From Electrophysiology to Chromatin

A Bottom-Up Approach to Angelman Syndrome

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ABSTRACT: Angelman syndrome is one of the most studied human diseases related to a gene that is expressed on the maternal chromosome only in at least some brain cells. It is caused by inactivation of the *UBE3A* gene in the brain due to various abnormalities of the 15q11-q13 chromosome inherited from the mother. It is characterized by severe developmental delay, seizures, virtual absence of speech, motor impairment, and a particular behavioral phenotype. Studies of cortical, electromyographic and cerebellar electrophysiology in patients with Angelman syndrome and a mouse model revealed unique rhythmic neurophysiological activities in the cerebral cortex, cerebellar cortex, and muscles. The oscillatory patterns may be linked to molecular pathophysiology of the syndrome involving dysregulation of synaptic neurotransmission through *UBE3A*-related modulation of functional $GABA_A$ receptor complexes.

KEYWORDS: genomic imprinting; DNA methylation; Angelman syndrome; chromosome 15; UBE3A; GABRB3; $GABA_A$; neurophysiology; Purkinje cells; electroencephalography

INTRODUCTION

Angelman syndrome (AS; Online Mendelian Inheritance in Man [OMIM]#105830) is a neurogenetic condition characterized by developmental cognitive, speech, and motor impairment, a peculiar behavioral phenotype, and seizures.[1] AS can serve as a model prompting broad questioning of genetic and epigenetic influences in neurology

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as well as of several concepts such as psychomotor development, cerebral palsy, behavioral phenotypes, and epileptic syndromes.[2] Distinctive electroencephalographic (EEG) abnormalities recognized in the original AS patients have proved to be very consistent in subsequent studies and are included in the clinical diagnostic criteria.[3] Genetic testing allows categorization of AS into six molecular classes, and subclasses have been defined on the basis of the mechanism giving rise to each molecular class.[4] These different mechanisms may result in phenotypes of varying severity, although the EEG features appear to be broadly similar across the different genotypes.

All patients with a molecular diagnosis have a functional absence of the maternally inherited *UBE3A* gene, a normally imprinted gene located on chromosome 15q11-13. Genomic imprinting is a process that determines the differential expression of genes according to either their maternal or paternal origin. In humans, ~50 genes are imprinted. About half of these are expressed when they are inherited from the mother and half when inherited from the father. Most of these genes seem to play roles in growth, development, or tumor suppression. At the concerned loci, selective imprinting of one allele (maternal or paternal) is signaled by DNA methylation at cytosine sites and/or histone modification.[5] DNA methylation has been studied more extensively than histone modification. After DNA synthesis has been completed, the addition of methyl groups to cytosine alters the major groove of DNA to which DNA-binding proteins attach. This may result in either a decrease or an increase in the rate of transcription, according to the position of the methylation change with respect to the transcription initiation site.[6] Such epigenetic markers can be copied postsynthetically, resulting in heritable changes in chromatin structure.

DNA cytosine methylation has been implicated as an important epigenetic determinant in human disease, particularly in cancer and developmental disorders (e.g., Rett syndrome; OMIM#312750). DNA methylation associated with genomic imprinting is also implicated in human disease, as in AS, Prader–Willi syndrome (OMIM #176270), and Huntington disease (OMIM*143100). AS is one of the most studied human diseases related to a gene that is normally imprinted. The molecular characterization of AS has allowed the development of animal models of the various mechanisms underlying the syndrome, based on the genetic homology between human chromosome 15q11-13 and murine chromosome 7C-D1. In this work, we used several paradigms to study cortical, electromyographic, and cerebellar electrophysiologic activities in AS patients and a mouse model, and we reviewed the available neurophysiologic evidence from both human and animal studies in light of recent molecular advances. This approach may have implications for our understanding of the functional effects of genetic and epigenetic mechanisms in neurology.

