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## Characterization of the role of E6-AP in Angelman Syndrome and Similar Neurodevelopmental Disorders

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**Characterization of the role of E6-AP in Angelman Syndrome  
and Similar Neurodevelopmental Disorders**

by

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## INTRODUCTION TO ANGELMAN SYNDROME

Angelman syndrome (AS) is a neurodevelopmental disorder first described by Harry Angelman in 1965 [8]. Incidence of the disease is estimated to be between 1 and 4 in 40,000, with an estimated morbidity rate of 1 in 15,000 [5, 8]. 50% of cases of the disease are initially misdiagnosed, therefore incidence rates may be higher than current estimates [32].

The disease is a genetic malformation associated with loss of function of the *UBE3A* gene; a gene located on chromosome 15q11-13 that is susceptible to genomic imprinting [5,8]. Genetic material (DNA) contains coding regions called genes which are arranged into chromosomes. Under normal conditions, humans have 46 chromosomes (23 inherited from each parent) present within the nucleus of each cell. The 46 chromosomes are defined as 23 pairs of chromosomes, numbered as 1 through 22. Two out of the 46 chromosomes are termed sex chromosomes meaning that they differ between males (XY) and females (XX). The chromosome itself is composed of a short arm (p) and a long arm (q) [12]. For specificity, the chromosomes are divided into several numbered bands [27]. For example, chromosome 15q11-13 mentioned above, specifies that *UBE3A* is located within chromosome 15 between bands 11 and 13 on the q arm of the chromosome [27].

Gene expression can be regulated on the DNA level by DNA methylation, addition of methyl groups to cytosine bases which has a negative effect on gene expression. Under normal cellular conditions, each inherited copy of a gene is active but some situations can cause only one of the copies to be active. Which is chosen to be turned on depends on the gene in question and the parent-of-origin, as the paternal

or maternal inherited gene copy may be silent [30]. This process is called genomic imprinting, one inherited copy of a *UBE3A* gene is silenced, while the other gene copy remains active in the embryo and adult [12]. It occurs at the germ line level, meaning at the level of egg and sperm cells before an embryo is formed. Imprinted genes are usually located in small to large clumps. A specific imprinted clump can contain expressed imprinted genes from both parents [31]. Within these clumps, there are CpG islands (short sequences of DNA near the transcription start site of genes containing large amounts of cytosine and guanine bases) that undergo DNA methylation only on one of the two parental chromosomes, this modification is often passed on to offspring [31]. Imprinting commonly appears as DNA methylation, but the process can also occur through modifications in histones, insulator proteins, and long non-coding RNAs [31].

A gene contains both coding and non-coding regions. Only the coding portion of the gene will undergo transcription and be translated into proteins. The *UBE3A* gene contains 16 exons with the coding region being from exon 8 to exon 16 [8]. The highly conserved HECT domain (homologous to the E6-AP carboxyl terminus) is at exon 16 and is composed of two lobes [8]. A catalytic site situated between the two lobes is a common site for frameshift, nonsense, missense, and splice site mutations associated with Angelman's syndrome patients [8]. In addition, point mutations across the coding region of *UBE3A*, but especially in exons 9 and 16, have also been identified in patients diagnosed with this syndrome [8].

Due to the importance of the *UBE3A* gene in Angelman's syndrome, it has been extensively characterized and found to be differentially expressed in neural tissues. Although it is predominately expressed in the cells of the hippocampus, an area of the

brain and Purkinje cells of the cerebellum, it is also expressed, but not imprinted in cultures of oligodendrocytes and astrocytes [8, 9]. The gene is not imprinted or expressed in neural glial cells, or peripheral tissues of humans and mice [23]. The paternal copy of *UBE3A* is silenced due to imprinting within neural tissues, specifically within cells of the olfactory bulb and hippocampus, and within Purkinje neurons [7,8,20]. Silencing is controlled by an anti-sense *UBE3A* transcript specific to neurons, which inhibits transcription of the gene from the paternal chromosome [9]. Imprinting of the gene is not fully completed in utero, so some paternal *UBE3A* expression occurs for a short period of time but declines as neurons mature [23]. The maternal copy of *UBE3A* can also be disrupted in Angelman syndrome [8]. Maternal expression of the *UBE3A* gene can be impacted by different mechanisms, and are currently classified into five classes, as described below, based on their mechanisms affecting the *UBE3A* gene. However, it should also be emphasized that in 5-10% of patients, no genetic defect in the gene can be identified [3].

