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REVIEW ARTICLE

Almeida et al: Molecular aspects of Angelman Syndrome

Molecular aspects of Angelman Syndrome: Defining the new path forward

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ABSTRACT

As a rare neuro-genetic disease, Angelman Syndrome (AS) affects about 15 to 500 thousand people worldwide. The AS is an imprinting genomic disease characterized by the loss of function of the maternal *UBE3A* gene, located in the 15q11-q13. This gene encodes a ~100 kDa protein, the Ubiquitin-protein ligase E3A (UBE3A), that participates in the ubiquitination process, one of the post-translational protein modifications. In the brain, under normal conditions, the paternal allele of the *UBE3A* gene is silenced, with only the maternal allele being active. However, in individuals with AS, the maternal loss of function of this gene leads to the complete absence of UBE3A expression, resulting in multiple pathological features. Clinically, children diagnosed with AS exhibit a characteristic behavioral phenotype, including a happy demeanor, frequent and unmotivated laughter, movement, speech impairment, severe intellectual disability, and sleep problems. Since its discovery in 1965, significant progress has been made in understanding the genetic and pathophysiological aspects of AS. However, despite these advances, the molecular mechanisms underlying the disease remain incompletely understood, and no effective treatment currently exists. Current therapies focus solely on symptom management, and no approach has yet succeeded in reactivating the silenced paternal *UBE3A* allele. Therefore, this review highlights the epigenetic aspects involved in the AS in order to provide a better understanding and clarification of the mechanisms, hopefully paving the way for future research to improve the treatment of affected individuals.

Keywords: Angelman Syndrome; AS; epigenetic repression; genetic imprinting disorders; neuronal plasticity; UBE3A silencing mechanism

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INTRODUCTION

Neurological conditions are the leading cause of illness and disability worldwide (1), in 2021 there were more than 3 billion people in the world suffering from some neurological disorders (2). Among these conditions, neurogenetic disorders represent one of the most significant and challenging groups, comprising also neurodevelopmental disorders (NDDs).

NDDs encompass a wide heterogeneous group of diseases that typically manifest early in life and are primarily linked to impairments in neurodevelopment. In 1965, a new disease had been adding to this list of NDDs, the Angelman Syndrome (AS), characterized by the loss of function of the *UBE3A* gene, inherited from the mother, located in the 15q11-q13 chromosomal region (3,4) **.** The gene *Ubiquitin-protein ligase E3A* (*UBE3A*) encodes a ~100 kDa protein with the same name that participates in the ubiquitination process, one of the post-translational protein modifications (3).

The loss of functional *UBE3A* gene causes directly and indirectly several pathological features. Despite the fact that children with AS have normal prenatal and birth history, also with normal laboratory parameters, the delay of the achievement of the milestones is perceived only around 6 months of life (5), and many clinical features are overlapped with other NDDs characteristics such as movement or balance disorder, speech impairment and behavioral abnormality, which leads a late diagnosis around 12-20 months of life (6).

Significant advances in the genetic aspects of this disease have been made since its discovery in 1965. Notably, in 1984, it was recognized that AS represents a striking example of genomic imprinting—an epigenetic phenomenon in which a gene is monoallelically expressed based on parental origin. Under normal conditions, the paternal allele of the *UBE3A* gene is silenced, with only the maternal allele being active. However, in individuals with AS, a maternal loss of function in this gene occurs, leading to the absence of *UBE3A* expression.

Genetic imprinting is only one example of many epigenetic phenomena, in this case, modulated by DNA methylation. In the *UBE3A* gene location, chromosome region 15q11-q13, an imprinting center, located 35kb upstream of the *SNURF-SNRPN* promoter bicistronic gene (4), regulates the imprinting area by DNA methylation in a mechanism that can be coordinated by the long noncoding antisense RNA *SNHG14* (7).The imprinted domain on human chromosome 15 consists of two oppositely imprinted gene clusters, which are under the coordinated control of an imprinting center (IC) at the 5' end of the *SNURF-SNRPN* gene. In this way, the maternal-only expression of *UBE3A* may be regulated indirectly through a paternally expressed antisense transcript. In particular, a processed antisense transcript of *UBE3A* starts at the IC. The *SNURF-*

SNRPN sense/ *UBE3A* antisense transcription unit contains at least 148 exons, including the previously identified IPW exons (i.e., HBII-13, HBII-85 and HBII-52 snoRNAs, as well as for four additional snoRNAs HBII-436, HBII-437, HBII-438A and HBII-438B) (3,7,8).