MATERIALS AND METHODS

Humans

Electromyography

We compared 14 AS patients aged 7–13 years (mean, 9.4 ± 2.2 years; 9 with 15q11-13 deletion, 1 with uniparental disomy, 2 with imprinting defect, and 2 with *UBE3A*

mutations) with 18 normal children aged 6–13 years (mean, 9.2 ± 2.2 years) performing two postural tasks: forward trunk bending and wrist extension. Surface electromyography (EMG) was recorded at a sampling rate of 1,000 Hz (TELEMG; BTS, Milan, Italy). For the lower limb task, electrodes were positioned over the rectus anterior, biceps femoris, tibialis anterior, and lateral gastrocnemius muscles of both lower limbs. For the upper limb task, two electrodes were placed over the extensor carpi radialis longus muscle. For two patients and two controls, the upper limb task was recorded using the Brainnet system (Medatec), so that EMG and EEG could be recorded at the same time for burst-locked back-averaging.

Electroencephalography

We retrospectively reviewed EEGs obtained for clinical purposes in AS patients with a cytogenetic or molecular diagnosis of AS from a personal joint series (Great Ormond Street Children's Hospital [London, U.K.] and Hôpital Universitaire des Enfants Reine Fabiola [Brussels, Belgium]) using conventional 10-20 electrode placement.[7] We also reviewed published studies of EEGs in AS since 1967. In this personal and published material, we analyzed the distinctive rhythmic EEG patterns recorded in AS:[7,8] pattern I consists of runs of high-amplitude 2–3/s rhythmic activity predominating over the frontal regions; pattern II consists of more diffuse runs of 4–6/s rhythmic activity; pattern III consists of bursts or runs of high-amplitude 3–5/s rhythmic activity, maximal over the occipital region, sometimes containing small spikes and facilitated by eye closure.

Mice

Cerebellar Recording

We recorded neuronal activity in the cerebellum[9] in eight mice with *ube3a* null mutation (*ube3a* m–/p+) developed as described previously[10] and nine wild-type (WT) C57Bl6 mice aged 10–13 months with a glass micropipette (1.5–5.0 MΩ impedance). Criteria for Purkinje cell (PC) recording and data analysis were the same as those used previously.[9] Signals were treated on-line and off-line (Spike 2 CED). Local field potentials (LFPs) were analyzed by wave-triggered averaging[11] and fast Fourier transform (FFT). PC pairs along the same parallel fiber beam were recorded by means of seven linearly arranged, quartz-insulated, platinum-tungsten fiber microelectrodes with 250-μm interelectrode spacing.[12] For drug microinjection, micropipettes (tip, 250 μm) were filled with either 27 mM SR95531 (gabazine, a γ-aminobutyric acid A [$GABA_A$] antagonist) or saline in control experiments.

Electrocorticography

We reviewed electrocorticographic data reported in the mouse models underlying AS. Electrocorticographic (ECoG) recordings were documented for mice with partial paternal uniparental disomy of chromosome 7A-C.[13] These were generated by intercrossing heterozygotes for a T(7;15)9H or a T(7;18)50H translocation. They showed mild gait ataxia, increased startle, and hyperactive behavior. ECoG data are also available for two models of *ube3a* m–/p+ mice. One was obtained using a targeting construct prepared to replace a 3-kb genomic DNA fragment containing exon 2, resulting in a deletion of 100 N-terminal amino acids and a shift in the reading

frame.[14] Although there was no obvious phenotypic abnormality in heterozygous mice with maternal deficiency, specific motor testing showed impaired coordination. The other *ube3a* m–/p+ model was constructed by deleting part of exon 15 and all of exon 16 and inserting a cassette containing an internal ribosome entry site and a *lacZ-neo*R fusion gene to permit the detection of allele knockout expression by lacZ staining.[10] These mice showed no obvious phenotypic abnormalities, but fine testing revealed impaired motor coordination and learning compared with paternal *ube3a*-deficient siblings. Of the transgenic models implicating other genes in the murine chromosomal region homologous for AS, the most relevant appears to be a mouse that is deficient in the *gabrb3* gene. Survivor homozygous knockout mice had seizures, hyperactive behavior, and coordination and learning impairments.[15] ECoG recordings have been documented.[15]

These projects were approved by the local ethics committee.

