and a substantial amount of the zippering energy may be dissipated while bringing the membranes together. Hence, it is plausible that assembly of the amino-terminal domain generates state 3, and what actually causes fusion is assembly of the carboxyl-terminal domain and linker domain, perhaps augmented by binding between the transmembrane domains and/or linker domain-membrane interactions.

A different scenario is suggested by other reconstitution data indicating that multiple SNARE complexes can be formed between membranes without inducing fusion (*12*). This finding could be explained if considerable repulsion between two membranes occurs only below ~4 nm (the intermembrane distance in state 2) and most of the zippering energy is used to produce state 2, yielding only the 8 k_BT of linker domain zippering available for fusion. These observations suggest the possibility that the energy of linker domain zippering, though substantial, is insufficient for fusion and needs to be augmented by factors such as the Ca^{2+} sensor synaptotagmin-1, which can also bridge two apposed membranes and might help bending them to initiate fusion (*13*). This feature could explain the Ca^{2+} dependence of synaptic vesicle fusion and would not be shared by other forms of membrane fusion that are Ca^{2+} independent.

The energy of carboxyl-terminal domain assembly, and perhaps even that of amino-terminal domain assembly, could be applied more efficiently to bend the membranes and induce fusion if the SNARE complex assembles while bound to bulky proteins such as Munc18-1 and/or Munc13s, which play crucial roles in neurotransmitter release. This could prevent the membranes from coming too close, thus favoring application of a torque by the SNAREs on the membranes (*2*). Repulsion between the SNARE complex and the membranes, or between the membranes at close distances, could also play a similar role. Moreover, multiple SNARE complexes could cooperate in fusion. Clearly, much research will be required to explore these ideas. Undoubtedly, the approach developed by Gao *et al*. will continue producing key advances in the field.

References

1. T. C. Südhof, J. E. Rothman, *Science* **323**, 474 (2009).
2. J. Rizo *et al*., *Trends Cell Biol.* **16**, 339 (2006).
3. J. B. Sørensen *et al*., *EMBO J.* **25**, 955 (2006).
4. X. Chen *et al*., *Neuron* **33**, 397 (2002).
5. K. Wiederhold, D. Fasshauer, *J. Biol. Chem.* **284**, 13143 (2009).
6. F. Li *et al*., *Nat. Struct. Mol. Biol.* **14**, 890 (2007).
7. W. Liu *et al*., *J. Nanoneurosci.* **1**, 120 (2009).
8. Y. Gao *et al*., *Science* **337**, 1340 (2012).
9. G. van den Bogaart *et al*., *Nat. Struct. Mol. Biol.* **17**, 358 (2010).
10. L. Shi *et al*., *Science* **335**, 1355 (2012).
11. F. S. Cohen, G. B. Melikyan, *J. Membr. Biol.* **199**, 1 (2004).
12. M. Kyoung *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **108**, E304 (2011).
13. D. Araç *et al*., *Nat. Struct. Mol. Biol.* **13**, 209 (2006).

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NEUROSCIENCE

The Emerging Biology of Autism Spectrum Disorders

Expression patterns of the diverse genes disrupted in autism spectrum disorders in the developing brain give a fresh perspective on the underlying biology.

Matthew W. State[1] and Nenad Šestan[2]

Autism spectrum disorders (ASD) are a genetically and phenotypically heterogeneous group of syndromes defined by fundamental impairments in social reciprocity and language development accompanied by highly restrictive interests and/or repetitive behaviors. Recent advances in genetics, genomics, developmental neurobiology, systems biology, monogenic neurodevelopment syndromes, and induced pluripotent stem cells (iPSC) are now offering remarkable insights into their etiologies and converging to provide a clear and immediate path forward from the bench to the bedside.