Despite all those steps forward in clarifying the complexity of the molecular aspects of this disease, it is still a puzzle, and unfortunately, there is no effective treatment for this disease, the current therapy is only based on managing the symptoms. Also, there is no effective treatment to reverse the imprinting paternal silenced gene so far. Thus, this review aims to summarize all the molecular aspects of the AS, highlighting the lack of sufficient epigenetic studies involving the AS, hopefully clarifying the road to future research to improve the treatment of affected individuals.

Clinical aspects

Neurogenetic disorders are a wide range of diseases that arise during the development of the nervous system. The overlap of clinical features among the neurodevelopmental disorders leads to a broad differential diagnosis with at least 13 neurological diseases, making it difficult to individualize a precise early diagnosis (9,10).

In 1965, a new neurological disease was added to this list, when a physician Harry Angelman, in England, observed three unrelated children with similar features described as flat heads, jerky movements, protruding tongues, and bouts of laughter. The physician, at the time, had taken a vacation in Italy and saw an oil painting called “A Boy with a Puppet” that reminded him of those three children, after that he published the first work about the disease and named those patients as “Puppet children”, lately known as Angelman Syndrome (AS) on his behalf (11).

AS is a rare neurogenetic disorder affecting about 15 to 500 thousand people worldwide (https://www.angelman.org/). The reports in the literature about the incidence are low, varying from 1:10 000 up to 1:24 000 (12, 13).

Despite the overlapping clinical features with other neurological disorders, the most typical clinical findings presented in the affected individuals with AS are severe developmental delay by age 6 to 12 months, delayed achievement of developmental milestones but without loss of abilities, lack of speech, epilepsy, sleep problems, gastrointestinal problems, fascination with water, consistent behavioral phenotype with a happy demeanor and an easily provoked laughter and hyper motoric behavior (Table 1) (4, 14–16).

The diagnosis of AS is not easy due to those shared clinical characteristics and a late diagnosis comes around 12-30 months (Table 1) (14–17, 17–21). The sensibility and the positive predictive value of non-invasive prenatal tests for microdeletions, especially for AS, are low and have some critical issues that make it difficult to reach an assurance in the early diagnosis of AS (22).

Genetic aspects

In 1987, in different works Lawrence Kaplan and Ellen Magenis (23,24), observed the deletion on the long arm of chromosome 15 in patients with AS pointing out the possible genetic cause of the disease, the same deletion already seen in another genetic disorder, the Prader-Willi Syndrome (PWS).

Soon enough, by the late 1980s, studies in a small cohort of patients suggested a possible maternal origin of AS (25–28). This was confirmed in 1992 by Smith and colleagues in a larger cohort of 25 individuals, with all of them showing the maternal pattern of inheritance, describing that PWS is caused by the loss of part of chromosome 15 from the paternal inheritance, while the AS is caused by the loss of the same portion of the same chromosome, but from the maternal inheritance (29). Finally, in 1997, Kishino and colleagues identified the gene involved in the AS, E6AP-E3 ubiquitin-protein ligase gene (*UBE3A*) (30).

The portion of 15q11-q13, which comprises all the genes involved in both syndromes PWS and AS, is a region likely to be regulated by genomic imprinting, and it is called the imprinting center. Genomic imprinting is an epigenetic phenomenon in which the gene is monoallelic expressed according to the parental origin. Those two syndromes are beautiful examples of imprinting disease, in which a paternal loss of the chromosome leads to the clinical features of PWS, while a maternal loss occurs it leads to the AS.

The gene *UBE3A* is biallelically expressed in non-neuron cells, while in neuron cells, it is expressed only in the maternal inherited allele (31). This imprinting expression is due to the action of the *UBE3A* antisense transcript (formerly *UBE3A-ATS*), now known as *SNHG14*, which silences the paternal allele only in neuron cells, which will be better discussed later in the next topic (31).