## **THE FIVE CLASSES OF ANGELMAN SYNDROME:**

### *Class I: Deletion in Chromosome 15q11-13*

This class is caused by interstitial de novo deletion of the maternal chromosome and is the most common mutation associated with development of AS. Deletions, typically 4-7 megabase pairs in size [4,8], are seen in approximately 70-75% of patients. The deletions appear to be due to misbalanced crossing-over events between low copy repeats within the 15q11-13 segment [8]. In addition to

neurodevelopment symptoms, this class is also associated with hypopigmentation of hair, eyes, and skin [17].

*Class II: Uniparental paternal disomy*

The second class is associated with uniparental paternal disomy (UPD) in chromosome 15 [8]. Uniparental disomy is when two copies of a chromosome from only one parent, rather than one copy from each parent, is present in the genome of a child [11]. This mutation occurs in approximately 2% of AS patients, and almost exclusively occurs when both copies of the *UBE3A* gene come from a paternal source [8]. This mutation is influenced by age as incidence of UPD is correlated with increased age of parents at the time of conception. Further research is needed to define the mechanism causing UPD in offspring from older parents [8].

*Class III: Methylation/imprinting defect*

The third class is associated with atypical methylation of chromosome 15, a defect present in approximately 3-5% of AS patients [8]. Through the process of genomic imprinting, one inherited copy of a gene is silenced, while the other gene copy remains active [12]. The imprinting centre located within chromosome 15q11-13 serves in regulating DNA methylation, gene expression, and chromatin structure through cis-acting elements [8]. Roughly 50% of patients with atypical methylation have a mutation located within the imprinting centre of chromosome 15q11-13 [8].

*Class IV: Mutation in E6-AP (i.e. UBE3A)*

This class of AS is associated with a mutation within the gene which codes for ubiquitin protein ligase E3A protein (*UBE3A*), also known as human

papillomavirus E6-associated protein (E6-AP) [8]. This mutation arises in 5-10% of AS cases, appearing as a random mutation in 20% of Class IV patients and 75% occurring through inheritance from defects in the parental genome [8]. All of these patients have reduced E3 ligase activity, which is associated with E6- AP function [17].

*Class V: Absence of detectable genetic abnormality*

The most controversial of the class designations is Class V, where there is no identified chromosomal abnormality but patients express the clinical phenotype of the disease. The existence of this class of patients is still under debate [8]. Some experts believe that these individuals have a mutation of *UBE3A* within a region that is not within the coding region or involves an unidentified gene within the ubiquitin pathway that disrupts expression of *UBE3A* [8]. No mutations related to Class V patients have been identified or defined, but the *ATP10C* gene is a potential suspect due to its location within 200 kb of *UBE3A* and the fact that it is maternally imprinted in the brain [8]. Individuals that lack a genetic abnormality in the *UBE3A* gene tend to have mild or no AS symptoms. Patients in this class are often described as having an Angelman-like syndrome [20].

Almost all mutations associated with AS can be detected through use of methylation-sensitive DNA probes, since imprinting of genes associated with AS involves the methylation of DNA [13].

## CLINICAL CHARACTERISTICS OF ANGELMAN SYNDROME

Angelman syndrome (AS) often results in developmental insufficiencies and severe learning disabilities. Traditionally, the disease is hallmarked by the presentation of frequent, inappropriate laughter. Motor and cognitive symptoms associated with AS tend to correlate to the severity of the associated genetic abnormality [4]. Some physical characteristics include hypotonia (low muscle tone in the upper limbs), hyperactive reflexes, ataxia (impaired muscle coordination), and a wide-based gait with feet placed farther apart than usual [8, 17]. The gait of affected individuals is often fitful with pronation at the ankles [17]. Many AS patients have distinct facial features such as deep-set eyes, a prominent chin, and macrostomia (a wide mouth). These facial features are noticeable because patients are known to have a cheerful demeanor and tend to smile frequently [8]. Other disease characteristics include symptoms of epilepsy (seizures), sleep deficiencies, and inability to speak [5, 8, 17]. Over 80% of AS individuals develop seizures but frequency of seizures decreases with age [17]. Brain morphology in patients generally appears normal on gross exam although a few cases of microcephaly do occur; There may be some minor structural abnormalities in the brain as well [11]. Individuals with smaller genetic abnormalities, such as point mutations, tend to have milder symptoms [4].

