Current Literature
In Basic Science

Combining Ubiquitin Deficiency and GABA-Mediated Inhibition Equals Seizures?

Altered Ultrasonic Vocalization and Impaired Learning and Memory in Angelman Syndrome Mouse Model With a Large Maternal Deletion From Ube3a to Gabrb3.


Angelman syndrome (AS) is a neurobehavioral disorder associated with mental retardation, absence of language development, characteristic electroencephalography (EEG) abnormalities and epilepsy, happy disposition, movement or balance disorders, and autistic behaviors. The molecular defects underlying AS are heterogeneous, including large maternal deletions of chromosome 15q11-q13 (70%), paternal uniparental disomy (UPD) of chromosome 15 (5%), imprinting mutations (rare), and mutations in the E6-AP ubiquitin ligase gene UBE3A (15%). Although patients with UBE3A mutations have a wide spectrum of neurological phenotypes, their features are usually milder than AS patients with deletions of 15q11-q13. Using a chromosomal engineering strategy, we generated mutant mice with a 1.6-Mb chromosomal deletion from Ube3a to Gabrb3, which inactivated the Ube3a and Gabrb3 genes and deleted the Atp10a gene. Homozygous deletion mutant mice died in the perinatal period due to a cleft palate resulting from the null mutation in Gabrb3 gene. Mice with a maternal deletion (m-/p+) were viable and did not have any obvious developmental defects. Expression analysis of the maternal and paternal deletion mice confirmed that the Ube3a gene is maternally expressed in brain, and showed that the Atp10a and Gabrb3 genes are biallelically expressed in all brain sub-regions studied. Maternal (m-/p+), but not paternal (m+/p-), deletion mice had increased spontaneous seizure activity and abnormal EEG. Extensive behavioral analyses revealed significant impairment in motor function, learning and memory tasks, and anxiety-related measures assayed in the light-dark box in maternal deletion but not paternal deletion mice. Ultrasonic vocalization (USV) recording in newborns revealed that maternal deletion pups emitted significantly more USVs than wild-type littermates. The increased USV in maternal deletion mice suggests abnormal signaling behavior between mothers and pups that may reflect abnormal communication behaviors in human AS patients. Thus, mutant mice with a maternal deletion from Ube3a to Gabrb3 provide an AS mouse model that is molecularly more similar to the contiguous gene deletion form of AS in humans than mice with Ube3a mutation alone. These mice will be valuable for future comparative studies to mice with maternal deficiency of Ube3a alone.

Spontaneous Seizures and Altered Gene Expression in GABA Signaling Pathways in a Mind Bomb Mutant Zebrafish.


Disruption of E3 ubiquitin ligase activity in immature zebrafish mind bomb mutants leads to a failure in Notch signaling, excessive numbers of neurons, and depletion of neural progenitor cells. This neurogenic phenotype is associated with defects in neural patterning and brain development. Because developmental brain abnormalities are recognized as an important feature of childhood neurological disorders such as epilepsy and autism, we determined whether zebrafish mutants with grossly abnormal brain structure exhibit spontaneous electrical activity that resembles the long-duration, high-amplitude multispike discharges reported in immature zebrafish exposed to convulsant drugs. Electrophysiological recordings from agar immobilized mind bomb mutants at 3 d postfertilization confirmed the occurrence of electrographic seizure activity; seizure-like behaviors were also noted during locomotion video tracking of freely behaving mutants. To identify genes differentially expressed in the mind bomb mutant and provide insight into molecular pathways that may mediate these epileptic phenotypes, a transcriptome analysis was performed using microarray. Interesting candidate genes were further analyzed using conventional reverse transcriptase-PCR and real-time quantitative PCR, as well as whole-mount in situ hybridization. Approximately 150 genes, some implicated in develop-
ment, transcription, cell metabolism, and signal transduction, are differentially regulated, including downregulation of several genes necessary for GABA-mediated signaling. These findings identify a collection of gene transcripts that may be responsible for the abnormal electrical discharge and epileptic activities observed in a mind bomb zebrafish mutant. This work may have important implications for neurological and neurodevelopmental disorders associated with mutations in ubiquitin ligase activity.

Commentary

A recent article by Jiang et al. (2010) (1) describes a new mouse model of Angelman syndrome (AS), a genomically imprinted neurodevelopmental disorder with epilepsy associated with reduced maternal gene expression on chromosome 15q, notably the gene UBE3A, that codes for an E3 ubiquitin protein ligase (3, 4). However, most clinical cases are produced by a large > 1.2 MB deletion in chromosome 15q11-13 that includes another gene ATP10a and a cluster of GABA_{A} receptor subunit genes, notably the β3 subunit, associated with epilepsy in human and mouse mutations (3, 5). Thus Jiang et al. (1) have now produced, by chromosome engineering, a mouse corresponding to the human deletion AS genotype and show that the phenotype of the maternal heterozygote 1.6 MB deletion animal is even closer to that of human deletion AS than mutations in either Ube3a or Gabrb3 alone.