Initially, the deletion of 15q11.2 seemed to be the only known cause of AS. However, in 1990, studies began to reveal that the deletion of chromosomal 15q11-q13 was not present in every patient, suggesting another molecular cause besides the deletion (32, 33).

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Nowadays it is known that AS, actually has four molecular possibilities of a cause involving the maternal loss of chromosome 15q11-q13: the most common is the de novo deletion of approximately 4 Mb of the 15q11-q13 with 70-85% of the cases (patients class I) (30,34), followed by the patients with intragenic mutations on gene *UBE3A* (patients class IV) with 10-30% of the cases (35–38). In contrast, less common causes are paternal uniparental disomy (UPD) with 2-5% (class II) and also defects of the imprinting process with 3-5% (class III) (4, 30, 39, 40). Nevertheless, a fifth class of patients does not fit in any other classes, without an established genetic cause but presenting all the main clinical features of the disease (40). In patients from classes IV-V, there is normal DNA methylation different from classes I-III that can be easily diagnosed by DNA methylation analyses of the 15q11-q13 imprinted center (40,41) (Figure 1).

The classification of the patients based on their molecular status appears to influence the disease's clinical course and progression. Considering all the molecular mechanisms involved in AS, accurate classification of the AS patient is crucial for clinicians to understand the clinical features better and to guide the scientific community in finding an effective treatment for AS patients. For this, an AS diagnostic algorithm has been used starting with the DNA methylation analysis of 15q11-q13, if it is normal the mutation test is performed to classify into IV (*UBE3A* mutation) or V patients (unknown cause). Otherwise, in case of abnormal DNA methylation, a FISH or microarray analysis is performed to seek microdeletion in case of class III patients (imprinting defect), or in case of microdeletion is not present a test for uniparental disomy (UPD) using DNA markers is done to reach the class II patients (UPD) (16).

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Molecular epigenetics of Angelman syndrome

Epigenomic signatures include histone variants and modifications, alterations in nucleosome positioning, DNA methylation, and non-coding RNAs (42). The first work to suggest the possible gender influence on the offspring's genetic inheritance was published in 1984 (43, 44). In this work, Davor (43) and James McGrath, as well as Azim Surani and coworkers (44, 45) independently, tested two types of embryos containing either two sets of chromosomes inherited exclusively from the father or exclusively from the mother, transferring them into pseudo-pregnant recipient females. However, the embryos failed to develop to term. This experiment demonstrated that, although the chromosomes were genetically identical, they were not functionally without the presence of the opposite parental origin. Thus, for normal embryonic development to occur, one set of each chromosome from each parent is required (46). This phenomenon is called "genomic imprinting", in which gender epigenetics inheritance dictates gene regulation, and parental offspring inherit an imprinted marker, named gametic differentially methylated region (gDMRs), this nomination was primarily described when the first imprinted genes were discovered, *Igf2r, Igf2* and *H19*, in 1991 (47–50). This "marked area", the differentially methylated region (DMR) inherited, will direct the parental-specific allelic expression from the mother or the father, and it is named imprinting center (IC). In the case of genomic imprinting disorders, such as PWS and AS, DNA methylation is essential to maintain the complexity of the imprinting phenomenon (8).

In 1992, it was revealed that the well-conserved area of the D15S63 locus in 15q11-q13 is methylated in the chromosome from the mother in PWS, while it is unmethylated in the father's inherited chromosome, on the other hand, in AS occurs the opposite (51, 52), serving as a diagnostic marker for classification of AS patients (40). The DNA methylation, in the IC of chromosome 15, leads to a suppressing gene expression, resulting in a silenced gene. Thus, identifying the gene or cluster of genes of the DMR is useful for understanding the epigenetics of imprinting diseases (7).

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In healthy individuals, the gene *UBE3A* is exclusively maternally expressed in the brain, while the paternal allele is imprinting silenced. This inactivation of the paternal copy of *UBE3A* is regulated by the *SNURF-SNRPN* bicistronic gene and orchestrated by the long noncoding antisense RNA *SNHG14* (formerly *UBE3A-ATS*) (7, 53). The maternal copy of chromosome 15q11-q13 is methylated on the region of PWS-IC. This epigenetic marker causes the gene to be silenced because the methylation prevents transcription factors from binding to the promoter and activating gene transcription. While in the paternal allele, non-methylated, the bicistronic gene *SNURF-SNRPN* is able to transcript the lncRNA *SNHG14* (54).

