surface area remain relatively unexplored. Several neuroimaging studies report significant increases in cortical thickness, particularly in frontotemporal regions, in children with ASD compared with age-matched controls.^{42,43} Furthermore, studies in adults with ASD typically show cortical thickening of the frontal cortex,^{44,45} whereas cortical thickness of the temporal lobe can be either increased or decreased in patients with ASD relative to controls.⁴⁶ Whether differences in cortical volume in ASD are predominantly driven by differences in cortical thickness, surface area, or a combination of both, is currently unknown. An increasing number of research groups are attempting to disentangle the respective influences of the distinct neuroanatomical features on differences in cortical volume to elucidate the cortical pathology associated with ASD. For example, the well-documented (and potentially accelerated) increase in total brain volume that occurs during early childhood (2–5 years of age)^{29,47} seems to be driven by a precocious and potentially nonuniform expansion of the cortical surface, rather than an increase in cortical thickness.²⁶ This finding is also supported by the results demonstrating that variations in cortical volume in adults with ASD are primarily driven by differences in surface area rather than cortical thickness.⁴⁸ These reports are potentially of great importance, as they shed light on the relative contributions of specific aspects of pathology to ASD—and hence narrow down the search for genetic and environmental factors that are the most important contributors to the risk of developing ASD.

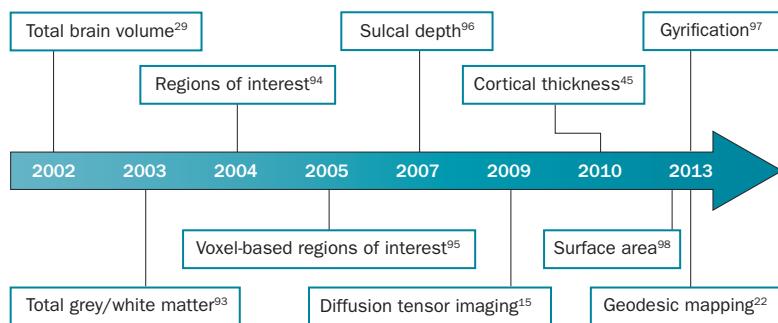


Figure 4 | The translatability of neuroimaging is directly related to its resolution. So far, the low specificity of neuroimaging techniques has hampered the development of translatable neuroimaging markers that could be meaningfully correlated with findings from animal models, and genetic and *in vitro* studies. Novel approaches to acquisition and analysis of neuroimaging data are being developed, which will increase the specificity of imaging markers and facilitate meaningful translation of neuroimaging measures across disciplines. This figure shows the progression of imaging approaches over time.

In terms of phylogeny, neurologists now widely believe that cortical thickness and surface area are determined by different types of progenitor cells, which divide in the ventricular zone to produce glial cells and neurons. Cortical thickness has been related primarily to intermediate progenitor cells (neurogenic transient amplifying cells in the developing cerebral cortex),⁴⁹ which divide symmetrically at basal (non-surface) positions of the ventricular surface. These progenitor cells only produce neurons,^{50,51} which then migrate along radial glial fibres to form ontogenetic columns arranged as radial units. According to the radial unit hypothesis,⁵² cortical thickness depends on the neuronal output from each radial unit amplified by intermediate progenitor cells and, therefore, reflects the number of neurons produced in each unit. By contrast, cortical surface area has mainly been related to 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.⁴⁹ The existence of distinct phylogenetic processes that drive cortical thickness and surface area also implies that at least two different genetic (or other) mechanisms might be involved in their aetiology and regulation.

Testing hypotheses in animal models

Genetic factors underpinning the dissociation of cortical thickness and surface area have been explored in both animal and human studies. In mice, mutations in the genes *Pax6*, *Lrp6*, *Neurog1* and *Neurog2* modify the abundance of intermediate progenitor cells and result in parallel increases in cortical thickness but not surface area.⁵³ In humans, mutations in *PAX6* and *EOMES* are associated with a reduction in cortical thickness relative to surface area. Moreover, the surface area but not the thickness of specific cortical regions, such as the cuneus and fusiform gyrus, is modulated by variations in *MECP2* that are associated with Rett syndrome.⁵³ Thus,

the genetic and molecular pathways that drive precocious and accelerated expansion of the cortical surface seem to contribute to the neuropathology of ASD, whereas the mechanisms underlying thickening or thinning of cortical sheet might be less important.