RESULTS

Human Electromyography

During the lower limb task, surface EMG recordings disclosed bursts of activity at frequencies of 6/s to 8/s in agonist–antagonist muscle pairs superimposed over persistent background tonic activity in patients with AS (FIG. 1A). These frequencies were confirmed by FFT. The bursts of activity were brief (15–25 ms) in duration. They appeared synchronous in homologous muscles of the right and left legs as well as in antagonistic muscles. Synchrony was confirmed by cross-correlation function analysis (significant [$P > 0.5$] maximal peak of cross-correlation coefficient at 0-ms lag). When extra mass was added (2 kg, ventrally), no significant change ($P > 0.1$) in the bursting pattern, duration, frequency, synchrony, or distribution was noted in the four patients in whom this was tested. No rhythmic bursting activity was seen in any of the controls. Occasional phasic activity was recorded in agonist–antagonist pairs, but these eventual bursts were longer in duration than the rhythmic bursts recorded in patients with AS, were mostly isolated, and never showed rhythmicity. With loading, the subjects tended to show tonic activity in the proximal muscles. No phasic activity was observed. In particular, no rhythmic EMG bursting appeared. During the upper limb task, extensor carpi EMG showed similar 6/s to 8/s bursting as recorded in lower limb muscles. It appeared over background tonic EMG. The frequencies were confirmed by FFT. This activity was not present at rest, during which virtually no EMG activity was seen. In the two patients who had an EEG during the task, the typical patterns of AS were observed, but no EEG change was seen during the task (FIG. 1B). In particular, no changes were correlated to the EMG bursting activity. Burst-locked back-averaging disclosed no spike or other graphic element before or during the bursts. In the controls, the task was associated with tonic activity of the muscle, with no bursts and in particular no rhythmic activity.

Human Electroencephalography

The interictal EEG findings were striking and distinctive (FIG. 2) and present in 98% of cases, although repeated recordings could be required to document the