Long noncoding RNAs, as their name implies, are not translated into a protein, and they have by definition a long length greater than 200 bp (8). The *SNHG14* lncRNA has a very long length of 3700 kb, and is also called a macro noncoding RNA (ncRNA). The lncRNA are present spliced in cytoplasm and/or present mainly unspliced in the nucleus, giving them a characteristic short half-life compared with mRNA (8, 55).

In mouse neurons, the *Snhg14* lncRNA is expressed only in the paternal allele while the *Ube3a* is expressed only in the mother. The proposed model of *Ube3a* silencing in the maternal chromosome in neurons is a collision model, it means that while a RNA polymerase is transcribing the *Snhg14,* the transcription area extends along the *Ube3a* termination area. This overlap of coding region and consequent transcriptional collision of RNA polymerases leads to a truncated elongation and subsequent degradation of *Ube3a* paternal transcript (8) (Figure 2). Thus, a *SNHG14* inhibitor would be a potential target treatment for the unsilence of the paternal copy of *UBE3A* (56). An American group observed that mice *Ube3a* (m-/p+) treated with topotecan, a Topoisomerase I inhibitor, had an upregulation of UBE3A expression compared with the wild-type (57). Showing that the inhibition of Topoisomerase I can disturb the transcriptional progression along the lncRNA *SNHG14* region and once *SNHG14* is not expressed on paternal chromosome, the UBE3A is no longer suppressed. However, these inhibitors of topotecans do not act only in chromosome 15q11-q13, so this lack of specificity may make this treatment approach less attractive for humans.

As evidenced by the above reported information, literature concerning epigenetic mechanisms in AS is still limited. However, elegant studies have been performed in neurodevelopmental disorders, showing clinical similarities with AS (reviewed in (42). Studies of children with neurodevelopmental defects indicate that DNA methylation and histone modification are crucial for normal brain development (58). Moreover, a right transcriptional regulation exerted

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by chromatin remodeling, as well as by the action of non-coding RNAs (ncRNAs; e.g., miRNAs and lncRNAs) have been showed to exert a crucial role in neurodevelopmental processes (53,59–62).

Nowadays, the most advanced approach for AS is using antisense oligonucleotides (ASO) targeting a conserved region of *SNHG14*, repressing its transcription, and allowing the expression of paternal *UBE3A* (63, 64). Dindot and colleagues obtained promising results using this ASO both in vitro and in vivo with monkey specimens (63). This therapeutic approach is currently in clinical trials (GeneTx NCT04259281; Roche NCT04428281). However, beyond the challenge of determining the optimal timing for re-establishing functional paternal *UBE3A* expression in human clinical testing, there is also the consideration of *UBE3A's* interactions with other proteins and pathways that may be disrupted by its absence in the brain (Figure 3a) (64). These interactions should be carefully evaluated when designing new therapies.

The homeostatic level of *UBE3A* expression is critical to maintaining normal neuronal function

The ubiquitin-proteasome system (UPS) is a large group of post-translational modification proteins responsible for the intracellular degradation in eukaryotic cells (3,65). The ubiquitination process is important to maintain cellular homeostasis by regulating several cellular functions such as proteasomal degradation, selective autophagy, cell signaling, endocytosis, receptor trafficking, DNA damage response, cell cycle control, and programmed cell death (3). The *UBE3A* gene encodes a large ubiquitin-protein E3 ligase, a ~100 kDa protein that participates in the three-step ubiquitination, including a cascade of three enzymes E1, E2, and E3 (3,15). Initially, E1 enzymes activate the Ub amino acid transferring them to E2, and then the E3 ligases recognize the E2 complex and facilitate the subsequent transfer of the Ub to the target protein (66).

The E3 ubiquitin-ligase is responsible for ensuring the specificity of the ubiquitination process, so is plausible to have a large amount of those (so far there are more than 800 proteins described) and only a small portion of E1 activating enzymes and E2 conjugating enzymes (65, 66). Those E3 ligases can be classified in four types: the most common Really Interesting New Gene (*RING*) finger type and the Homologous to the E6-AP Carboxyl Terminus (*HECT*) type, and also the less common U-box type and the RBR type (66).