The highly specific nature of these results, in terms of focusing on a particular aspect of ASD neuropathology, substantially enhances their translatability across disciplines by providing novel and precise hypotheses that can be explored and validated in animal and/or *in vitro* models. For example, several animal models are currently available to study the effects of autism susceptibility genes, which enable detailed characterization of the molecular pathways underlying ASD.⁵⁴ An important next step will be to develop human neuroimaging markers that provide specific predictions, which could be used to validate animal models of ASD and test hypotheses. Increasing the specificity of neuroimaging measures for ASD (Figure 4) will also enhance the translational research potential of neuroimaging techniques in general, and facilitate their integration into the translational research cycle.

Combining imaging and genetic data

Traditionally, ASD has been considered to be a highly heritable neurodevelopmental disorder,⁵⁵ with more recent heritability estimates ranging from 60%⁵⁶ to 80%,⁵⁷ but the number of common genetic variants underlying ASD seems surprisingly small. An increasing number of genetic investigations are focusing on the importance of distinct, individually rare genetic variants in the aetiology of ASD. For example, copy number variations (CNVs), meaning large-scale genomic deletions and duplications, occur in 5–10% of patients with ASD, and can either be inherited or occur *de novo*.² Several groups are now investigating the effects of genetic variants associated with ASD using animal models and human neuroimaging in order to link CNV to specific aspects of brain pathology. These findings constitute a conceptual shift from a ‘common disease, common variant’ model to a ‘common disease, multiple rare variants’ model, in which ASD can be caused by a wide range of causative genetic variants, each of which is individually rare (found in only a few people).⁵⁸

The genetic background of ASD is complex: more than 100 disease genes and genomic loci are potentially implicated in this disorder.⁵⁹ Autism is also frequently observed in a number of autism-related monogenic syndromes (syndromic autism), which share certain genetic variants with individuals with idiopathic, nonsyndromic autism. These disorders include Fragile X syndrome and Rett syndrome,² which both display specific defects in synaptic plasticity⁶⁰ that might be shared between syndromic and nonsyndromic autism.⁶¹ Furthermore, many of the genetic variants that underlie idiopathic ASD occur in genes primarily associated with synaptic development, axon targeting and neuron motility, and are thus functionally related.^{58,62} For example, many rare CNVs associated with ASD also have a major role in cell adhesion molecule (CAM) pathways.^{2,58} CAMs are

involved in the formation and maintenance of synaptic contacts, and include molecules responsible for initiating contact between presynaptic and postsynaptic cells, maintaining synaptic adhesion, and providing ‘anchors’ for scaffolding proteins that assemble signalling molecules, neurotransmitter receptors, and proteins in the cytoskeleton.⁶³ During neuronal development, CAMs also guide the growth cone at the tip of the developing axon and, thereby, support development of neuronal networks even before synapse formation.⁶⁴ ASD-linked variations in genes encoding CAMs suggest that synaptic development and plasticity might be perturbed in ASD, which could affect the way that neurons and brain areas are connected.⁵⁸ Research efforts are currently directed towards establishing the molecular pathways affected by these genetic variants in animal models of ASD, and combining these findings with *in vivo* neuroimaging research.