Leading the charge has been the emergence of reliable genetic findings. Multiple studies have confirmed that rare and de novo point mutations and submicroscopic variations in chromosomal structure contribute to a considerable number of cases and have identified a growing number of specific genomic intervals and genes conferring risks (*1*–*9*). These advances provide a critical foundation for the development of a more refined understanding of the biological underpinnings of ASD. The earliest findings in the genetics of idiopathic (nonsyndromic) autism highlighted the role of synaptic adhesion molecules and postsynaptic density proteins (*1*, *10*). A set of newly discovered ASD proteins (see the figure) expands this view, highlighting a role for chromatin modifiers (CHD8), and DNA binding proteins (POGZ), ion channels (SCN2A), microtubule-associated proteins (KATNAL2), neurotransmitter receptors (GRIN2B), and phosphorylation-regulated tyrosine kinases (DYRK1A) (*5*–*9*).

As success in discovery has accelerated, the number of genes predicted to carry risk for ASD has steadily increased, now reaching well into the hundreds (*5*–*9*), with no single locus accounting for more than 1% of cases. This "many-to-one" relationship suggests that future studies need to go beyond isolated molecular dissections of single mutations. Another surprising and conceptually challenging observation from recent genetic studies has been the considerable overlap of risks for distinct disorders. Identical highly penetrant variants in different individuals carry large effects but for a wide range of outcomes, including, but likely not limited to, ASD, epilepsy, intellectual disability, and schizophrenia. This "one-to-many" phenomenon, combined with the biological pleiotropy of genes that have so far been implicated in ASD and the presence of core phenotypes that are distinctly human, such as impairments in language development, make the study of ASD in model organisms especially challenging. While modeling mutations in animals or cell culture will reveal perturbations in conserved biological pathways, the key challenge will be to determine which molecular, cellular, and neural circuit–level phenotypes are particularly relevant for ASD.

Ironically, the staggering degree of locus heterogeneity may hold the key to translating genetic findings into a new generation of treatments. The growing set of newly discovered ASD genes should provide a powerful means to use convergence among molecular pathways and cellular processes to identify relevant biological and therapeutic targets. As gene discovery progresses for schizophrenia, epilepsy, and other neurodevelopmental and neuropsychiatric disorders, the ability to study both convergence and divergence of mechanism across conditions will become

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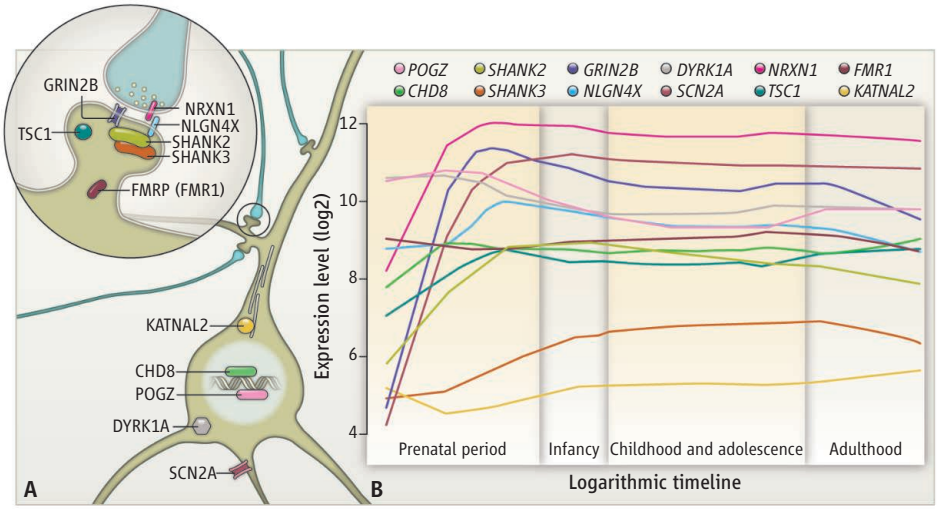
increasingly feasible. There is already convincing evidence that molecular pathways converge in ASD (*9*, *10*). What is particularly exciting, however, is the new-found opportunity to address not just the question of which molecules and pathways intersect but to specify when and where these events occur within the developing human brain.