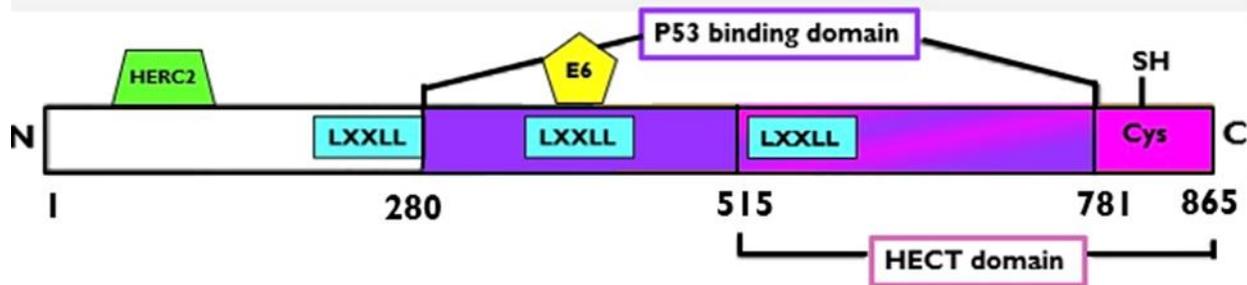
Of interest is the observation that developmental symptoms of AS do not tend to appear until 6-12 months of age [11]. This is hypothesized to be because paternal *UBE3A* may be accessible during the initial stages of neurogenesis prior to inhibition of the paternal allele, permitting regular brain development and cellular function for a brief period of time [11]. This also suggests that imprinting of the *UBE3A* gene is incomplete neonatally due to an incompletely silenced paternal allele [11]. Specifically, recent

studies have shown that the paternal *UBE3A* allele is not silenced within the brain cortex at birth, while it is silenced in the rest of the brain [11].

## **INFLUENCE OF MUTATIONS IN E6-AP ON MOLECULAR PATHWAYS & CELLULAR FUNCTIONS**

### **1. DEFECTS IN HUMAN PAPILLOMAVIRUS E6-ASSOCIATED PROTEIN (E6-AP)**

Defects in human papillomavirus E6-associated protein (E6-AP, or also known as ubiquitin protein ligase E3A (UBE3A)) are thought to be the primary cause for the pathogenesis of Angelman syndrome [17]. E6-AP serves many cellular functions; it controls translation in protein synthesis, replication of DNA, intracellular trafficking through manipulation of the PI3K/Akt pathway and other protein kinase cascades, in addition to being reported to play a role in the pathogenesis of other diseases including cervical cancer, prostate cancer, Prader-Willi syndrome, Dup15q syndrome, and Autism spectrum disorders [15]. The E6-AP protein is 865 amino acids in length [2, 5, 10], functions as a HECT E3 ubiquitin ligase protein (on the carboxyl terminus), and is encoded by the *UBE3A* gene on chromosome 15q11-13 in humans and chromosome 7 in mice [3, 2, 5]. The neuroactivity of the gene is determined by monoallelic expression of the maternal allele, but its function is dependent on the parent-of-origin, as the paternal or maternal inherited gene copy may be silent [23]. The gene contains five functional domains: a p53 binding domain, catalytic HECT domain, an E6 binding domain, three nuclear receptor response domains (LXXLL) and an activation domain near the end of the amino acid chain [5]. The structure of E6-AP is depicted in *Figure 1*.



*Figure 1.* Structure of E6-AP. Location of the p53 binding domain, HECT domain, and E6 binding site from Talis (1998). Location of HERC2 binding site, nuclear receptor response domains (LXXLL), and catalytic site cysteine from Mortensen (2015).