Some of the most consistently replicated CNVs associated with ASD occur in genes encoding synaptic molecules, such as the neuroligins (NLGNs)—a family of CAMs involved in the formation and consolidation of inhibitory and excitatory synaptic contacts in a subtype-specific manner.⁶³ Notably, the ASD-linked neuroligin 3 (encoded by *NLGN3*) seems to primarily promote excitatory synaptogenesis,^{65,66} whereas neuroligin 2 (encoded by *NLGN2*) preferentially induces the formation of inhibitory contacts.^{67,68} *Nlgn3*-knockout mice exhibit disrupted heterosynaptic competition and perturbed metabotropic glutamate receptor-dependent synaptic plasticity,⁶¹ which implicates the glutamatergic system in the pathogenesis of ASD and supports the suggestion that ASD is associated with an altered excitation–inhibition (E–I) balance, favouring increased excitation.⁶⁹ The hypothesis of a perturbed E–I balance in ASD is also supported by several PET studies that have provided converging evidence for atypical inhibitory synaptic transmission in ASD, suggesting involvement of γ-aminobutyric acid (GABA) in the pathophysiology of ASD (discussed below).^{70,71}

Besides neuroligins, the neurexins might contribute to an altered E–I balance and affect the development of brain connectivity in ASD. For example, variants of contactin-associated protein 1 (encoded by *CNTNAP1*, also known as *NRXN4*) are associated with ASD. *CNTNAP2* encodes contactin-associated protein-like 2, another member of the neurexin family. *CNTNAP2* protein is localized at the nodes of Ranvier, where it is involved in clustering K⁺ channels within differentiating axons⁷² and mediates interactions between neurons and glia during neurodevelopment.⁷³ Moreover, mice with deletion of *CNTNAP2* show reduced numbers of cortical GABAergic neurons and abnormal neuronal migration, as well as deficits in the three core behavioural domains of ASD.⁷⁴ Finally, *CNTNAP2* variants are also closely linked with epileptic seizures, which have been reported to be more prevalent among individuals with ASD compared with controls.⁷⁵ For example, genetic syndromes known to be associated with a deletion of *CNTNAP2* are accompanied by severe and frequent seizures, and

include cortical dysplasia–focal epilepsy syndrome⁷⁶ and Piff–Hopkins-like syndrome 1.⁷⁷

The above findings suggest that although genetic variation in *CNTNAP2* may contribute to the risk of developing ASD, such variants seem to be neither specific nor causal for developing ASD, and may also be observed in individuals without a clinical diagnosis of ASD (that is, healthy carriers). To investigate how a particular genotype affects brain structure and function, it is important, therefore, to examine genotypic and phenotypic interactions across multiple conditions with a common genetic architecture, in addition to studying healthy carriers of a risk gene. For example, although no neuroimaging studies have investigated brain anatomy and connectivity in individuals with ASD who have known variations in *CNTNAP2*, several reports have focused on healthy carriers of a common variant of *CNTNAP2* that is linked to ASD. Healthy carriers of the *CNTNAP2* ASD risk allele show altered structural and functional brain connectivity,^{78,79} which is a general hallmark of ASD. Similar studies linking genetic and brain imaging findings will be of high importance in the future, because they could enable specific aspects of brain characteristics to be linked to specific genetic variations that increase susceptibility to ASD.

A number of studies have examined the effects of variants in *SHANK3* that are associated with ASD as well as schizophrenia.⁸⁰ *SHANK3* encodes SH3 and multiple ankyrin repeat domains 3, which is primarily located in the postsynaptic density and functions as a postsynaptic scaffold protein that connects receptors, CAMs and signalling molecules required for synaptogenesis and synaptic transmission.^{81,82} In human neuronal cell cultures, overexpression of *SHANK3* protein significantly increases the number of dendritic spines, affects their morphology (in terms of length and size), and reduces synaptic transmission by modulating the frequency of miniature excitatory synaptic currents in mature neurons.⁸³ These findings complement the results of studies in *SHANK3*-knockout mice, which exhibit increased complexity of dendritic arborization, volumetrically enlarged striata, and defective corticostratal circuits.⁸⁴ Atypical corticostratal circuitry and enlarged striata have also been reported in imaging studies of children and adults with ASD,^{6,37} which suggests a link between variants of *SHANK3* and ASD-related pathology in corticostratal systems.

