

Drugmakers dance with autism

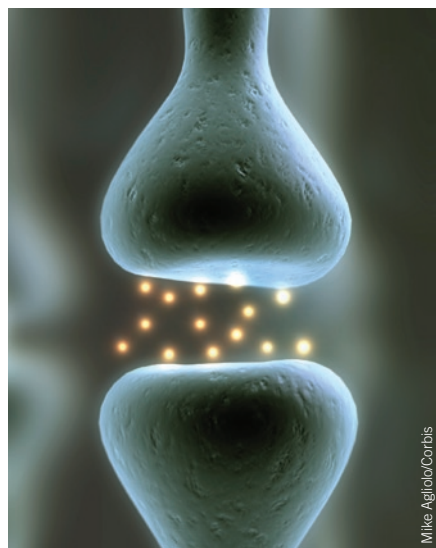
With monogenetic neurodevelopmental disorders similar to autism serving as starting points for several drug discovery programs, smaller biotechs are now joining big pharma in pursuing therapies to tackle this perplexing condition. Sarah Webb reports.

In June, the Autism Research Project published the largest genetic study of autism so far, identifying 226 gene mutations that are found in people with the syndrome¹. Children with autism are 20% more likely to carry one of these rare mutations, though they are not inheriting them; they are present in less than 6% of the parents of autistic children. This study adds to the growing list of genes that could serve as starting points for research on autism therapies.

Whereas the pharmaceutical industry increasingly has been shying away from psychiatric disorders, such as schizophrenia and depression, interest in autism has intensified. Together with an increasing number of autism cases diagnosed each year, there is a dearth of effective treatments. As a result, “autism seems to be a relatively hot area,” says Manuel Lopez-Figueroa of Bay City Capital, a venture capital firm in San Francisco, and scientific liaison for the Pritzker Neuropsychiatric Disorders Research Consortium. Not only is the pharmaceutical sector ploughing R&D resources into the condition, but several smaller companies are pioneering therapies, one of which is an enzyme replacement therapy already in phase 3 human testing (Table 1 and Box 1). What’s more, progress in drug discovery programs aiming to target proteins associated with Mendelian neurodevelopmental disorders may pave the way for expansion into broader spectrum autism conditions.

Repurposed drugs

Current estimates indicate that 1 in 110 children in the United States have an autism spectrum disorder defined by three core symptoms: deficits in social interactions, problems with communication and repetitive behaviors. Although twin and family studies have established a strong genetic basis for autism, no clear genetic cause has emerged. In addition to complex genetics, the disorder is phenotypically diverse: individuals with an autism spectrum diagnosis may be intelligent and high functioning (e.g., those with Asperger’s syndrome) or have severe mental deficits. The large variation in phenotypes and



Trouble at the synapse. The genetics of autism is pointing toward malfunctioning at the synapse.

high concordance in monozygotic twins suggests many genetic and environmental biasing factors are involved.

A diagnosis of autism brings along a slew of unmet medical needs, including anxiety, sleep disturbances, and metabolic and gastrointestinal issues. Initial moves by industry into autism therapeutics have involved applying existing drugs to alleviate some of these symptoms, says Sophia Colamarino, vice president for research at Autism Speaks, a patient advocacy group based in New York. “In the short term, that’s where many of the pharmaceutical companies will be able to have an immediate impact,” she says. Two atypical antipsychotics have been approved by the US Food and Drug Administration (FDA) for treating irritability in autistic children. Johnson & Johnson’s Risperdal (risperidone) was approved in late 2006, followed by Abilify (aripiprazole) from Bristol-Myers Squibb in New York, and Otsuka in Princeton, New Jersey, in 2009. Selective serotonin reuptake inhibitors such as low-dose Prozac (fluoxetine) are approved for use in adults and children for obsessive compulsive disorder and have been tested in children with autism. Anticonvulsives such

as valproate (Stavzor, Depakene, Depacon) may serve the same sort of purpose for some patients, says Eric Hollander, director of the Compulsive, Impulsive and Autism Spectrum Disorders Program at Albert Einstein College of Medicine and Montefiore Medical Center in New York.

Treating these related symptoms gives patients and their caregivers an improved quality of life, making it more likely that an individual with autism can live at home rather than in a care facility, Hollander adds. Improving those related symptoms can also make patients more responsive to behavioral therapies, says Robert Ring, who is heading up Pfizer’s autism research unit in Groton, Connecticut.