The UBE3A protein was initially also known as E6-associated protein (E6-AP) because it specifically acts together with the Human Papillomavirus E6 oncoprotein to degrade the cell

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cycle protein p53 (67). Nevertheless, later it was revealed that this degradation occurs only in the presence and association with E6 viral oncoprotein (68).

In 1998, Jiang and colleagues established a mouse model for AS (69), generated by a completed knocked-out maternal gene *UBE3A* on exon 2. Those mice presented the main clinical features of AS such as motor disability, seizures, sleep disturbance, and a learning-memory deficiency but also showed an increased cytoplasmatic p53 in postmitotic Purkinje cells in m-/p+ mice. Considering the findings of Cooper in 2003 (68), it is possible that E6-AP can play an important role in the regulation of the amount of p53 in vivo, by using some substitute molecule for E6, as previously suggested by Jiang (69).

The UBE3A protein plays an important role in the target protein recognition providing specificity in the ubiquitination process. Thus, the absence or deficiency of this protein, and transcriptions, in the nervous system would be extremely detrimental to neuron cells. While the deficiency of UBE3A leads to AS, its increased levels cause autism spectrum disorder (ASD) (41). The duplication of the portion 15q11-q13 chromosome leads to an increase of UBE3A protein raising the symptoms characterized by ASD, a phenomenon seen in rodents (69), showing that the correct amount of UBE3A dictates the clinical course of the affected individual.

The gene *UBE3A* also plays an important role in gene expression by generating several transcriptional factors that interact with various molecules. Ferdousy and colleagues, in 2011, in an experiment in Drosophilas flies, showed that *UBE3A* (Dube3A) transcriptionally coactivates and upregulates GTP cyclohydrolase I (GCH1). Therefore, the absence of Dube3A in Drosophilas results in increased levels of dopamine and its precursors (70).

In addition, there are evidences that *UBE3A* transcriptions are essential to maintain circadian clock by regulating the transcriptional factor *BMAL1* (brain and muscle Arnt-like 1). Gossan and colleagues have shown that the levels of *UBE3A* (*in vivo*) are critical for regulating the circadian system in mammals and flies, showing that in the absence of UBE3A, the levels of BMAL1 protein are higher in wild type rodents (71). *UBE3A* also interacts with the factors ECT2 (Epithelial cell transforming factor) and Ephexin V (E5). Those molecules interact with Rho GTPases, which in turn are essential to maintain the correct dendritic spine density, contributing to the neuronal plasticity in the brain. The lack of UBE3A expression leads to a dysregulation of those two molecules and could consequently cause memory and learning impairment (72).

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The UBEA3 protein also acts as a coactivator of steroid hormone receptors such as progesterone, estrogen, androgen, glucocorticoid, retinoic acid receptor-α, and thyroid hormone receptors (73). The lack of a functional protein UBE3A in the proper levels in the brain leads to an accumulation of its targets, which would probably contribute to the pathogenesis of AS (Figure 3b).

The impact of UBE3A deficiency on cellular pathways

Studies demonstrate that the levels of UBE3A influence important cellular pathways such as cAMP, MAPK, c-Jun-N-terminal Kinase (JNK), and extracellular-signal-regulated kinase (ERK) levels. Filonova and colleagues (2015) showed that in an AS mouse model (Ube3a m-/p+) the p44/ p42 extracellular signal-regulated kinase (ERK1/2) activation is impaired after neuronal depolarization, demonstrating that the absence of UBE3A reduces MAPK activation in the brain (74), which also influence in the synaptic plasticity and memory formation. The lack of UBE3A leads to increased JNK activity, a stress signaling pathway, and a decreased p-ERK/ERK ratio in heterozygotes (m-/p+) mice versus wild-type (75). The activation of JNK in the brain could participate in the neurodegenerative process, by phosphorylating c-Jun, in consequence activating the neuronal death process, suggesting that inhibitors of JNK signaling in the brain could be a good treatment target (Figure 3b). Since UBE3A is an important ubiquitin-protein, partially responsible for the degradation of the intracellular proteins, their lack would generate an accumulation of several substrates that would directly affect cell signaling.