These studies demonstrate how genotypic information can be combined with data from imaging studies, in both individuals with ASD and healthy carriers of specific autism risk alleles, in order to link various aspects of brain pathology to individual CNVs associated with ASD. The development of human neuroimaging markers that are motivated by genetic investigations and enable novel hypotheses to be addressed will be crucial to further disentangle the complex neurobiology of ASD. Findings from genetic studies need to be translated into usable neuroimaging markers that can subsequently be used to link specific genetic variants to particular aspects of pathology. In the future, such approaches will be an

important component of efforts to translate insights from genetic investigations into novel neuroimaging markers to further elucidate the complex association between ASD genotypes and phenotypes.

Markers for drug development

Stratification of individuals with ASD on the basis of their individual neurochemical make-up is important for the development of new pharmacotherapies. Current evidence suggests that three neurotransmitter systems are particularly suited for defining subgroups of individuals with ASD: the 5-hydroxytryptaminergic (5-HT), GABAergic and glutamatergic systems. These systems can be assessed *in vivo* using state-of-the-art magnetic resonance spectroscopy or PET. For example, about 30% of individuals with ASD are thought to have hyperserotonemia,^{85,86} (that is, increased levels of 5-HT in whole blood, relative to those of controls),⁸⁷ reduced 5-HT_{2A} receptor binding⁸⁸ and decreased numbers of 5-HT transporter molecules.⁸⁹ Advanced neuroimaging techniques might, therefore, be used in the future for selection individuals with ASD who would benefit the most from pharmacological manipulation of the serotonergic system. Similar techniques could be used to identify potential interindividual variations in GABAergic and glutamatergic systems within the ASD population.

As mentioned above, disturbance of the E-I balance in the brain, favouring increased excitation, could be an important aspect of the pathophysiology of ASD.⁶⁹ This notion is supported by some preliminary human neuroimaging data suggesting that some individuals with ASD have upregulation of the glutamatergic system, such as higher neurotransmitter density,⁷⁰ leading to increased excitation (termed the hyperglutamatergic hypothesis of autism⁹⁰). They also show downregulation of the GABAergic system, such as reduced expression of GABA receptors, in brain regions associated with ASD.^{71,91} By contrast, other individuals with ASD (and/or different brain regions in the same individual) can show the opposite profile.⁹² As such, an imbalance in glutamatergic and GABAergic regulation could be present in people with ASD, but its aetiology—and hence treatment—might differ between individuals. The extent to which individuals with ASD differ from neurotypical controls with regard to an E-I imbalance is currently unknown,

but these early studies highlight the potential translational value of neurochemical markers assessed by various imaging techniques in the development of targeted treatments and interventions, and in designing an individually tailored treatment strategy.

Conclusions

Over the past two decades, neuroimaging approaches have had a crucial role in identifying the large-scale neural substrates and transmitter systems that underlie autistic symptoms and traits. The role of neuroimaging studies in mental health research, however, is currently transitioning from a basic scientific tool to an integral part of the translational research cycle. So far, the development of translatable biomarkers for ASD has been hampered by the low specificity and resolution of neuroimaging techniques. A strong need remains, therefore, for novel proxy measures for assessing specific aspects of the neuropathology underlying ASD, not only to elucidate the neurobiological background of the disorder, but also to generate hypotheses that can be meaningfully tested in *in vitro* and *in vivo* models. Such novel markers might be based on genetic insights into the aetiology of ASD, making it possible to combine findings across disciplines. Last, novel analytical techniques could be used to facilitate the translation of neuroimaging findings from bench to bedside, which is particularly important for the stratification of patients for clinical trials, and the development of individually tailored treatment strategies. Taken together, these efforts are important first steps towards a personalized approach to the diagnosis and treatment of ASD in the future, and highlight the need to employ an integrative approach to the study of ASD.

Review criteria

A search for original articles published between 1960 and 2013 and focusing on autism was performed in MEDLINE and PubMed. The search terms used were “autism”, “genetics”, “neuroimaging”, “brain functioning”, “connectivity”, “biomarkers” and “brain anatomy”, alone and in combination. All articles included were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

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Author contributions

Both authors contributed to researching data for the article, discussion of the article content, writing of the article and to review and/or editing of the manuscript before submission.