The AS phenotype includes severe mental retardation with no speech, motor and sensory deficits, and epilepsy. The epilepsy includes atypical absence seizures, myoclonic seizures, and sometimes tonic-clonic convulsions. The seizures often respond to ethosuximide, but many AS patients are refractory to antiepileptic medications, though seizures in AS tend to improve with age (3, 5). The AS gene UBE3A codes for a ubiquitin protein ligase that catalyzes the covalent attachment of ubiquitin to specific target proteins, leading to their degradation by the cellular proteasome. It is unknown how UBE3A deficiency leads to the AS phenotype or even what proteins are targeted for destruction by the UBE3A product. Presumably, some target protein, possibly p53, builds up and confers a negative outcome on neuronal growth and development. Mouse knockouts, or rather, heterozygotes lacking the normally derived copy of the gene for Ube3a, exhibit many symptoms of AS, including cognitive deficits and abnormal EEG but not spontaneous seizures (6, 7). Additional studies on deficient mice, Drosophila, and Caenorhabditis elegans have shown impairment of numerous neurodevelopmental features, including excitatory dendritic spine morphology (8), glutamate receptor trafficking/plasticity (9), and sensory input-dependent maturation of the neocortex (10).

However, the human AS is more complicated than a one-gene explanation, and 80% of probands exhibit a large 1.2 MB deletion in chromosome 15q11-13 including genes for the ATPase 10a and a cluster of three GABA_{A} receptor subunits, β3, α5, and γ3 (3). This region contains a variety of imprinting genes and exhibits genomic rearrangements at several breakpoints that often result in deletions and duplications of these genes. Parent-of-origin, age, tissue, gender-dependent, and also epigenetic regulation of gene expression are described in the area, possibly associated with neurodevelopmental disorders including Rett syndrome and autism spectrum disorders, and often presenting with epilepsy (5). AS is imprinted, with only maternal mutations conferring the disease phenotype. In contrast, individuals with Prader-Willi syndrome (PWS), resulting from deficiency of paternal genes from the same region, exhibit a different phenotype, with little or less severe epilepsy. Nevertheless, epilepsy is prominent in a significant fraction of deletion PWS cases but in virtually none of the point mutation or imprinting center PWS cases. Furthermore, the 10% of AS probands that harbor only a mutation in UBE3A are less severely affected than individuals with the full 15q11-13 deletion. Such patients typically display only mild epilepsy and a lower recurrence risk, suggesting that the severe epilepsy in deletion cases appears to be caused by the lack of maternal GABRB3 in addition to the AS gene, UBE3A (3). This observation is consistent with the epilepsy present in human GABRB3 and mouse Gabrb3 mutations (3, 5).

The currently favored hypothesis for the mechanism of imprinting (4) is that the paternal allele of UBE3A is silenced by a cis-acting antisense RNA transcript whose expression is regulated epigenetically by an as yet undiscovered mechanism that is tissue-specific and occurs only in the brain. This situation leads to production of disease phenotypes from normally autosomal recessive mutations (occurring on only maternal UBE3A) in an unstable chromosomal region subject to breaks, deletions, and duplications and including important neuronal GABA receptor genes. New findings in the mouse deletion AS model (1) include verification that Ube3a is expressed only maternally in mouse brain while ATP10a and Gabrb3 are expressed biallelically in the brain regions and ages tested in both sexes. The maternal but not paternal heterozygote deletion mice exhibit spontaneous tonic-clonic seizures and EEG abnormalities. This occurs despite the partial expression of Gabrb3 in the heterozygotes. This important observation suggests that the more severe epilepsy in deletions results from a combination of the total lack of Ube3a product and a partial lack of Gabrb3 product compared with the lack of Ube3a alone, which did not exhibit spontaneous seizures but could be induced to exhibit strain-dependent seizures (6, 7). The authors note the unlikelihood of ATP10a contributing to the phenotype (1). It remains possible that Gabrb3 expression is regulated in a parent-of-origin, age, or sex-dependent manner in a specialized population of cells undetected here. However, it is likely that Ube3a deficiency has some contribution to the epilepsy, consistent with the important role of ubiquitin and the
family of E3 protein ligases in neurodevelopment and the suggestion that the confluence of ubiquitin deficiency and GABA pathways frequently leads to epilepsy.