Vatsa and colleagues showed, also in a mouse model of AS, that in rodents *Ube3a* (m-/p+) the miRNA-708 is downregulated in the brain of those mice. Since miRNA-708 is involved in the regulation of intracellular calcium homeostasis, essential for neuronal function, once it is deregulated there is an abnormal rising of calcium signaling in AS mice. This disruption could affect synaptic plasticity in the AS context (76).

It is seen that in AS mice model (m-/p+) there is a disruption in the neuroplasticity process, specifically long-term potentiation (LTP) in their hippocampus (69, 77). To maintain basal conditions of synaptic plasticity and transmission, there is an orchestrated process among the G protein-coupled receptor (GPCR) of the Adenosine, especially adenosine A2A receptor (A2AR) and also adenosine A1 receptor (A1 R) (78–80). In basal conditions in brain A2AR expression is low compared with A1R, but when high-frequency-induced synaptic is present A2AR is upregulated, which means that this receptor is recruited only during higher

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frequencies of nerve stimulation-inducing synaptic changes, such as LTP (79). In light of this information, there is evidence that A2AR may be involved in the physiopathology of the AS disease. A Portuguese group, in 2020, tested if A2AR blockade could improve memory dysfunction and synaptic plasticity. They observed that in AS mice model (*Ube3a* m-/p+) there was an inability to use hippocampal-dependent strategies for learning and memory in the Morris Water Maze with an upregulation of A2AR expression in the hippocampus tissue. Those mice were chronically treated with a selective A2AR antagonist and the function of hippocampal-dependent learning strategies and LTD deficits were restored (81).

So if the absence of *Ube3a* in rodents leads to an accumulation of A2AR it is plausible that the lack of UBE3A in humans could also interfere with the expression of adenosine receptors in the brain. As a matter of fact, the A2BR is known to play an important role in energy regulation in the brain, participating in cAMP signaling in astrocytes to tune the metabolic activation of these glial cells via cAMP–PKA signalling pathway, and also there is an upregulation of this adenosine receptor in the brain to support this function (82). Therefore, investigation of the role of adenosine receptors in UBE3A models would be valuable to understanding the physiopathology of AS and hopefully open new ways to a combined treatment approaches.

Future perspectives

Genetic imprinting is one of the most fascinating aspects of molecular genetics, and AS is one of the imprinting disorders affecting up to 500,000 people worldwide. Over the past 60 years, significant scientific progress has been made in understanding the molecular and genetic aspects of the disease. However, an effective treatment remains elusive. Currently, the most studied therapeutic approach targets the inhibition of *SNHG14*, either directly through antisense oligonucleotides (ASOs) or indirectly via topotecan-mediated inhibition. Nevertheless, concerns persist regarding the specificity and efficacy of this strategy in in vivo models, as well as the optimal timing for re-establishing functional paternal *UBE3A* expression in human clinical trials.

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CONCLUSION

In conclusion, elucidating the molecular mechanisms underlying the silencing of the paternal *UBE3A* allele is crucial for addressing the root cause of AS and restoring functional UBE3A protein expression in affected individuals. However, given UBE3A's interactions with numerous other proteins in the brain, it is also essential to consider the modulation of abnormal signaling pathways to achieve a more effective combination therapy. Furthermore, investigating the imbalance in receptor expression in the neuronal cells of AS models appears to be key to unlocking one of the many promising avenues for treatment targets.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Overview of clinical features and genetic cause of AS patients, from studies with large cohort published in the last 5 years.

Characteristics| Authors	Du et al., 2024 (PMID: 36011358)	Carriero et al., 2024 (PMID: 38930051)	Bindels-de Heus et al., 2019 (PMID: 31729827)	Den Besten et al., 2020 (PMID: 33108066)	Manoubi et al., 2024 (PMID: 38322471)
Total of patients	695	62	100	95	50
Mean or range of age (months)	6.34 ± 2.94	8.0 ± 17.7	5.7±4.8	31.6±12.6	12-84 months
Country of the study	China	Italy	Netherlands	Netherlands	Tunisia
Age at diagnosis (months)	31.7±24.14	24±11.4	30±27.6	NR	NR
Symptoms					
Epilepsy	554 (79.7%)	51 (82,2%)	82 (82%)	84 (89.4%)	44 (88%)
Sleep problem	613 (88.2%)	43 (69,4%)	91 (91%)	81 (88%)	45 (90%)
Feeding problems	564 (81.2)	40 (64,5%)	45 (45%)	45/91 (49%)	47 (94%)
Speech impairment	695 (100%)	49 (79%)	NR	95 (100%)	40 (80%)
Strabismus	375 (54%)	42 (67,8%)	40 (40%)	30 (32%)	NR
Behavioral features	647 (93.1%)	57 (92%)	NR	NR	48 (96%)
Genetic cause					
Deletions	577 (83%)	36 (58%)	62 (62%)	56 (58.9%)	NR
Non-deletions	118 (17%)	26 (42%)	38 (38%)	39 (41.1%)	NR
Mutations					7 (14%)