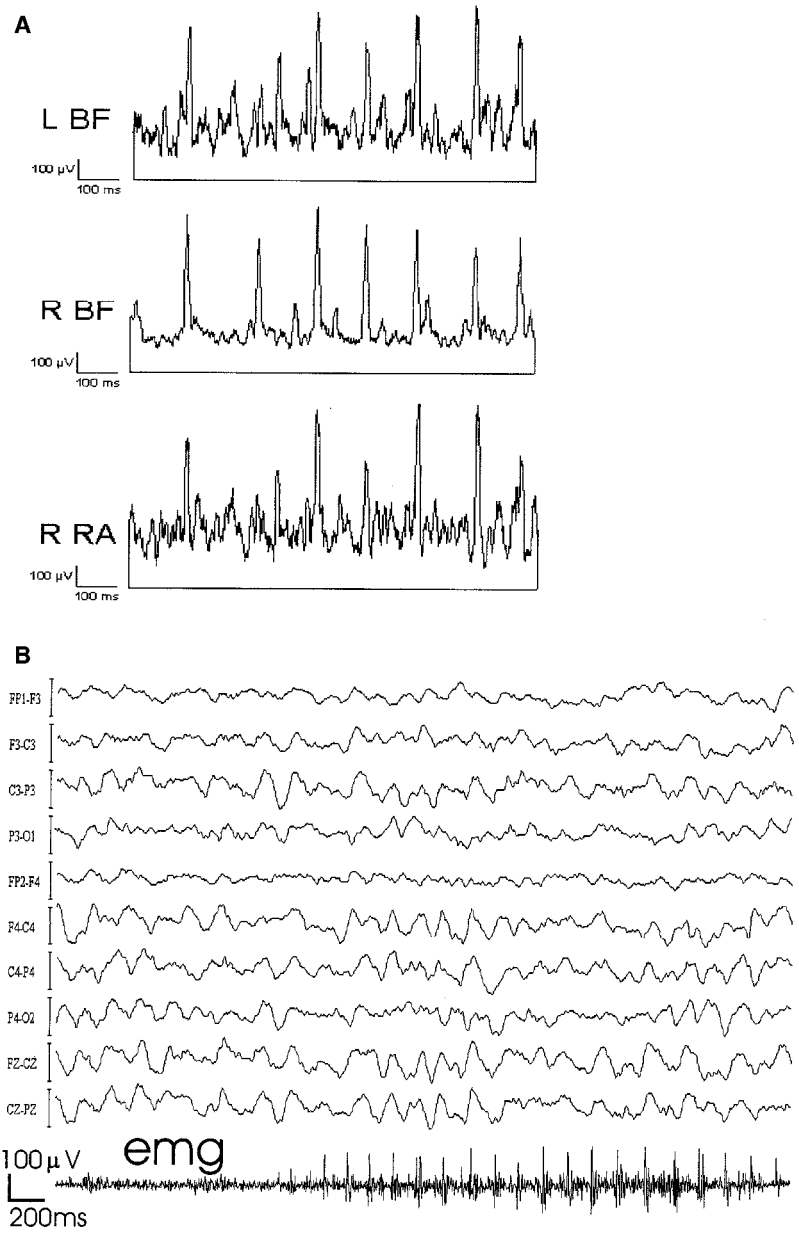

FIGURE 1. Postural rhythmic muscle bursting activity in Angelman syndrome. (**A**) Lower limb task. Rectified integrated EMG of the left biceps femoris (L BF), right biceps femoris (R BF), and right rectus anterior (R RA) during the lower limb task in a patient with Angelman syndrome. R BF has a periodogram peak at 2.09 for 7 Hz, with a spectral density of 1.31, and cross-correlation coefficient peaks at 0.68 and 0.66 at 0 ms with L BF and R RA, respectively. (**B**) Upper limb task. EEG and extensor carpi radialis longus muscle raw EMG before and during the upper limb task in a patient with Angelman syndrome. Note the absence of EEG change associated with the task and the rhythmic EMG bursting.