At least one repurposed drug is targeting the imbalance between excitatory and inhibitory signaling suspected to be part of the basis of autism. New York-based Forest Laboratories is testing Namenda (memantine), an Alzheimer’s drug and *N*-methyl *D*-aspartate receptor (NMDA) receptor modulator, in a phase 2 trial in autism patients.

Abnormal synaptic connectivity

Because this spectrum of disorders has a clear genetic basis but no clear genetic cause, researchers are chewing on the question of how so many different mutations could lead to a similar phenotype, says Luca Santarelli, head of Roche’s central nervous system exploratory development in Basel.

Genetic studies are important, but they don’t tell a complete story. “Identifying genes and coming up with gene candidates is really just a first step in gaining confidence in a potential genetic target that could be druggable,” says John Spiro, a research director at the Simons Foundation Autism Research Initiative in New York City. “There are not many genes that you can be really, really confident are accounting for any significant portion of autism.” Though researchers remain hopeful that the genes might converge into a single meaningful pathway, he adds, “for the most part in autism, it’s not clear yet that’s going to be the case.”

Nonetheless, some patterns are emerging that may help researchers devise new therapeutic strategies. A genome-wide survey of a group of autistic and mentally retarded individuals revealed a set of mutations (point mutations and copy number variants) in a gene, *SHANK2*, that controls synaptic structure, defects in which could lead to problems in neuronal communication².

Mutations in another family of genes involved with synapse formation, the neuroligins, which code for adhesion molecules that cluster on the receiving side

Box 1 Enzyme replacement for autism?

Unlike other emerging treatment strategies for autism that target genes or neurochemical pathways, Rye New York's Curemark is working on an enzyme replacement therapy comprising a mixture of several digestive enzymes (Table 1). In clinical work with children who showed symptoms of autism, Curemark's founder and CEO, Joan Fallon, noticed that several of these patients restricted their diets by their own choice, preferring carbohydrate-laden foods such as crackers and pasta. Searching for an explanation, she found that these patients had low fecal levels of the protease chymotrypsin (fecal chymotrypsin levels have also served as a diagnostic indicator of cystic fibrosis). Children with autism without a known genetic cause, often had these low enzyme levels, Fallon says.

Administering high-protease enzymes, the physicians observed behavioral changes in the children. Fallon filed patents in 1999 and formed a biotech company in 2005. The company's protease-based treatment, CM-AT, is currently being tested in a phase 3 study with 170 children ages 3–8 in 12 locations around the United States.

with the fragile X mutation, Bear and his colleagues found that knocking down expression of mGluR5 to 50% rescued the learning deficits, stopped seizures and increased other measures of plasticity in the brain.

Confident that they're targeting the appropriate pathways, Seaside Therapeutics has licensed a series of small-molecule compounds from Merck to target glutamate signaling in general and mGluR5 signaling specifically, Carpenter says. They recently completed a phase 2 clinical trial of a general γ -aminobutyric acid (GABA) B agonist, STX209, in fragile X patients, and will soon complete a phase 2 trial of the same compound in individuals with autism spectrum disorders. A specific antagonist of the mGluR5 receptor is currently in repeat-dose phase 2 trials, and Seaside expects to start phase 2 trials with fragile X patients by early 2011.

Mutations in glutamate receptor genes *GRIN2A* and *GRIK2* and multiple GABA receptor genes have been associated with autism. Two pharma companies also see promise in the mGluR5 receptor strategy for treating fragile X patients. Novartis in Basel recently completed a phase 2 clinical trial of their compound AFQ 056 at sites in Europe and is planning their next study, which is scheduled to open later in 2010, says spokesman Jeffrey Lockwood in an e-mail. Roche's small-molecule mGluR5 antagonist is being tested in phase 2 clinical trials in five locations in the United States, says Santarelli. Their results are "encouraging so far," he says. This growing understanding of these specific, related genetic disorders, Santarelli adds, provides a pathway to think about possible extrapolations to the more sporadic types of autism.

Peptide hormone targets

The peptide oxytocin and its related receptors are emerging as a pathway that could prove useful for treating a variety of neuropsychiatric disorders including autism. Animal studies have pointed to the importance of oxytocin in social behavior; in voles, for example, oxytocin and its counterpoint hormone vasopressin appears to have a role in pair bonding. Karen Parker and her colleagues at Stanford University in California observed seasonal differences in the way females and males who are raising young interacted. In the laboratory, they tracked these differences, caused by purely environmental cues to the locations of oxytocin receptors in the animals' brains. Changes based on environmental cues have led researchers to consider oxytocin therapies for treating social dysfunctioning in humans.

