Nature Reviews Neuroscience | Published online 5 Jan 2017; doi:10.1038/nrn.2016.184

> Many of the differential splicing events in ASD cortex are regulated by neuron-specific splicing factors

Many genetic factors are thought to contribute to autism spectrum disorder (ASD); however, whether the effects of these factors converge on common functional pathways is not clear. Now, Geschwind and colleagues use RNA sequencing (RNA-seq) of human brain samples to characterize differences in the transcriptomes of the cortex and cerebellum between individuals with ASD and typically developing controls, and to investigate the phenotypic relevance of these differences.

The authors carried out RNA-seq on post-mortem samples of frontal cortex, temporal cortex and cerebellum from 48 individuals with idiopathic ASD and 49 control individuals. They found 584 and 558 genes that were differentially upregulated and downregulated, respectively, in cortical samples from people with ASD compared with controls. Notably, cell-type analysis revealed that the upregulated genes were enriched for those expressed by microglia and astrocytes, whereas the downregulated set was enriched for neuron-specific genes. By contrast, differences in gene expression between ASD and control cerebellar samples were much less pronounced, suggesting that the cortex is more vulnerable to genetic perturbations in ASD. To prioritize and organize the data, the researchers conducted weighted co-expression analyses, identifying that changes in 6 of 24 total gene co-expression modules were associated with ASD, with 3 modules (related to development, inflammation and glial function) being upregulated and 3 modules (associated with synaptic and neuronal function) downregulated in the disorder.

Of the genes found to be differentially expressed in the cortex of individuals with ASD, 60 encode long non-coding RNAs (lncRNAs). Of these lncRNAs, 20 interact with microRNA–protein complexes, and a similar number are primate specific. Moreover, the authors found that two primate-specific lncRNAs, *LINC00693* and *LINC00689*, which are usually downregulated with development, are expressed at higher levels in ASD cortical samples.

Next, the authors investigated differences in splicing in ASD. In the cortex, but not the cerebellum, there were considerable differences in splicing between the two groups, with 1,127 differential splicing events across 833 genes. Many of the differential splicing events in ASD cortex are regulated by neuron-specific splicing factors, controlled by neuronal activity, and were associated with exclusion of neuron-specific exons

In addition, the authors confirmed previous research showing that, in ASD, there is an attenuation of the differences in expression of certain genes between different regions of the cortex (known as cortical patterning of gene expression); specifically, 523 genes expressed at different levels in the frontal cortex versus the temporal cortex in controls showed less cortical patterning in individuals with ASD. These were found to be enriched for neuron-specific genes. One gene in this set encodes SOX5; given that a considerable number of other genes in the set are regulated by this transcription factor, a lack of cortical patterning of SOX5 expression in ASD might contribute to other deficits in cortical patterning of gene expression.

Last, the authors investigated whether there was an enrichment of genes known to be commonly mutated in ASD in particular gene co-expression modules. One module showed such enrichment for rare ASD-associated mutations and encodes many lncRNAs; another module was associated with common genetic variation linked to ASD and showed enrichment for genes that exhibited less cortical patterning in the disorder.

Together, this genetic characterization of transcriptomic changes in ASD points to shared cortical alterations that, despite the considerable heterogeneity of the disorder, converge to result in profound changes in neuronal gene transcription, lncRNA transcription, cortical patterning and glial function and inflammation. The timeline of these changes also suggests potential for intervention over the first decade of life.

Natasha Bray

ORIGINAL ARTICLE Parikshak, N. N. *et al.* Genome-wide changes in lncRNA, splicing and regional gene expression patterns in autism. *Nature* **540**, 423–427 (2016)
FURTHER READING Bourgeron, T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nat. Rev. Neurosci.* **16**, 551–563 (2016)