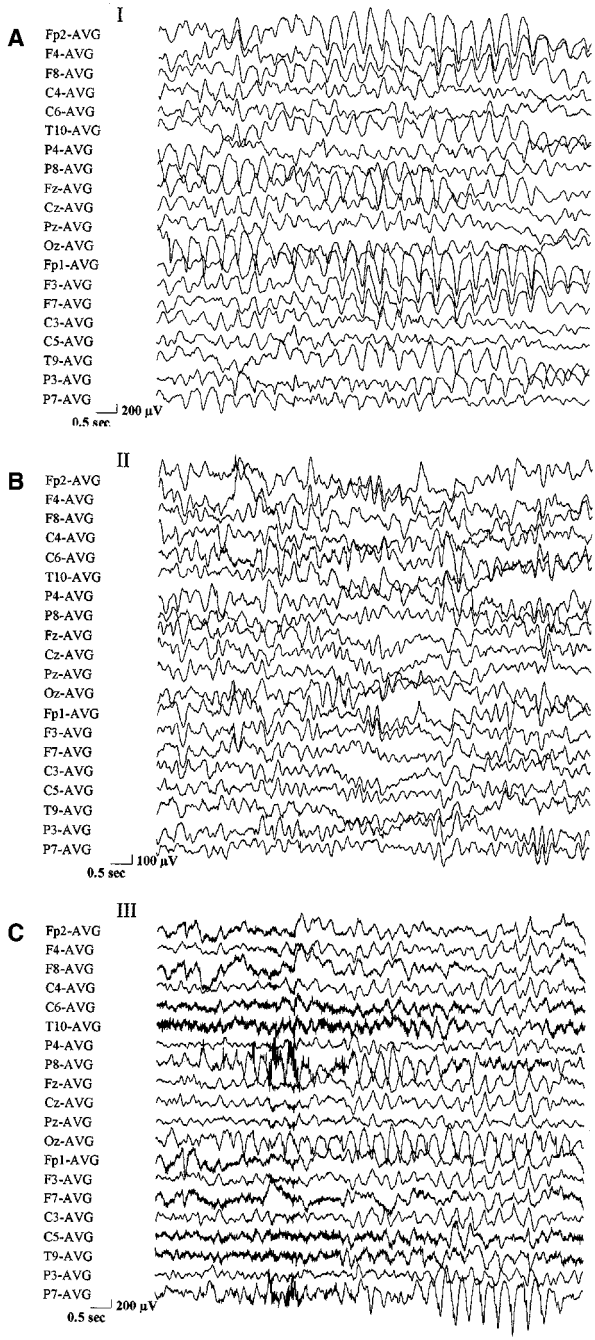

FIGURE 2. Characteristic EEG patterns associated with Angelman syndrome. (**A**) Pattern I: high-amplitude rhythmic 2/s to 3/s activity seen anteriorly. (**B**) Pattern II: runs of rhythmic 4/s to 6/s theta activity. (**C**) Pattern III: 3/s to 5/s rhythmic activity maximal over the occipital region.

different patterns. Patterns I, II, and III (FIG. 2)[7] were found equally across all genotypes, despite differences in clinical severity between molecular classes, patients without a deletion having a milder phenotype and less severe epilepsy. They appeared in the first year of life, uncommonly before 6 months. There was a marked paucity of physiological EEG rhythms. Sleep recordings showed prominent spindles with usual distribution and timing. Sporadic interictal discharges could be seen. By contrast, nonconvulsive status epilepticus was relatively common, manifesting as quasi-continuous generalized 1/s to 2/s sharp-slow complexes, usually accompanied by neurological deterioration. Epileptic seizures were commonly associated with their usual EEG manifestations, such as 3/s spike-wave complexes in myoclonic absences.

Mouse Cerebellar Recording

Spontaneous spindle-shaped LFP oscillations (LFPOs) were found throughout the explored cerebellar regions (vermis, uvula, and nodulus) of all seven alert *ube3a* m–/p+ mice, with frequencies of 158.9 ± 30.1 Hz and maximal amplitude of 0.45 ± 0.22 mV. Fast LFPOs were not recorded in WT mice. The LFPO amplitude increased when the electrode approached the PC layer, reaching its maximum just beneath the PC bodies, and disappeared abruptly when the electrode tip reached the fourth ventricle or medulla. LFPO was synchronized along the coronal plane (i.e., along the same parallel fiber beam). In contrast, paired sites aligned in the sagittal plane did not show synchronization (cross-correlation coefficient of 0.02 ± 0.11; $n = 8$). The spontaneous rate of simple spikes (SSs) was much higher in mutants than in WT mice. The rate of complex spikes (CSs) showed no statistical difference between groups. However, durations of CSs, and of the pause in SS firing after CSs, were significantly reduced. The SS and CS discharges were phase-locked to the LFPOs. SS discharges occurred at the depth of the LFP wave, whereas the CS discharge appeared during the LFP ascending phase. Given the role of $GABA_A$ transmission in fast brain rhythms, we studied the effect of microinjection of gabazine. This $GABA_A$ blocker reversibly reduced the LFPO amplitude.

Mouse Electrocorticography

In mice with uniparental disomy,[13] ECoG showed prolonged runs of bilateral high-amplitude (up to 800 μV) rhythmic activity (2/s to 5/s), in marked contrast to the normal low-amplitude irregular fast activity seen in WT mice. In *ube3a* m–/p+ mice, ECoG showed almost continuous runs of rhythmic 3/s activity mixed with polyspikes and slow waves[14] or runs of high-amplitude (750 μV) 4/s to 5/s spike-wave discharges, lasting 5–12 s and recurring every 2–3 min.[10] Both ethosuximide and valproic acid abolished the latter activity. Homozygous *gabrb3* knockout mice showed developmental changes in their ECoG recordings consisting of progressive slowing and subsequent appearance of irregular high-amplitude (500–600 μV) slow and sharp waves.[15] They also showed frequent myoclonic jerks and generalized clonic seizures with associated spiking. Ethosuximide abolished the spiking but not the slow activity. Carbamazepine exacerbated ECoG abnormalities. Valproic acid and clonazepam had no effect. *Gabrb3*–/+ mice had similar ECoG abnormalities and seizures.[15]

