by sorting through the X-ray photons in the deepest images taken by the Chandra X-ray Observatory.

X-rays are the ubiquitous observational signature of gas falling into black holes. X-ray emission more easily penetrates dense gas and dust clouds than does optical or ultraviolet (UV) light, and so it is considered to be one of the cleanest methods of finding active black holes. Chandra's image of the 'Deep Field South' patch (Fig. 1) indeed contains hundreds of active black holes, mostly in quasars at $z = 1–3$ (ref. 6). However, no black holes at z greater than 6 have hitherto been found in X-ray observations. The Chandra images are too small to contain rare 'monsters' such as the Sloan quasars, and are not sensitive enough to detect more typical supermassive black holes at these high redshifts. However, we know from Hubble observations of the same field that, in the area between detected Chandra sources, there are hundreds of galaxies at z higher than 6.

In their study, Treister *et al.*[5] cross-correlated the Chandra X-ray photons with the locations of the Hubble galaxies and found a positive signal. The observed X-ray signal is very low — less than five X-ray photons per galaxy — and is detectable only because it was averaged over almost 200 high-z galaxies, yielding an effective exposure time of 23 years. But the statistical significance of this detection is high (nearly 7 sigma, to use the technical jargon), so we have a confident detection of X-ray emission from a population of typical supermassive black holes hosted by high-z galaxies.

A far-reaching finding of this analysis[5] is the X-ray 'colours' of these high-z galaxies. The observed X-ray emission spectrum peaks at energies greater than 3 kiloelectronvolts (keV). Because the Universe's expansion stretches an object's light waves by a factor of $1 + z$, this corresponds to a spectral peak at energies greater than 20 keV in the galaxies' reference frame. This observation strongly indicates that the central black holes in nearly all high-z galaxies within the Chandra field are blanketed in large amounts of cold gas that absorbs softer (lower-energy) X-rays. This same gas would completely absorb the optical and UV emission, making these black holes detectable only in the X-ray band.

The authors estimate the total mass of the newly discovered black-hole population using the notion[7] that about 10% (within a factor of a few) of rest energy of infalling matter is radiated away, and by further assuming that the black holes have been accreting at the same rate for a substantial fraction of the age of the Universe at their redshift. A limiting factor in such an estimate is that only a small fraction of the black-hole luminosity is in the hard (high-energy) X-ray regime directly observed by Chandra. Another caveat is that more than 95% of the black holes' luminosity, emitted mostly in the UV band, never reaches outside the galaxies because of absorption.

The reader might therefore conclude that the authors' estimate of the total mass of the high-z black-hole population, which is based solely on Chandra's X-ray emission, is highly uncertain, even though such a high uncertainty is usually unavoidable for ground-breaking astronomical observations. With these reservations in mind, it is still intriguing that Treister *et al.* conclude that the estimated black-hole masses are consistent with a hypothesis in which the relationship between galaxy mass and black-hole mass that is observed in the local Universe[8,9] is already established a billion years after the Big Bang.

Treister and colleagues' results[5] have implications for many studies of the early Universe. Unfortunately, however, answers to some key questions — such as how the progenitors of these early supermassive black holes were generated, or the exact mechanism that underlies the coevolution of the black holes and their host galaxies — will probably have to wait for the next generation of telescopes. These telescopes should be capable of detecting the objects individually in the X-ray band and of observing their absorbed and re-radiated emission in the sub-millimetre and far-infrared regimes. But Treister *et al.* have taken the first, most difficult, step: the detection of a typical population of supermassive black holes near the end of the cosmic dark ages. ■

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GENE EXPRESSION

The autism disconnect

Separating primary from secondary changes in the autistic brain has long been a research goal. With knowledge of wide-ranging molecular deficits, identification of the best therapeutic targets becomes a priority. SEE LETTER P.380

ŽELJKA KORADE & KÁROLY MIRNICS

Autism spectrum disorder is a complex neurodevelopmental condition. It is characterized by altered social interactions, communication difficulties and repetitive patterns of behaviour. There is no known single cause of autism, but it is believed that genetic predisposition together with environmental influences lead to molecular changes in brain cells, altering normal brain development[1]. On page 380 of this issue, Voineagu *et al.*[2] present the first appropriately powered and comprehensive gene-expression analysis of autistic brains using cutting-edge technologies and excellent data-mining approaches.