The catalytic HECT domain located at the carboxyl terminus of E6-AP, contains two lobes (C and N), and is roughly 45 kDa in size [15,20]. The catalytic domain is conserved within the family of HECT E3 ligases [15, 20]. The C-lobe of the HECT domain contains a catalytic cysteine, which forms a thioester bond with the E2-ubiquitin complex when the E2 enzyme binds to the N lobe of the domain [15, 20]. The exosite region of E6-AP, which is a secondary binding site outside of the active site, is critical for formation of isopeptide bonds and elongation of ubiquitin chains, and can impact binding of ubiquitin to substrates [15]. E6-AP also contains three Leu-Xaa-Xaa-Leu-Leu (LXXLL) motifs, which serve as nuclear receptor interaction domains and are important for protein-protein interactions related to regulation of transcription, activation of steroid receptors, and regulatory control of proteins such as HPV E6 protein preventing p53 degradation and tumorigenesis [20, 33]. Because of its role in regulation of cellular activities, modification of the E6-AP protein can alter function within cells, particularly within the neural system.

## 2. E6-AP & THE UBIQUITIN-PROTEASOME PATHWAY

In a complex with E6-AP, ubiquitin plays an important role in targeting proteins for degradation within the proteasome of cells. Binding of E6-AP to ubiquitin requires the linkage of two ubiquitin molecules catalyzed by E2 or E3 enzymes. One ubiquitin molecule (“donor”) is covalently linked to the active cysteine of the E2 enzyme or bound to the HECT domain of the E3 enzyme. This allows the donor ubiquitin to attach to one of seven lysine residues on the accepting ubiquitin molecule [16]. The hydrophilic region located near Lys-48, is necessary for the acceptor ubiquitin’s to interact with E6-AP [15]. Binding between ubiquitin and the HECT domain of E6-AP is by a thioester bond is catalyzed by NEDD4 (neural precursor cell-expressed, developmentally downregulated 4)-type ligases, a subtype of HECT ligases [15, 20]. Ubiquitination is highly conserved throughout the HECT protein family [2].

An 18-amino acid region (amino acids 391-408) in E6-AP serves as a binding site for the E6 protein, and is necessary for the formation of the E6/E6-AP complex [2, 6]. The carboxyl terminal end of E6-AP and ubiquitin’s “canonical” hydrophobic patch both contain Leu-8, Ile-44, and Val70 residues, which aid the ubiquitin chain elongation function of E6-AP [2, 14, 15]. After formation of the E6/E6-AP complex, the hydrophobic patch of ubiquitin is no longer necessary because a conformational change allows substrates to bind, thus E6-AP serves as an allosteric activator [20].

The E6/E-AP complex uses Lys48 to add polyubiquitin chains to specific proteins to target them for degradation by proteasomes [2, 6, 15]. Ubiquitination of proteins requires three cellular enzymes (E1, E2, and E3). E1 functions as a ubiquitin-activator which forms a thioester bond between cysteine and the C-terminus of ubiquitin [20]. E2 (ubiquitin-conjugating enzyme) and typically E3 (ubiquitin protein ligase) catalyze the

formation of an isopeptide bond between the C-terminus of ubiquitin and a lysine residue of the targeted substrate [20]. The combined activity of the three enzymes leads to polyubiquitination of proteins, allowing them to be recognized for degradation by the 26S proteasome [2, 15]. The Lys48-linked polyubiquitination catalyzed by E6-AP also serves an important role in stimulation of p97, an ATPase associated with various cellular activities [16]. P97 recognizes Lys48-linked polyubiquitin chains by an unknown mechanism, resulting in tagged substrates being transferred to the proteasome [16] and also plays a cellular role in the breakdown of misfolded proteins within the endoplasmic reticulum [16]. Weak enzymatic interactions are required for transfer of ubiquitin and E6-AP recognition of substrates targeted for degradation [15].

Examples of substrates targeted for ubiquitination and degradation by E6-AP include Mcm-7 (minichromosome maintenance protein 7), p27 (a cell cycle protein kinase inhibitor), IL- $\beta$ 1 (a cytokine synthesized by mononuclear macrophages), Sox9 (a gene associated with controlling cartilage generation in early fetal development) [5], and the p53 tumor suppressor protein [12]. For the P53 protein, E6-AP forms a high-energy thioester bond between the C-terminus of ubiquitin and the active site of the ligase [15]. A E6-AP-E6-p53 enzyme-substrate complex is formed and additional ubiquitin is added in the presence of the human papillomavirus (HPV) E6 gene product to target p53 for degradation by the proteasome [2, 10]. Loss of p53 function is associated with many cancers including HPV-induced cervical carcinogenesis, as genetically damaged cells avoid programmed cell death and continue to proliferate, driving cervical cancer [12, 15]. E6-AP has also been shown to aid in monoubiquitination, which is important for the

regulation of transcription through the modification of histones and transcription factors [6, 4].

