The epitopes in the IEDB database were identified in numerous studies that used differing methodologies, and the database includes peptides recognized by epitopes in individuals with latent infection, active disease or both. Thus, these epitopes might consist of a mix of those that are beneficial for the host and those beneficial for the pathogen. Although, overall, the epitopes showed conservation, a small number of them showed evidence of increased diversity, consistent with the idea that not all antigens are under the same selective pressures in all individuals. In addition, many of the epitopes selected from the IEDB database were initially discovered by testing peptides derived from a single bacterial strain against T cells from individuals infected with strains of different lineages. This process would bias toward more evolutionarily stable

peptides and could also explain at least part of the conservation of the epitopes observed by Comas et al.[4]

The attenuated live vaccine *Mycobacterium bovis* BCG is given to infants in many parts of the world as a preventative against tuberculosis. Unfortunately, this strain offers adults very poor protection against developing the disease. The work of Comas et al.[4] suggests that some epitopes, including many present in *M. bovis* BCG, might augment transmission rather than protect against disease. If this work can be extended to define epitopes that are protective, it could provide a valuable tool for developing new, more efficacious vaccines. Given the limited variation among natural MTBC isolates, this is likely to require better characterization of existing epitopes, the discovery of new immune recognition sites and, perhaps,

harnessing the known variation among *M. bovis* BCG strains.

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Another piece of the autism puzzle

Matthew W State

A new study has identified rare *de novo* mutations in *SHANK2* in individuals with autism and/or mental retardation. *SHANK2* encodes a scaffolding protein present in excitatory synapses. This finding sheds some light on the pathophysiology of social and cognitive disability.

Autism is an often-devastating neurodevelopmental disorder characterized by impairments in social communication and language development, accompanied by highly restricted interests and/or repetitive stereotyped behaviors. It is the prototype of a continuum of syndromes referred to as autism-spectrum disorders (ASD) that have a prevalence of approximately 1% in the general population. Despite strong evidence for a genetic contribution, the identification of genetic variants contributing to ASD susceptibility has proven elusive, owing in part to a high degree of allelic and locus heterogeneity. However, recent discoveries from studies that have identified rare genetic variation in individuals with ASD, together with findings from investigations of Mendelian mental retardation syndromes, are beginning to illuminate molecular and cellular mechanisms of disease. On page 489 of this issue, Gudrun Rappold and colleagues[1] report the discovery of rare *de novo*, apparently

Matthew W. State is at the Program on Neurogenetics and the, Departments of Child Psychiatry, Psychiatry and Genetics, Yale University School of Medicine, New Haven, Connecticut, USA.
e-mail: matthew.state@yale.edu

loss-of-function mutations in *SHANK2* in individuals with ASD, mental retardation or both.

SHANK2 in autism
Berkel et al.[1] report a genome-wide analysis of copy number variation in 396 individuals with ASD and 184 individuals with mental retardation. They identify two *de novo* deletions of coding segments of *SHANK2*, one seen in each disease cohort. Sequencing of *SHANK2* within the same groups identified a *de novo* nonsense mutation in an individual with ASD. Six additional transmitted missense mutations and one duplication were found in probands that were not present in controls. However, as Berkel et al.[1] note, the transmitted variants are of uncertain importance, as the authors' approach did not allow for a comparison of the burden of deleterious mutations in cases compared to controls. That variants were seen only in probands also provides limited support, as any very rare allele, whether found in a case or a control, is unlikely to be seen again in a study of this size.

The identification of multiple *de novo* mutations at *SHANK2* in Berkel et al.[1] is of particular biological interest. SHANK2 is one of three homologous scaffolding proteins (SHANK1–3)

highly expressed at the postsynaptic density (PSD), a functionally specialized, electron-dense structure found at the postsynaptic membrane in excitatory, glutamatergic synapses. Berkel et al.[1] provides an important point of convergence with previous findings implicating PSD molecules in the etiology of social disability. The first rare functional mutations identified in idiopathic ASD were reported in *NLGN4X* (encoding Neuroligin 4X) and *NLGN3* (encoding Neuroligin 3) in 2003 (ref. 2). Shortly thereafter, a unique rare nonsense mutation in *NLGN4X* was mapped in a multigenerational pedigree affected with mental retardation and ASD[3]. Subsequently, rare loss-of-function mutations have been found in *SHANK3* in individuals with ASD and mental retardation, and multiple groups have identified structural variations disrupting *Neurexin 1* (*NRXN1*) in affected individuals[4].

Connections to the synapse
Notably, these molecules all show no more than one degree of separation from each other (**Fig. 1**). NRXN1 is a transynaptic binding partner for NLGN proteins at the PSD which, in turn, complex with SHANK-family molecules. The precise contribution of these proteins, and their varied isoforms, to nervous system

development and function remains a matter for investigation. However, there is already evidence that these interactions are required for normal synaptic function[5], that they mediate plasticity and that they ensure an appropriate balance of inhibitory and excitatory synapses[6,7].