The authors measured messenger RNA levels for more than 30,000 genes in three regions of post-mortem brains from 19 patients with autism and 17 controls. They identified 444 genes that were differentially expressed between the cerebral cortices of the autistic and control brains. They then replicated most of the data with a second, independent cohort of post-mortem brains.

Brain regions differ in cellular composition, connectivity and molecular signatures, which, together, lead to functional specialization. In humans, for example, the prefrontal cortex is primarily responsible for higher cognitive processes such as working memory, whereas the temporal cortex is crucial for auditory perception and semantics. Voineagu *et al.* report that the differential patterns of gene expression that normally distinguish the frontal and temporal cortices are significantly attenuated in the autistic brain. This disappearance of differential gene expression — which may also occur in other regions of the autistic brain — is very intriguing. Loss of cortical patterning may impair connectivity between the brain regions and, ultimately, weaken the specialized functions of the cortical areas.

To investigate the functional relationship between gene-expression changes, the authors[2] used two distinct data-mining methods. One method groups genes together on the basis of their known cellular, molecular or functional characteristics[3]; the other builds functional gene modules according to the observed co-expression relationships[4]. The authors identified two distinct gene-expression modules associated with autism that might act in concert to disrupt typical brain development. One of the modules, which mediates synaptic communication between neurons, showed

decreased expression in the autistic cortices. The other was related to immune-system activation in the brain and showed increased expression. These observations are consistent with previous findings[5,6] that the brains of patients with autism show altered expression of genes relating to normal synapse development and increased expression of genes mediating inflammation.

The central gene in the synaptic module was *A2BP1*, which functions in a pathway that has been implicated[7] in autism. The A2BP1 protein controls what portions of a gene are included in the mature, functional mRNA during the process of alternative splicing. Through genome-wide assessment of autistic brains with decreased *A2BP1* expression, Voineagu *et al.* found *A2BP1*-dependent deficits in the RNA-splicing assembly of many genes. So it is possible for reduced expression of a single gene to affect a huge number of other genes that are responsible for normal brain development. Because diminished patterning of the cortex might arise from altered brain development, one could hypothesize that a general developmental dysregulation of transcript splicing leads to a more uniform development of various cortical areas, preventing proper functional specialization of these brain regions. This is an interesting and little-studied concept.