DISCUSSION

Using different paradigms, we characterized unique rhythmic neurophysiologic activities in the cerebral cortex, cerebellar cortex, and muscles of patients with AS and mouse models of this condition. In addition to the rhythmic patterns that were present equally in all AS molecular classes, cerebral cortical epileptiform discharges were found to be over-represented in patients and mice with *GABRB3/gabrb3* gene abnormality. EEG features in both AS patients and animal models suggest two separable categories: distinctive rhythmic patterns that are unrelated to epilepsy and less specific epilepsy-related discharge activity. The distinctive rhythmic EEG patterns are found with great consistency and show no relation to genotype or clinical phenotype, and in particular to the presence and severity of seizure disorder.[4,7] Recent developments in neurophysiology have indicated the central importance of rhythmic characteristics of neuronal firing in cerebral function.[16] EEG rhythms result from the synchronization of oscillatory activities between neural populations. Physiological EEG rhythms and some spike-wave discharges arise in the cortex as a result of thalamocortical interactions.[17] These interactions have been studied with particular reference to sleep spindles and childhood absence epilepsy. The waveform semiology of AS pattern III (FIG. 2C) is similar to that seen interictally in childhood absence epilepsy. Polymorphism studies have shown significant association in the transmission of *GABRB3* and childhood absence epilepsy.[18] The oscillatory firing in this condition is known to involve thalamocortical neurons, including those in the thalamic reticular nucleus.[19] In *gabrb3–/–* mice, studies of reciprocal inhibitory connections demonstrated virtual abolition of GABA-mediated inhibition specifically in the thalamic reticular nucleus as well as a dramatic increase in oscillatory synchrony.[20] Despite this, no rhythmic EEG activities were observed and only irregular discharges related to seizures were recorded from the cortex in these mice.[15] These epileptic features were abolished by ethosuximide, and this may link to similar effects in AS patients[2,7] and suggests a role for transient voltage-dependent calcium channels in this condition.

The postural rhythmic muscle bursting activity may be linked, as are most rhythmic muscle activities, to oscillations in the central nervous system involved in motor control, and in particular in the processing of motor commands.[21] The facts that the bursting EMG activity was time-locked in the right and left lower limbs and unaffected by loading suggest that it is driven centrally. Faster rhythmic activities than those we recorded in AS have been reported in normal adults performing various motor tasks, notably including isometric muscle contraction. Neurophysiological study of this phenomenon showed that oscillatory activity in the primary motor cortex is coherent, or phase-locked, to 15- to 30-Hz activity in contralateral muscles involved in a voluntary motor task.[22] However, lower levels of coherence were found during isometric contractions than in compliant conditions. Rhythmic isometric 12- to 18-Hz muscle contractions during postural activity also occur in primary orthostatic tremor, a rare and poorly understood condition of cerebellar origin. In this condition, the tremor appears to be best expressed in weight-bearing muscles and in muscles active in sustained isometric contraction, facilitated when loaded, and EMG discharges are synchronous between homologous muscles. On the basis of multiple-electrode recordings of PCs in rats, Welsh and Llinás[21] have postulated that phase-locking of 6- to 10-Hz oscillations arising from the inferior olive enables the dynamic linking

of groups of olivary outputs to the cerebellum. In AS, cerebellar dysfunction contributes to the motor impairment,[23] as already suspected by Angelman.[1]

The spontaneous 160-Hz LFPO we found throughout the cerebellar cortex of *ube3a* m−/p+ mice was never recorded in WT mice. However, it is physiologically similar to the 160-Hz LFPO previously described in mice lacking calretinin or calbindin.[9] In these three groups of knockout mice, the emergence of high-frequency LFPO is related to an increase in SS firing, a decrease in CS duration, shortening of the pause in post-CS SS firing, and increases in rhythmicity and synchrony. The depth recording profile, the synchronization between LFPO and both SS and CS, and the LFPO synchronization along the parallel fiber beam suggest that PC populations are the major generator of the fast 160-Hz LFPO. LFPO suppression by gabazine could indicate the contribution of inhibitory molecular interneurons. In the molecular layer, stellate cells form a densely connected network through fast $GABA_A$ receptor synapses.[24] PCs are regulated by the output of the molecular interneuron network, which provides the dominant inhibitory input to PCs through fast GABAergic synapses.[25] Golgi cells can be excluded as generators of the oscillation because the $GABA_A$ synapses they make on granule cells, their principal targets, induce inhibitory postsynaptic currents with a slow decay.[25]