The recent emergence of comprehensive maps of spatiotemporal gene expression (*11*) in the human brain, the ability to construct similar maps of gene-regulatory interactions and chromatin states, and the availability of a growing list of definitive ASD point mutations sets the stage for a powerful developmentally informed approach to studying ASD biology. For example, genes conferring risk can be assessed for spatially and temporally defined gene coexpression and coregulatory networks during human brain development. The identified networks can be evaluated with respect to molecular, anatomical, and developmental convergence, and mechanistic hypotheses can be generated and tested. Confirmation at the bench can leverage model systems, postmortem human tissue, and iPSC-derived neural cells, including isogenic lines carrying engineered mutations and cells generated from patients carrying the mutation(s) of interest. Overall, this type of integration offers immediate opportunities to define relevant molecular and cellular processes and, as more and more genes are identified, will increasingly point to higher-level neural system properties relevant to ASD.

In fact, the available published data on ASD gene expression in the developing human brain already points to some intriguing etiological hypotheses and plausible explanations for the observation that a single genetic risk variant can lead to highly divergent phenotypes, including autism and schizophrenia. Many of the ASD genes noted above exhibit distinctive spatiotemporal expression patterns in the developing brain, including dramatic increases in the cerebral cortex during mid-gestation (see the figure), a developmental period crucial for the formation of early neural circuits (*11, 12*). The assembly of related cortical neural circuits progresses in an orderly spatiotemporal pattern such that, in general, neurons in the anterior regions are chronologically older, and form synapses earlier, than neurons in the posterior regions. Indeed, signs of early cortical neuronal differentiation and synapse formation are present in the regions of the early and mid-fetal prefrontal and temporal cortices (*11*, *12*) that ultimately give rise to circuits underlying executive control, social affective processing, and language—all functions that are altered in ASD (*13*). These early regional differences in the timing of synaptogenesis may help explain why the ontogenetically older circuits involved in these processes are particularly vulnerable in ASD, whereas other cortical processes, such as vision, are less affected. The rarity and functional immaturity of nascent synapses in the early and mid-fetal cortex may make them especially susceptible to perturbed function of ASD-related genes, which appear to be dynamically regulated during the same developmental window.



Developmental neurobiology of ASD risk genes. (**A**) Diverse subcellular distribution and pleiotropic roles for syndromic and idiopathic ASD proteins. (**B**) Expression profiles of select previously established and newly identified ASD genes during development of the human neocortex. See supplementary materials for methods.

Several lines of evidence indicate that cortical areas and their circuits mature at different rates during postnatal development (*14*). Higher-order association cortices, including prefrontal and temporal cortices, do not fully mature until late adolescence and early adulthood (*14*), and some of their maturational trajectories are altered in ASD and schizophrenia (*13*, *14*). Their extended period of maturation may increase the sensitivity of the ontogenetically older frontal and temporal circuits involved in executive control, social affective processing, and language, to both ongoing alterations in the molecular landscape, and interceding environmental insults. In short, the identical genetic risk could lead to variable phenotypes via early developmental and ongoing functional alterations in temporally defined molecular interactions in neural circuits that show both early vulnerability and an extended period of maturational sensitivity.

Although integrated genetic, molecular, and circuit-level analyses are critical for identifying more refined mechanistic hypotheses, important questions will remain unresolved. For instance, what is the nature of the male predominance in ASD? How and why do particular individuals show resilience in the face of highly penetrant genetic risks? What roles do genetic background, somatic mutation, epigenetic modifications, the microbiome, environmental factors, and stochastic events play in determining the emergence and trajectory of symptoms? Fortunately, as the overall genetic landscape of ASD becomes clearer, these difficult questions appear increasingly tractable.

The most pressing issue for patients, families, and physicians at present is what recent findings portend for prevention and treatment. In this regard, demonstrations that aspects of the phenotypes accompanying monogenic neurodevelopmental syndromes are reversible in model organisms (*10*) provides promise that key features of human neurodevelopmental disorders involve the contribution of dynamic, and therefore potentially treatable, derangements in neural function. Moreover, although the picture of one mutation leading to a diverse array of disorders is conceptually challenging, the model we have proposed suggests that there may be useful analogies to treatment strategies from other areas of medicine. For example, heart disease and stroke prevention both rely in part on the management of hypertension. It may well be that ASD and schizophrenia will increasingly be thought of in a similar light, reflecting divergent manifestations of a shared pathophysiological liability. This would further underscore the importance of clarifying the nature of the molecular and neural circuit–level perturbations underlying these disorders. It would also suggest that future treatment trials will need to be organized around these shared mechanisms, as opposed to traditional psychiatric diagnostic categories. Finally, while the molecular diversity underlying ASD presents a con-

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siderable challenge in the search for convergence, it may well also portend, just as it has in cancer, the development of both more personalized and more effective therapies.