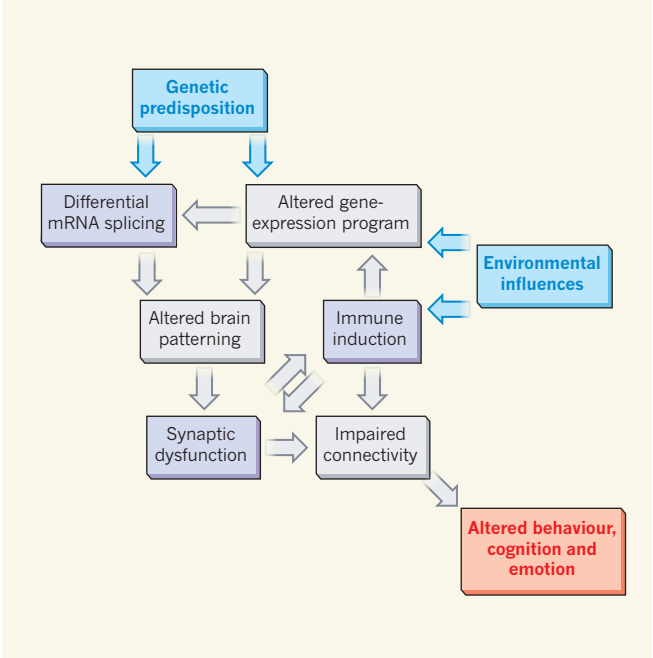

Figure 1 | The complexity of autism. In autism, genetic predisposition and environmental influences disrupt typical gene-expression patterns; this, in turn, alters brain development. Voineagu and colleagues[2] suggest that whereas alterations in messenger RNA splicing and synaptic disturbances are primarily controlled by genetics, immune responses in the autistic brain are either adaptive or environmental. Together, these deficits cause impaired connectivity — and, ultimately, altered behaviour, cognition and emotion.

Are the gene-expression differences reported here primary (genetic) or environmentally induced? In search of an answer, the researchers[2] analysed DNA data from a published genome-wide association study of autism[8] to test whether expression changes in the genes of synaptic and immune modules were due to specific sequences in the patients with autism. Compared with controls, genes that were involved in synaptic function (and showed altered RNA expression in post-mortem samples) were also strongly associated with a genetic predisposition to autism, suggesting that these differences are probably strong contributors to the development of this disorder.

But perhaps the authors' most intriguing finding concerns the immune module. Immune dysfunction has been suggested[6] to occur in autism, although not by an unbiased genome-wide assessment. Voineagu *et al.* now show that — unlike expression changes in synapse-related genes, which seem to be due to genetic predisposition to autism — the immune response of the autistic brain is probably a non-genetic, adaptive or environmental process.

These data should, however, be interpreted cautiously. And they do not diminish the importance of immune-system activation in autism as a therapeutic target. Classification of primary and secondary changes in complex brain disorders is somewhat artificial, and one cannot be certain which set of changes mainly contributes to the symptoms. But this is not necessarily a problem. After all, some of the most common therapies — those that treat fever in influenza, cough in upper respiratory infection or pain in arthritis — target disease symptoms (and not causes), providing relief for the patients. Besides, owing to high genetic predisposition and heterogeneity among patients, causal treatments for autism might not be possible in the foreseeable future, whereas therapies targeting the common, symptom-related molecular pathways might be within reach.

But how can we establish which gene-expression alterations are most critical and how they relate to the symptoms of autism? Is a defect in alternative splicing more important than disturbances in synaptogenesis or immune-system induction? Can we even assess them independently, or are the various impairments intertwined? Unlike our notable ability to uncover autism-related molecular/genetic changes, our ability to make clinical sense of the observed and validated changes is limited. Linking molecular abnormalities to disease symptoms and manifestations is particularly challenging in the absence of true animal models — as is the case for autism.

One of the strengths of Voineagu and colleagues' study[2] is that it provides a framework for a testable model (Fig. 1). The main priority is to determine the extent and origin of the differential splicing in the autistic brain, and its effect on the development of various brain regions. We must also establish whether splicing dysregulation is limited to A2BP1-targeted genes alone or is more widespread. The causality of the various changes is another fascinating issue: do the genetics-driven, converging synaptic alterations activate a detrimental immune response, or does the immune response have a more pronounced and potentiated effect when the synaptic genes show genetic vulnerability? As the authors suggest, how the changes they report relate to other neurodevelopmental disorders such as schizophrenia and attention deficit hyperactivity disorder should also be tested, given the emerging evidence[9] of genetic overlap.

Finally, the molecular changes ought to be correlated with the core features of the disease. The available data suggest that autism-related genetic and molecular changes are not present in all patients. However, there has not been a combined molecular–behavioural classification of autism spectrum disorder into subgroups — such as 'splicing' autism, 'synaptic' autism and 'inflammatory' autism. Should such a molecular classification become achievable (and linkable to specific symptoms of the disorder), it would revolutionize autism research, and open the door to developing more targeted and individualized therapies. ■

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