The discovery of the GABRB3 gene in the 15q11-13 region[26] made it a strong candidate for explaining the molecular pathogenesis of AS. All $GABA_A$ receptor subtypes contain a β subunit, and the β3 subunit is the only one present early in life.[27] However, this cannot account for all of the features of AS. Patients with cytogenetic alterations in this region (e.g., deletions [Prader–Willi syndrome], pericentromeric inversions and duplications [inverted duplication of chromosome 15, or inv-dup(15) syndrome], or other types of duplications found in some cases of autism, either isodicentric or intrachromosomal) have distinct clinical phenotypes. Moreover, ~30% of patients with features of AS do not have a deletion involving the GABRB3 gene (i.e., patients with imprinting defect, paternal uniparental disomy, UBE3A mutation, or no detectable 15q11-13 abnormality). Furthermore, a patient with a deletion involving the GABRB3 gene but not the critical region for AS did not show the clinical characteristics of AS, although he did have seizures.[28]

The mechanism by which UBE3A inactivation results in AS is still unclear. This gene is specifically imprinted in the brain, with different region-specific levels of silencing of the paternal allele. In different mouse models, evidence of expression of ube3a has been found predominantly in the hippocampus, olfactory bulbs, and cerebellar PC. The product of the ube3a gene, ubiquitin-protein ligase E3A (abbreviated ube3a), influences the trafficking of other proteins between cellular compartments through its protein ligase activity along the ubiquitin pathway. Ube3a plays a role in ubiquitin-mediated protein catabolism. Ubiquitination pathways have been implicated in the pathophysiology of other neurologic disorders,[29] such as early-onset autosomal recessive Parkinson disease (OMIM#600116) (due to abnormalities of the gene coding for parkin, another E3 ubiquitin-protein ligase).

It may be speculated that the effect of the functional absence of the UBE3A gene gives rise to AS through several mechanisms. It has been proposed that disruption of a step in the ubiquitin pathway of proteolysis results in the accumulation of intracellular proteins.[30] These proteins could then interfere with the function of different tissues, and perhaps more specifically with the expression of other genes. This phenomenon might be enhanced in the brain because of a lack of compensation by other

proteolysis mechanisms that do not include the specific functional aspects of UBE3A and also because neurons do not divide after differentiation. However, ubiquitination has other roles in specific protein labeling than targeting for proteolysis. It was recently demonstrated that UBE3A interacts with ubiquitin-like Plic proteins.[31] This is of particular interest because Plic-1 selectively binds to $GABA_A$ receptors containing the β3 subunit and regulates the number of these receptors in the cell membrane.[32] This process is critical for the regulation of GABAergic synaptic strength.[33]

This would provide a more complete mechanism for AS whereby the functional absence of UBE3A would impair the regulation of $GABA_A$ receptors, likely leading to stoichiometric rearrangements. Decreased binding of ligands for the benzodiazepine site of $GABA_A$ receptors has been demonstrated in mice with knockout gabrb3 genes and AS patients with GABRB3 deletion, but not in mice with deficient maternal ube3a gene or in one patient with a UBE3A mutation.[34,35] This may be explained by the biallelic expression of GABRB3 in the brain in humans[30] and its murine homolog gabrb3.[15] The differential effect of benzodiazepines on the typical EEG patterns of AS suggest that $GABA_A$ receptor subunit rearrangements also involve α and γ subunits, since their action does not depend on the β subunit subtype of the $GABA_A$ receptor.[36]

Pharmacological agents that interact with the $GABA_A$ receptor complex, such as benzodiazepines, barbiturates, and corticosteroids, and other drugs that increase GABA level at GABAergic synapses have been abundantly used to control the seizure disorder associated with AS. However, several cases of clinical deterioration have been recorded after treatment of epilepsy with vigabatrin. As this medication potentiates GABAergic neurotransmission by inhibiting GABA transaminase, it seems paradoxical that it would aggravate a condition thought to be due to a deficiency in $GABA_A$ receptors. Nevertheless, $GABA_A$-ergic drugs have not been proposed to alter other aspects of the syndrome expression.