Such tests are already being done in humans. Hollander has given intravenous oxytocin

of the synapse, may account for up to 6% of autism cases, according to Nils Brose, director of the Department of Molecular Neurobiology at the Max Planck Institute of Experimental Medicine, in Göttingen, Germany. Neuroligins 3 and 4 localize to glutamatergic synapses, and loss-of-function mutations in these genes segregate in certain pedigrees with mental retardation, autism and Asperger's syndrome. These molecules are likely operating as the organizational point for information coming into the postsynaptic space, recruiting signaling receptors. In mouse knockouts of two of these neuroligins, Brose says, "the synapses are intrinsically operational, but they lack normal receptors and as a consequence don't function properly."

But just noting a connection between these genes and synaptic structures isn't enough for developing drug candidates, Spiro adds. "You don't know. Is it too much? Is it too little? Are [the structures] in the wrong place during development? There are just a million questions that need to be ironed out before you can think about a pharmaceutical intervention."

Santarelli's group at Roche is trying to get at some of these questions, in collaboration with Peter Scheiffele, a professor of cell and developmental neurobiology at the University of Basel and a leader in the neuroligin research area. "We'd like to understand the common downstream effects of different genetic alterations that lead to autisms and whether there are common mechanisms that could lead to treatments," Santarelli says.

Clues from rare single-gene disorders

The increasing understanding of some of the molecular mechanisms of autism is providing one avenue forward. The second breakthrough, according to Colamarino, is coming through animal studies of single-gene disorders such as fragile X³ and Rett's syndromes⁴, which are

found in a disproportionate number of individuals who meet the criteria for autism spectrum disorders. Since 2007, a handful of studies of animal models with inducible mutations have shown that animals can develop to adulthood with these disorders, and then recover after proper gene function is switched back on.

That ability to reverse the symptoms in animals with advanced disease has been a major breakthrough, says Spiro. With clear genetic causes coupled with the opportunity to build animal models of these disorders, "it may be very reasonable to say that the pathway to drug discovery in autism may be paved by a careful focus on these rarer syndromes," Ring says.

Fragile X syndrome provides a case study in this approach that weds treatment strategies for a rare disorder with the possibility of understanding the underpinnings of autism. This genetic disorder, which affects 1 in 4,000 males and 1 in 6,000 females (<http://www.fraxa.org/>), leads to learning disabilities and even mental retardation, anxiety and seizures. Up to 20% of individuals with fragile X also meet the criteria for an autism diagnosis. As a result of a single gene mutation, these individuals do not make the fragile X mental retardation protein (FMRP). Mark Bear of the Massachusetts Institute of Technology in Cambridge and his colleagues found that the lack of FMRP leads to dysregulation of signaling through the metabotropic glutamate receptors (mGluR). The mGluR5 receptor is highly expressed in regions of the brain critical for learning and memory.

FMRP serves as a brake on this signaling pathway, says Randall Carpenter, CEO and president of Seaside Therapeutics, a Cambridge, Massachusetts, biotech company co-founded by Bear. "When it's not there then there's overactivation of the signaling pathway. The brain can't discriminate between important information and noise and it doesn't develop normally." In mice

to higher functioning patients with autism and Asperger's syndrome and has observed improved social cognition. Patients were better able to lay down social memories or recognize emotions in spoken language, he says. Such treatments also decreased the severity of repetitive behaviors and self-stimulatory behaviors such as hand clapping, rocking and head banging.

Patients treated with intranasal oxytocin showed similar improvements. Earlier this year, researchers at the Center for Cognitive Neuroscience in Bron, France, found that adults diagnosed as high functioning on the autism spectrum who received doses of intranasal oxytocin were better able to recognize cooperative play than adults with a similar diagnosis who had not received oxytocin. Those who had received oxytocin also spent more time looking at the face of their virtual playmates⁵.

But teasing out the importance of oxytocin isn't easy. The French study shows variation in individual responses to oxytocin. "We don't have good biomarkers of oxytocin levels," Parker says. Funded by a grant from the Simons Foundation, she and her colleagues are trying to measure plasma oxytocin levels, various mutations and social phenotypes among individuals with autism and their siblings and compare them with controls matched for age and gender.

Oxytocin and the related response pathways represent "one of the most exciting biologies in the autism space today," says Pfizer's Ring and could have implications for other psychiatric areas as well. In research Ring carried out at Wyeth, he developed the first nonpeptide oxytocin receptor agonist⁶. "The oxytocin receptor is a priority target for the field, but a very challenging target to develop traditional small-molecule chemistry for."