The maternal deletion AS mouse model exhibits, in addition to seizures, significant impairments in motor function, learning and memory tasks, and anxiety-related measures. Interestingly, the mice show increased ultrasonic vocalizations as newborns, indicative of impaired signaling between pup and mother. This finding corroborates the impaired communication seen in human AS (1), and further supports this model as representative of AS. It will be useful to compare some of the characteristics implicated in the Ube3a knockout mouse mentioned above with the new deletion animal model of AS, in particular, altered excitatory synaptic structure and experience-dependent refinement (8–10). In light of the connection to GABA-mediated inhibition implied for AS and neurodevelopmental aspects of epilepsy presented in the companion article (2), it is warranted to overcome the excitatory plasticity bias exhibited by workers in the learning and memory field and examine the GABA as well as glutamate aspects of the AS model.

The other study discussed here (2) involves a zebrafish developmental mutation with epilepsy and downregulated GABA function genes, with numerous interesting similarities to the AS phenotype. First, the development of the early brain in zebrafish shows important similarities to mammals in secondary neurogenesis, such as forebrain and other topologically localized homeobox, proneural, and other transcription factor gene expression. Migration and regional differentiation patterns, including the onset of the GABA/GAD expression or lack thereof, show strong parallels in the subpallium and telencephalic areas (11).

The zebrafish mutant employed in this report (2) is the mind bomb mutation in an ubiquitin protein ligase that regulates notch signaling, via regulation of protein trafficking, either proteasomal degradation or endocytosis. The connection with ubiquitin E3 protein ligases and autism spectrum disorders has been noted above (2, 5). The stated objective for the zebrafish mind bomb mutant study is to find pathways relevant to the epilepsy phenotype that these authors have characterized behaviorally and electrophysiologically in a fish (an excellent genetic and anatomical model). Using microarray analysis, quantitative real-time PCR, and whole animal mount in situ hybridization, they identified 150 genes that are differentially regulated in the mutant and wild-type transcriptome, with differing protein substrates for ubiquitination, and the zebrafish gene is not the homologue of the AS gene UBE3A/UBE3A. But the substrate for ubiquitination is not known in either case; thus they could be related in function or pathway, if not in the same gene family. In AS, the GABA_A receptor β3 subunit gene GABRB3 happens to be situated very near UBE3A on chromosome 15q11, but there is no evidence for interaction at the protein level; that is, the β3 subunit is not ubiquitinated by this enzyme. Rather, in both cases, the ubiquitin protein ligase shows a complex and poorly understood role in neurodevelopment, a process with which GABA function will be intimately involved. Although the epigenetic imprinting situation with the UBE3A gene and AS is probably unique among ubiquitin E3 protein ligases, it is possible that they are so important that any genetic problems make them susceptible to neurological disorders because of the epigenetic involvement.

by Richard W. Olsen, PhD

References

2011

AMERICAN EPILEPSY SOCIETY

65TH ANNUAL MEETING

IMPORTANT DEADLINES

October 28............. Early Bird Discount
October 30............. Hotel Reservations
November 17............ Pre-Registration

www.AESNET.org

Baltimore, MD
Baltimore Convention Center
December 2 - 6, 2011

Future Annual Meeting Dates

2012
San Diego, CA
San Diego Convention Center
November 30 - December 4

2013
Washington, D.C.
Washington Convention Center
December 6 - 10

2014
Seattle, WA
Washington State Convention and Trade Center
December 5 - 9

2015
Philadelphia, PA
Pennsylvania Convention Center
December 4 - 8

2016
Houston, TX
George R. Brown Convention Center
December 2 - 6
The American Epilepsy Society announces the call for nominations for the 2011 Epilepsy Research Recognition Awards. This program honors individuals whose history of professional excellence in epilepsy research has advanced the understanding, diagnosis and/or treatment of epilepsy.

AWARDS
One Basic Scientist and one Clinical Investigator each receive a $10,000 award and are recognized at the Society’s Annual Meeting in December.

ELIGIBILITY
Nominations are open to active scientists or clinicians around the world whose research impacts an aspect of epilepsy. Candidates are nominated by their peers and must hold a professional degree.

NOMINATION
All nomination materials must be received in the American Epilepsy Society office by the end of the day, August 5, 2011. Electronic submission is encouraged. Complete instructions and details for the nomination are on the AES Web site at www.aesnet.org/go/research/research-awards.
Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

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American Epilepsy Society
Epilepsy Currents Journal
Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today’s Date: ____March 2, 2011_____________________________

2. First Name __Richard_______ Last Name Olsen _________________ Degree PhD __________

3. Are you the Main Assigned Author? __X__ Yes ____ No

If no, enter your name as co-author __________________________________________________

4. Manuscript/Article Title: __Combining Ubiquitin and GABA-Mediated Inhibition can Equal Seizures?

_____________________________________________________________

5. Journal Issue you are submitting for: _11.3________________________________________________________

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

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<th>Money to Your Institution*</th>
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* This means money that your institution received for your efforts on this study.
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Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

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* This means money that your institution received for your efforts.
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