The heterogeneity of $GABA_A$ receptor subunits determines not only functional differences between subtypes but also differential interactions with drugs. For AS, it would be interesting to develop a pharmacological agent that specifically augments $GABA_A$ receptors containing a β3 subunit. In this context, it is of interest that lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] has been recognized as an effective anticonvulsant in AS. Although this drug has no direct effect on $GABA_A$ receptors,[37] it was recently shown to increase *GABRB3* gene expression.[38]

The effect of methylation of the *UBE3A* gene on its activity has also fostered hopes that epigenetic modulation might lead to restoration of its function. The larger part of the paternal chromosome 15q11-13 is unmethylated, and most of the genes located in this region are expressed. DNA methylation can be affected by dietary levels of methyl-donor components, such as folic acid.[39] Empirical oral supplementation of folinic acid normalized 5-methyltetrahydrofolate levels in the cerebrospinal fluid and led to partial clinical improvement in four patients with Rett syndrome,[40] a condition that shows a clinical overlap with AS.[41] Given the possible phenotypic similarities between AS and methylenetetrahydrofolate reductase deficiency (OMIM #607093),[42] Beaudet and Bacino[43] hypothesized that low folic acid might decrease *UBE3A* gene expression and high folic acid might increase it. Formulating the serial hypotheses that dietary manipulation might increase DNA methylation, that this eventual increase would favor expression of the paternal *UBE3A* gene, and that even-

tual, even late, expression of this gene would improve the patient's condition, this team proposed to treat AS patients with a diet enriched with high doses of folic acid and betaine. Folic acid was expected to increase DNA methylation, and betaine was expected to enhance the effect of folic acid. Early clinical results of a double-blind trial conducted in children have not shown clear changes, although the possibility of moderate benefits has not been analyzed.[43]

Another avenue to alter *UBE3A* gene methylation involves epigenetic mechanisms that contribute to the regulation of imprinting, such as modification of the chromatin structure that constitutes the functional mark distinguishing the two alleles at imprinted domains. Developmentally, this may be generated through a number of different molecular pathways, including differential DNA methylation or asynchronous replication timing.[44] The role of histone methylation in the maintenance of parent-specific DNA methylation[45] suggests a role of histone methylation in establishing DNA methylation patterns and has direct relevance to *UBE3A* gene expression. The Angelman syndrome–shortest region of deletion overlap (AS-SRO) has been shown to be packaged with acetylated histone H4 and methylated histone H3(K4) only on the maternal allele, and this imprinted epigenetic structure is maintained through cell divisions despite the absence of clear differential DNA methylation.[46] Pharmacological approaches that could induce histone modification or more generally interfere with chromatin packaging might promote the demethylation or remethylation of imprinted genes or of imprinting control regions.

CONCLUSION

Unique rhythmic neurophysiological patterns can be found in AS, a condition caused by the lack of *UBE3A* expression in the brain. In physiological conditions, the maternal allele of this gene is imprinted. Lack of *UBE3A* expression may result from several mechanisms, including deletion of the 15q11-13 region where this gene and *GABRB3* are located, paternal uniparental disomy, an imprinting defect, or *UBE3A* mutation. Animal models corresponding to the different molecular classes have been generated. An integrative hypothesis for the molecular pathophysiology of this syndrome suggests dysregulation of synaptic neurotransmission through *UBE3A*-related recruitment of functional $GABA_A$ receptors and the *GABRB3*-related amount of β3 subunit in these receptors. This would account for the developmental changes as well as the differences in severity between deletion and nondeletion cases. In addition to rehabilitation programs adapted to the patient's potential, promising treatment approaches may include pharmacological agents that interfere with $GABA_A$ receptors, increase *GABRB3* expression, or alter DNA methylation.

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