Cellceutix, a biotech company in Beverly, Massachusetts, is also testing a preclinical compound for autism, KM-391, in a rodent model of autism developed by researchers at the Kennedy Krieger Institute in Baltimore. The autism-like symptoms are induced by injecting the chemical 5,7-dihydroxytryptamine (5,7-DHT) into the forebrain of newborn rat pups, leading to neonatal serotonin depletion, reduced brain plasticity and abnormal behaviors. In an initial study, KM-391 given over 90 days restored normal behaviors, and near-normal serotonin levels and increased brain plasticity relative to a nontreatment group and a group given Prozac. Another study measuring serotonin levels in three regions of the rat brain has confirmed the restoration of normal serotonin levels.

Another small study added an oxytocin antagonist to the mix. The antagonist alone intensified the autism-related behaviors, such as

Table 1 Selected companies with autism targets in clinical development

Company	Target	Drug candidate	Stage of development
Curemark	Protease deficiency	CM-AT (a mixture of amylase, protease, chymotrypsin, trypsin, papain and papaya in a 4–10:1 ratio with lipase, derived from animal, plant, microbial or synthetic sources)	Phase 3
Novartis	mGluR5	AFQ 056 (small molecule)	Phase 2
Roche	mGluR5	RO4917523 (small molecule)	Phase 2
Seaside Therapeutics	GABA B mGluR5	STX209 (R-isomer of baclofen) STX107 (2-methyl-1,3-thiazol-4-yl) ethynylpyridine)	Phase 2 Phase 1
Forest Laboratories	NMDA receptor modulator	Namenda (memantine)	Phase 2

repetitive behaviors and sensitivity to touch, but when given with KM-391, the frequency and intensity of these behaviors were reduced.

Measuring outcomes

Fueled by academic research and increased funding from the US National Institutes of Health, nonprofit and advocacy organizations, the field is moving forward. But even as some drug candidates are moving into the clinic, a number of challenges remain for the field as a whole. Above all is the problem of the heterogeneity of the disorder, according to Colamarino. "We're calling it one thing when it's really probably more than one." That heterogeneity can pose a challenge in choosing appropriate study subjects. The field is also struggling with finding appropriate outcome measures, particularly those that can be measured within the time frame of a clinical study. Without sensitive measures of changes in the core symptoms, researchers need to identify what the focus should be within a particular trial. In many cases researchers have depended on parental reporting of behavioral changes, Colamarino says, leading to a large placebo effect. Although no biomarkers have been established for autism, some sort of biological measure of change in connection with autism's core symptoms, would be particularly attractive. Some clinical trials have failed because of methodological issues, she adds. "That's why we need to address this sooner rather than later."

To bring researchers together to discuss these challenges, Autism Speaks and Pfizer are co-sponsoring a translational research meeting to improve clinical study methodology and design, tentatively scheduled for later this year. "There's no better investment for us externally than to bring together all the key experts in this area and have a discussion with FDA present and try to iron out a framework to address this challenge together," Ring says. The development of the *Diagnostic and Statistical Manual of Mental*

Disorders (DSM-V), the bible for neurological diseases, scheduled for release in May 2013, could complicate the development of trial endpoints, Bay City's Lopez-Figueroa adds, depending on how autism disorders and symptoms are classified.

A second meeting in early 2011 will look at clinical targets—both their identification and validation—in an attempt to reach a consensus on where therapeutics can bring the most initial benefit to patients. This is something the field is still struggling with, Ring says. "If we had one shot today to demonstrate that this would work, what would be the clinical target that we should take on?"

Pfizer and Roche are also developing an autism proposal for the Innovative Medicines Initiative, which coordinates European Union-based public-private partnerships in drug discovery and development. The idea is for companies to join forces to work on research that is not generating intellectual property, Santarelli says, such as the development of animal models, understanding disease mechanisms and physiology, finding biomarkers and developing clinical methodology.

Unquestionably, developing therapeutics for a developmental neuropsychiatric disorder with such an early onset presents several challenges. But Autism Speaks' Colamarino is encouraged by the growth in the field. "Three to five years ago, we wouldn't have been talking about clinical trials, certainly with respect to novel drug discovery," she says. Pfizer's Ring expects industry involvement to continue to grow: "It's just too large an unmet medical need for companies not to see the opportunity to enter into this research space."

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