References and Notes

1. S. Jamain *et al.*, *Nat. Genet.* **34**, 27 (2003).
2. J. Sebat *et al.*, *Science* **316**, 445 (2007).
3. P. Szatmari *et al.*, *Nat. Genet.* **39**, 319 (2007).
4. M. W. State, P. Levitt, *Nat. Neurosci.* **14**, 1499 (2011).
5. B. J. O'Roak *et al.*, *Nat. Genet.* **43**, 585 (2011).
6. S. J. Sanders *et al.*, *Nature* **485**, 237 (2012).
7. B. M. Neale *et al.*, *Nature* **485**, 242 (2012).
8. B. J. O'Roak *et al.*, *Nature* **485**, 246 (2012).
9. I. Iossifov *et al.*, *Neuron* **74**, 285 (2012).
10. H. Y. Zoghbi, M. F. Bear, *Cold Spring Harb. Perspect. Biol.* **4**, a009886 (2012).
11. H. J. Kang *et al.*, *Nature* **478**, 483 (2011).
12. K. Y. Kwan *et al.*, *Cell* **149**, 899 (2012).
13. C. A. Walsh *et al.*, *Cell* **135**, 396 (2008).
14. J. L. Rapoport, N. Gogtay, *Neuropsychopharmacology* **33**, 181 (2008).

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MATERIALS SCIENCE

Building Research Equipment with Free, Open-Source Hardware

A rapidly increasing selection of laboratory equipment can be fabricated with open-source three-dimensional printers at low cost.

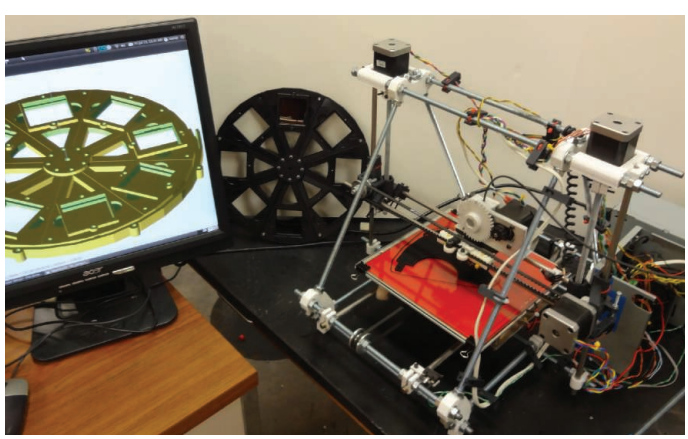
Joshua M. Pearce

Most experimental research projects are executed with a combination of purchased hardware equipment, which may be modified in the laboratory and custom single-built equipment fabricated in-house. However, the computer software that helps design and execute experiments and analyze data has an additional source: It can also be free and open-source software (FOSS) (*1*). FOSS has the advantage that the code is openly available for modification and is also often free of charge. In the past, customizing software has been much easier than custom-building equipment, which often can be quite costly because fabrication requires the skills of machinists, glassblowers, technicians, or outside suppliers. However, the open-source paradigm is now enabling creation of open-source scientific hardware by combining three-dimensional (3D) printing with open-source microcontrollers running on FOSS. These developments are illustrated below by several examples of equipment fabrication that can better meet particular specifications at substantially lower overall costs.

The FOSS movement emerged as a decentralized, participatory, and transparent system to develop software, in contrast to commercial software, which tends to be written anticipating user needs and does not allow modifications to the code, which is often proprietary (*2*). Although FOSS is a collaborative effort driven by user demands, this decentralized innovation process is still efficient and has been implemented in areas such as nanotechnology (*3*) and medicine (*4*), and the open and collaborative principles of FOSS have been readily transferred to hardware (*5*). A key enabling open-source hardware project is the Arduino electronic prototyping platform (*6*–*8*). The $20 to $30 Arduino is a versatile yet easy-to-learn microcontroller that can run a number of associated scientific instruments, including Arduino Geiger (radiation detector), pHduino (pH meter), Xoscillo (oscilloscope), and OpenPCR (DNA analysis). However, Arduino's most impressive enabling application is 3D printing. Open-source 3D printers can perform additive-layer manufacturing with polymers, ceramics, and metals. Such approaches have been popular in microfluidic lab-on-a-chip architectures, where flow paths are created layer by layer, but are adaptable to a much wider array of devices.

