



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.

Jiang YH, Pan Y, Zhu L, Landa L, Yoo J, Spencer C, Lorenzo I, Brilliant M, Noebels J, Beaudet AL. *PLoS One* 2010;5(8):e12278.

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.

Hortopan GA, Dinday MT, Baraban SC. *J Neurosci* 2010;30(41):13718-13728.

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 multispikes 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 $\beta 3$ 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 maternally 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, $\beta 3$, $\alpha 5$, and $\gamma 3$ (3). This region contains a variety of imprinted 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-q13 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 electrographically 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, including many candidates for abnormal electrical excitability, such as the genes for Glutamic Acid Decarboxylase-1 (GAD 65), and the GABA_A receptor $\alpha 1$ subunit. For comparison purposes, it is unfortunate that the authors did not examine the GABA_A receptor $\beta 3$ subunit, *GABRB3*. The zebrafish mutant shows an upregulation of the neurotrophic factor BDNF, and reduced levels of neuropeptides calbindin and parvalbumin, all associated with inhibitory synaptic function. This report is a very exciting, if preliminary, attempt to identify genes and pathways involved in seizures, an approach used recently in several epilepsy models.

However, despite its exciting potential, these two studies share only a superficial connection. Although an ubiquitin protein ligase is involved in both, there is a family of such enzymes with differing protein substrates for ubiquitination, and the zebrafish gene is not the homologue of the AS gene *UBE3A*(5). 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 $\beta 3$ 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 $\beta 3$ 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

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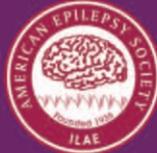
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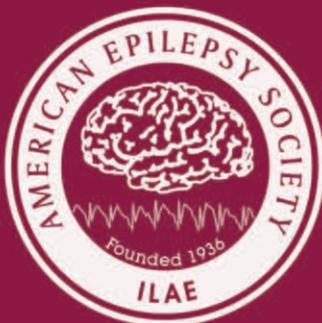
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All nomination materials must be received in the American Epilepsy Society office by the end of the day, August 5, 2011. Electronic submission is encouraged. Complete instructions and details for the nomination are on the AES Web site at www.aesnet.org/go/research/research-awards.





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