NR = Non reported

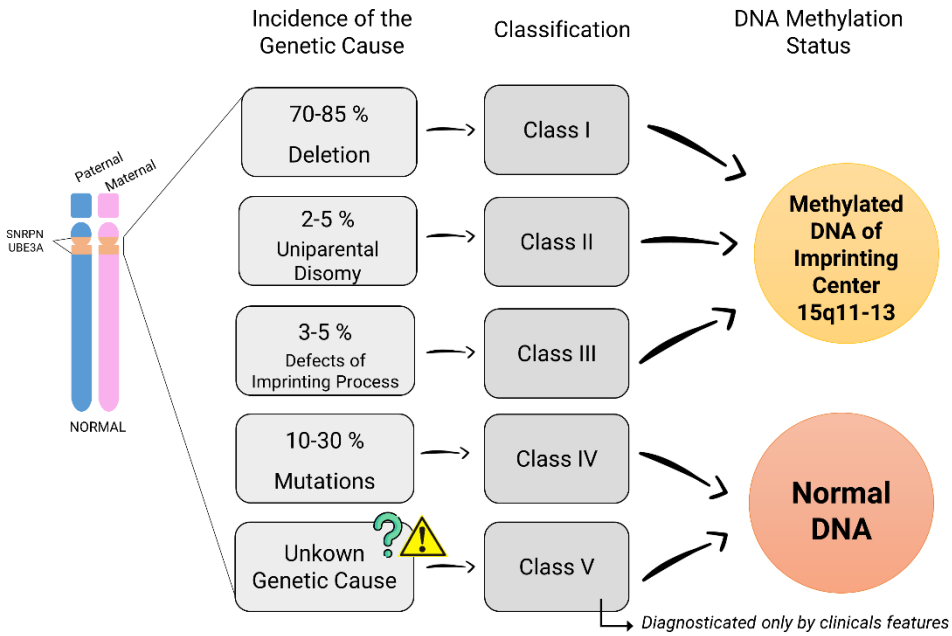

Figure 1. Genetic cause distribution in Angelman Syndrome with their clinical classification by the DNA methylation status: Classes I-III typically show abnormal DNA methylation, while Class IV and V present normal methylation patterns.

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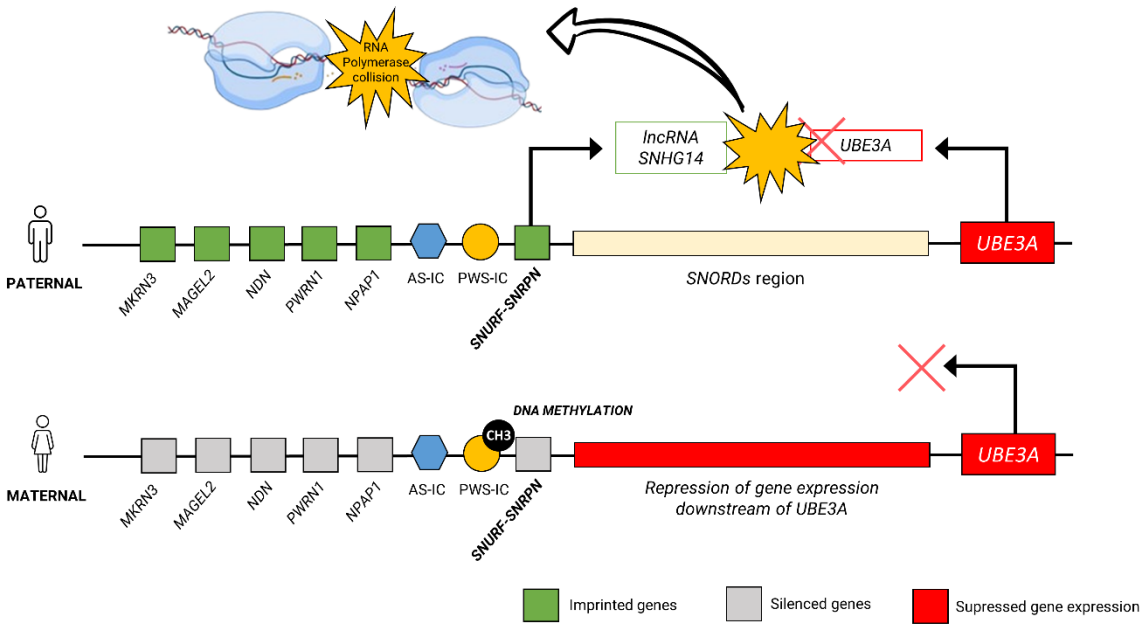