The observation by Berkel et al.[1] that mutations in *SHANK2* may lead to ASD, mental retardation or both is also in line with previous studies noting overlap of genetic loci in susceptibility to these disorders. Such shared genetic risks are suggested by overlapping phenotypes. About half of individuals presenting with ASD have intelligence quotients less than 70, and there is a well-documented increased risk for ASD among individuals with some Mendelian forms of mental retardation. What is perhaps more intriguing is the observation that individuals carrying identical mutations may show either ASD or mental retardation without evidence of social disability. Such findings are unlikely to reflect diagnostic uncertainty because, except in the case of profound mental retardation, social and cognitive impairment are readily distinguishable. In fact, these observations suggest that functionally equivalent mutations in the same gene may lead to clearly divergent phenotypes. Similarly, the recent characterization of *de novo* mutations in *SHANK3* among individuals with schizophrenia[8] adds to the growing evidence that a range of neuropsychiatric disorders once considered entirely distinct may share underlying genetic mechanisms[9].

Potential for therapeutics

Findings of genetic loci associated with susceptibility to both ASD and mental retardation have recently provided important clues into shared mechanisms underlying both social and cognitive impairments and into possibilities for therapeutic intervention. Recent studies of the fragile X mental retardation protein (FMRP) demonstrate that it, like NLGN3 and NLGN4, SHANK2 and SHANK3, and NRXN, has a critical role at excitatory synapses[10], that alterations of FMRP increase the risk for ASD as well as mental retardation and, most intriguingly, that a deficit of FMRP, at least in model organisms, is reversible via antagonism of metabotropic glutamate receptors or via manipulation of related signaling cascades[11,12]. A similar story is unfolding with regard to the PI3K-AKT-mTOR pathway. There is strong evidence that mutations in NF-1 and TSC-1 or TSC-2 may lead to both mental retardation and ASD. These molecules, along with PTEN, which is also implicated in ASD[13], converge on the rapamycin-sensitive mTOR-raptor
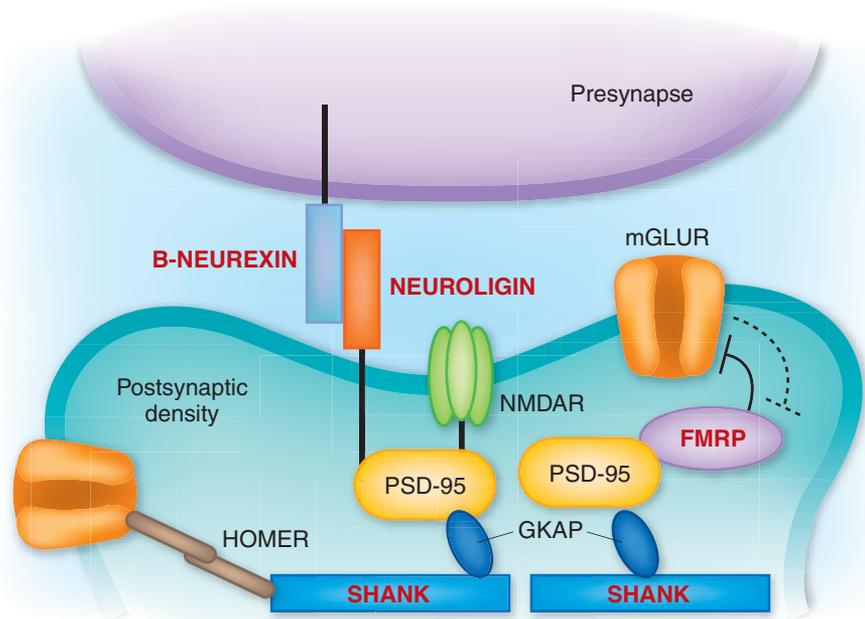
Figure 1 Molecular network at the synapse includes molecules implicated in social disability. Presynaptic neuronal adhesion molecules in the B-neurexin family, including neurexin 1, interact with postsynaptic neuroligins, which in turn bind intracellularly to postsynaptic density protein 95 (PSD-95). SHANK proteins form a matrix that is a scaffold for postsynaptic density molecules, including HOMER and guanylate kinase–associated proteins (GKAPs), which link to PSD-95. The postsynaptic density is characterized by glutamate-responsive neurotransmitter receptors such as *N*-methyl-D-aspartate receptors (NMDARs) and metabotropic glutamate receptors (mGluRs). Group 1 mGluRs mediate activity-dependent protein synthesis, and fragile X mental retardation protein (FMRP) is a key negative regulator of this process. mGluR signaling activates the PI3K-AKT-mTOR pathway, which is similarly implicated in ASD, and this in turn negatively regulates FMRP phosphorylation. Proteins implicated in the pathogenesis of ASD are in red lettering.

complex, a key regulator of protein synthesis and cell growth. Here again, the elaboration of molecular mechanisms has led for the first time to the investigation of rationally derived therapies[14,15]. For those working in the area of social and cognitive disabilities, these developments are breathtaking. The notion that ASD and mental retardation to some degree reflect dynamic, reversible and targetable processes represents an extraordinary paradigm shift.

Of course, many questions remain, among them: what are the distinct functions of *SHANK2* and *SHANK3* and why do heterozygous mutations in either lead to highly penetrant behavioral phenotypes? Is there a point of convergence among these pathways and others recently implicated in ASD[4], or do alterations in widely diverse aspects of neuronal development and function underlie social and cognitive disability? How are differences in outcomes in individuals carrying similar mutations mediated by environmental, epigenetic or genetic modifiers across development? What proportion of the genetic risk for idiopathic ASD will be accounted for by rare mutations? Just how far will genetic discoveries go in challenging

current psychiatric diagnostic nosology? Berkel et al.[1] provide an important piece of a complex puzzle, one that will require considerable effort to complete. Thankfully, the emerging picture is one that is already providing a glimmer of hope for those affected with disease, their families and the physicians who treat them.

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