Fine-tuning mGluRs

By Lev Osherovich, Senior Writer

Studies by two American teams have converged on a family of metabotropic glutamate receptors as players in attention deficit hyperactivity disorder and autism spectrum disorder.^{1,2} The findings build an argument for agonizing the receptors in patients with either ADHD or a form of ASD associated with tuberous sclerosis complex and antagonizing the targets to treat a different form of ASD associated with fragile X syndrome.

Determining which patients could benefit from which approach will require new diagnostics.

Metabotropic glutamate receptors (mGluRs) are a family of G protein-coupled receptors that influence the sensitivity of neurons to excitatory signals. Previous human genetic studies implicated mGluR mutations in various neuropsychiatric illnesses including schizophrenia, depression and anxiety.³ At least 10 companies are developing mGluR modulators for neuropsychiatric and neurodegenerative indications.

The new studies by a consortium led by the **University of Pennsylvania** and **The Children's Hospital of Philadelphia** and a separate team at the **Massachusetts Institute of Technology** suggest that agonizing the receptors could be beneficial for a subset of ADHD and ASD patients.

The consortium led by the UPenn and Children's Hospital researchers ran a genomewide association study and identified deletions and mutations in several *mGluR* genes in ADHD patients. The MIT team used mouse models of two common ASD-associated genetic conditions—fragile X syndrome and tuberous sclerosis complex (TSC)—to test how modulating mGluR signaling affects brain activity and behavior.

G'day, glutamate

The ADHD study, led by Hakon Hakonarson, associate professor of pediatrics at UPenn and director of the Center for Applied Genomics at Children's Hospital, used high-density microarrays to identify genomic copy number variants that occurred more frequently in patients compared with healthy controls.

Among 2,493 patients with ADHD and 9,222 controls, deletions in the *mGluR5* (*GRM5*) gene were found in 10 ADHD cases and 1 control ($p=1.36 \times 10^{-6}$). Deletions in genes for two related receptors, mGluR7 (*GRM7*) and mGluR8 (*GRM8*), were found in six and eight cases compared with zero controls ($p=3.52 \times 10^{-6}$ and $p=8.14 \times 10^{-5}$, respectively).

Results were published in *Nature Genetics*.

Hakonarson said the findings are in line with previous genomewide association studies that found mGluR mutations in ASD and schizophrenia patients, many of whom also have ADHD.⁴

Although previous studies had implicated mGluRs in neuropsychiatric indications, “nobody has previously shown that deletions in these genes are significantly associated with ADHD,” said Hakonarson.

He thinks various mGluRs identified by his team could perform similar functions in keeping brain activity in balance, so mutations in any of them could lead to disturbed glutamate signaling and behavior. Thus, it may be possible to correct the defect caused by the mutations in certain mGluRs by agonizing the remaining related functional receptors.

Hakonarson plans to start an investigator-led Phase I ADHD trial of an mGluR agonist his team licensed from an undisclosed Japanese pharma.

He said the compound, which agonizes mGluR5, mGluR7 and mGluR8, previously failed Phase III testing for Alzheimer's disease (AD). The trial is expected to start this year.

Hakonarson's team also is creating cell culture models of ADHD using induced pluripotent stem cells from patients with neuropsychiatric disease who have deletions and duplications in various *mGluR* genes.

Children's Hospital has filed for patents on Hakonarson's findings, and the IP is available for licensing.

Up and down

A team led by Mark Bear, professor of neuroscience at MIT, examined the involvement of mGluR5 in mouse models of fragile X syndrome and TSC. Previous work by Bear and others has shown that antagonizing mGluR5 could decrease fragile X electrophysiological and behavioral pathology in mice,⁵ but the involvement of glutamate signaling in TSC was unknown.

Bear's team found that brain slices from mice with low levels of tuberous sclerosis complex tumor suppressor 2 (*Tsc2*), a translational regulator that is mutated in some TSC patients, had defective mGluR5 signaling and electrophysiological functioning compared with brain slices of wild-type controls.

Compared with vehicle-treated controls, the TSC mouse brain slices treated with a positive allosteric modulator of mGluR5 had improved brain functioning *in vitro*. Also, TSC mice treated with the compound had less freezing behavior in response to new stimuli, a hallmark of ASD in the animals.

Finally, the team crossed TSC mice, which have too little mGluR5 activity, with fragile X syndrome mice, which have too much. The resulting mice had normal levels of mGluR5 signaling and resembled a wild-type mouse in behavioral assays.

Results were published in *Nature*. The patent and licensing status of Bear's findings is undisclosed.

Altogether, Bear's findings show that imbalanced mGluR activity leads to ASD-like behavior in mice.

The study “shows that you have to operate in a certain zone of mGluR activity, and if you go out of this zone to the left or right, you get an ASD-like phenotype,” said Hakonarson.