Figure 2. Schematic of the epigenetic imprinting regulation in Angelman Syndrome, located in chromosome 15q11-q13 of neuron cells and the plausible theory of silencing mechanism of paternal UBE3A gene.

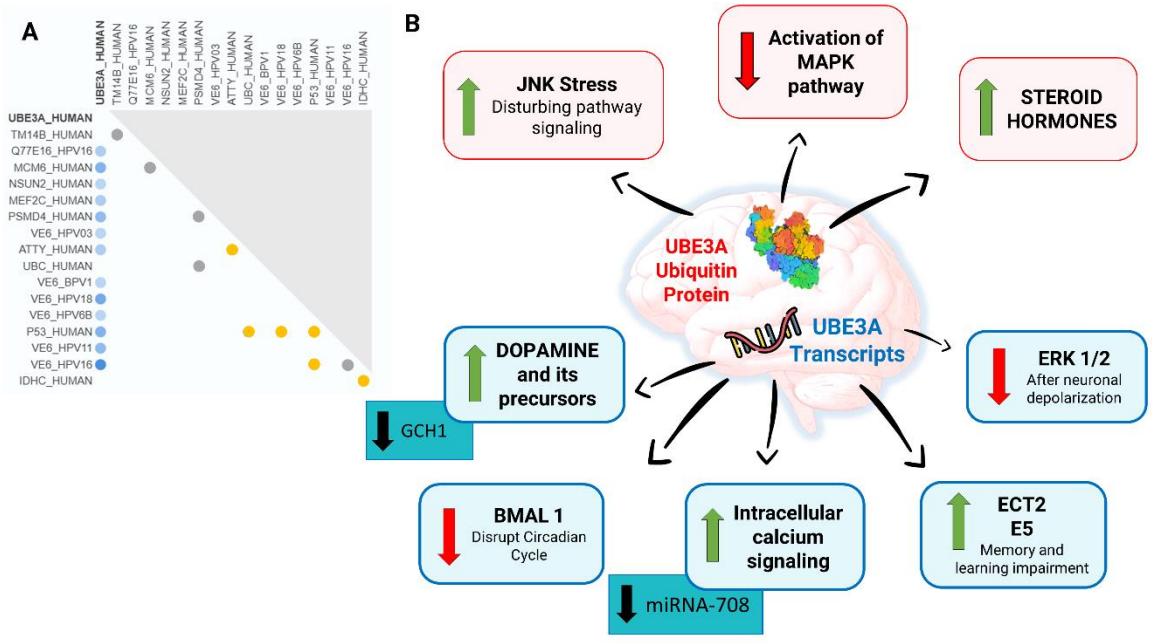

Figure 3 (A): UBE3A protein interactions based on UniProt data. Blue circles indicate interactions associated with Angelman Syndrome, yellow circles indicate associations with other diseases, and gray circles indicate interactions with no known disease association. (Modified from: https://www.uniprot.org/uniprotkb/Q05086/entry#interaction). **(B)**: The absence or deficiency of UBE3A ubiquitin protein and transcripts in the nervous system disrupts several cellular functions and negatively affects neuronal cell physiology.

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