The most popular fabrication tool is the RepRap, named because it is a partially self-replicating rapid prototyping machine. Currently, the <$1000 RepRap can fabricate about 50% of its own parts from acrylonitrile butyl styrene or polylactic acid polymers with no postprocessing and a 0.1-mm spatial precision. This ability for self-replication has resulted in an explosion of both RepRap users and evolutionary design improvements (*9*). Scientists with access to RepRaps have found many examples where it is less expensive to design and print research tools rather than buy them. A number of simple designs are flourishing in Thingiverse, a free and open repository for digital designs of physical objects (*10*). These include single-component prints such as vial racks (thing:25080), Buchner funnels (thing:25188), and microtiter plates (thing:11621). 3D printers have also been used to print custom chemical reactionware (*11*). For example, 3D printers can be outfitted with syringes to print with materials like acetoxysilicone to quickly make reactionware capable of in situ spectroelectrochemistry or easily alter reactor architecture to gauge the effects on chemical synthesis (*11*).

The 3D printers can also be coupled with existing hardware tools such as the portable cell lysis device for DNA extraction (thing:18289), a 3D printable adapter that converts a Craftsman automatic hammer into a bead grinder for use with DNA extraction, or the DremelFuge chuck (thing:1483), a printable rotor for



Factory for one. A parametric (easily customized) filter-wheel holder is shown in the OpenSCAD program on the monitor (**left**), with the completed inside of the Arduino-controlled automated filter wheel (**center**). An Arduino-controlled RepRap 3D printer (**right**) is printing out a component of a case design. All of the hardware and software for both the filter wheel and the RepRap are open source.

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Supplementary Materials for

The Emerging Biology of Autism Spectrum Disorders

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This PDF file includes:

Methods

We selected six representative genes reflecting prior discoveries in both idiopathic and syndromic ASD (*NLGN4X*, *NRXN1*, *TSC-1*, *SHANK3*, *FMR1*, and *SHANK2*) and six newly identified ASD risk genes (*CHD8*, *GRIN2B*, *DYRK1A*, *SCN2A*, *POGZ*, and *KATNAL2*) based on a threshold of at least two independent de novo loss-of-function mutations identified in unrelated affected individuals, as described in four recent whole-exome sequencing studies (*1*–*4*). To evaluate expression profiles, we used a previously published human brain data set (*5*) generated by exon arrays. The raw data is available from the Human Brain Transcriptome database (http://www.humanbraintranscriptome.org) and the NCBI Gene Expression Omnibus (accession number GSE25219). The entire data includes 1340 samples and covers 16 human brain regions (including 11 neocortical areas), evaluated at 15 developmental periods ranging from embryonic development to late adulthood. The methods for evaluating gene expression in each region and at each period are detailed in Kang *et al*. (*5*). For panel B of the figure, the expression data for each of the genes noted above was evaluated across the 11 neocortical areas for all developmental periods. We used a loess function in the R software package (http://www.r-project.org) (*6*) to perform a local polynomial regression fitting and then plot the smoothed curves across all developmental periods. In the figure, the different-colored curves represent distinct autism-related genes, as noted at the top of the figure. The *x* axis is labeled with the developmental periods on a logarithmic time scale. The *y* axis is the log2-transformed exon array signal intensity.

References

1. S. J. Sanders *et al*., *Nature* **485**, 237 (2012).

2. B. M. Neale *et al*., *Nature* **485**, 242 (2012).

3. B. J. O'Roak *et al*., *Nature* **485**, 246 (2012).

4. I. Iossifov *et al*., *Neuron* **74**, 285 (2012)

5. H. J. Kang *et al*., *Nature* **478**, 483 (2011).

6. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.