The findings “lend support for our basic working hypothesis that optimal brain function and circuit activity are tightly regulated—too much or too little activity in a particular circuit can get you off balance,” said Randall Carpenter, cofounder, president and CEO of **Seaside Therapeutics Inc.**

Seaside's STX107, an mGluR5 antagonist, is expected to enter Phase II testing for fragile X syndrome and other forms of ASD in the first half of this year. The company's lead compound, arbaclofen (STX209), is a GABA_B receptor antagonist that is in Phase III testing for fragile X syndrome. Bear is a Seaside cofounder.

Carpenter said mGluR5's newly found roles in TSC and ADHD could broaden the therapeutic opportunities for the target.

Novartis AG's AFQ056 is the most advanced mGluR5 antagonist for fragile X syndrome. The compound is in Phase III testing, and Novartis plans to seek approval this year.

Common cause?

ASD, ADHD and other psychiatric disorders are often found together in the same patients. The new studies point to common genetic underpinnings for ASD and ADHD, potentially explaining the high comorbidity of these clinically distinct conditions.

In ADHD patients "where there are gene deletions, there's a case for agonizing the functional copy of the receptor," said Joseph Buxbaum, a professor of psychiatry, neuroscience, genetics and genomic sciences at **Mount Sinai School of Medicine**. He is a coauthor of Hakonarson's study.

However, in patients with duplications and polymorphisms that lead to unpredictable effects on mGluR function, "you wouldn't know whether agonism or antagonism is appropriate," he said.

Carpenter thinks the next step is to develop diagnostics that determine which patients would benefit from mGluR agonists or antagonists.

He suggested that proteomic or gene expression profiling of mouse models of ASD could identify biomarkers of excessive or insufficient mGluR activity.

"If you could identify proteins that are most increased in the fragile X mouse and are most decreased in the TSC mouse, you could have a profile of peripheral markers" for different forms of the disease, said Carpenter.

Buxbaum noted that it is not yet clear whether abnormalities in mGluR signaling play a role in idiopathic cases of ASD or ADHD, which are far more common than cases of TSC, fragile X syndrome or ADHD associated with mGluR mutations. Thus, he said, it remains to be seen whether modulating mGluRs would be useful in ADHD or ASD patients who do not have mutations in *mGluR* genes.

Whereas Seaside is pursuing mGluR therapeutics for ASD, **Addex Pharmaceuticals Ltd.** is holding off on the indication until it becomes clear which way to modulate the targets.

Addex has a portfolio of mGluR modulators for a range of neurological indications not including ASD or ADHD.

"We don't have any programs specifically targeting ASD," said Sonia Poli, head of CNS and nonclinical development at Addex. "We are going for indications where you can get to registration quickly."

"As a small biotech, we shouldn't rush to apply these compounds in autism," said Mikhail Kalinichev, group leader of *in vivo* pharmacology

at Addex. "We're at the early stages of understanding the neurobiological underpinnings and various subpopulations" of ASD.

The company's most advanced candidates are the Phase II compounds dipraglurant (ADX48621), a negative allosteric modulator of mGluR5 for Parkinson's disease (PD) and hyperkinetic movement disorder, and ADX1149, a positive allosteric modulator of mGluR2 (GRM2) for schizophrenia that is being developed in partnership with **Johnson & Johnson**.

ADX63365, a positive allosteric modulator of mGluR5, is in preclinical development for schizophrenia and cognitive dysfunction.

Kalinichev and Poli were more convinced about the prospects for using mGluR modulators to treat ADHD, an indication with more clearly defined clinical endpoints than ASD.

In addition to Hakonarson's human genetic study, "there are preclinical data that link mGluRs to certain models of ADHD, so we were already aware of the

relevance" of mGluRs, said Kalinichev.

Kalinichev and Poli think there may be an opportunity to target mGluR7 in ADHD.

"mGluR7 is the least investigated of these receptors, despite being the most abundant one in the CNS," said Kalinichev. "It's expressed in the key areas involved in emotional reactivity and learning, so it's not surprising to see the genetic link to ADHD."

"We are keen on mGluR7 because there are no other compounds available to target it besides ours," added Poli. "We have compounds that can potentiate or inhibit its activity."

Addex has an unnamed negative allosteric modulator of mGluR7 in discovery for depression and post-traumatic stress disorder (PTSD).

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e-mail: hakonarson@email.chop.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Addex Pharmaceuticals Ltd. (SIX:ADXN), Geneva, Switzerland
The Children's Hospital of Philadelphia, Philadelphia, Pa.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Massachusetts Institute of Technology, Cambridge, Mass.
Mount Sinai School of Medicine, New York, N.Y.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Seaside Therapeutics Inc., Cambridge, Mass.
University of Pennsylvania, Philadelphia, Pa.

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**—Mikhail Kalinichev,
Addex Pharmaceuticals Ltd.**