Altered E6-AP function could influence the ubiquitin pathway of protein degradation leading to accumulation of proteins within cells, alteration of physiological processes, and ultimately cause metabolic dysfunction at the cellular level. Because of the concentration of E6-AP activity in neural cells, this could contribute to the pathophysiology that causes the clinical symptoms of AS.

#### INTERACTION BETWEEN HERC2 & E6-AP

The E3 ubiquitin ligase activity of E6-AP is impacted by HPV E6 oncoprotein and HERC2, a large HECT and RCC1-like E3 ubiquitin protein ligase. The HERC2 protein contains three RCC1-like (Regulator of Chromosome Condensation 1) domains, and a HECT domain at its C-terminus allowing for E3 ligase function [14, 17,20]. Both HERC2 and HPV E6 proteins bind near the N-terminus of E6-AP to enhance the protein's E3 ubiquitin ligase activity [12]. HERC2 serves as an allosteric activator of E6-AP and drives a conformational change to make the protein more or less active [14]. Mutations in *HERC2*, can significantly decrease expression of the HERC2 protein resulting in dose-dependent reductions in E6-AP function. This interaction has been shown to result in a neurodevelopmental disease phenotype in humans in an Amish community that is similar but of reduced clinical severity as compared to AS. These patients present with hypotonia and behavioral effects, but less extreme language difficulties [17]. Of interest was the observation that half of the patients with HERC2-deficiency

were missing part of their posterior corpus callosum, an area of the brain associated with motor, sensory, and mental functions [17]. As decreased E6-AP levels have been linked to *HERC2* gene mutations that cause enhanced break down of the HERC2 protein, this type of mutation may also be related to AS symptoms [12, 14].

#### INFLUENCE OF E6-AP ON THE PI3K/AKT PATHWAY

E6-AP is an important regulator of protein kinase cascades, specifically the PI3K-Akt-GSK3 signaling pathway, which controls functions of the endoplasmic reticulum actions through phosphorylation thereby promoting neuronal cell growth and survival during development [20]. PI3K is an important regulator of transcription and translation. Both PI3K and the MAP kinase pathway are frequently disrupted in neoplastic cells suggesting a role in promoting carcinogenesis [20]. Although the mechanism its influence on the activation of the PI3K/Akt pathway is unknown, it has been hypothesized that E6-AP directly activates RhoA or increases concentrations of reactive oxygen species that activate the PI3K/Akt pathway. RhoA is a small GTPase that stimulates actin filament formation and functions in contraction of the myosin, actin filament ring located between cells during the cytokinesis stage of the cell cycle, allowing for separation into two individual cells [12]. However, additional studies are needed to define the mechanistic interaction between E6-AP and the PI3K/Akt pathway [20].

#### EFFECTS OF INTERACTION BETWEEN E6-AP's & EPHEXIN5

E6-AP serves an important role in regulating excitatory synapse frequency in the cortex and hippocampus through Ephexin5 [5, 7]. Ephexin5 (E5) inhibits creation of excitatory synapses and is controlled and targeted for degradation by E6-AP, [7, 4]. E5 function requires binding of EphrinB and EphB (a receptor tyrosine kinase), which results in the phosphorylation of the N-terminal end of E5 at Tyr-361 [7]. In contrast to E5, EphB promotes excitatory synapse development [7]. The interaction of E5 and EphB are thought to be important for contact between incoming axons and postsynaptic dendrites that lead to creation of excitatory synapses during brain development [7]. This can greatly impair learning, memory, and overall development as communication between neurons is critical for many different processes throughout the human lifespan. Normally Ephexin5 is able to be degraded by EphrinB, which acts to oppose the action of Ephexin5. But, when E6-AP is knocked out in mouse models, EphrinB treatment is unable to degrade or oppose the action of Ephexin5, thus the disruption in development of synapses remains [7].

#### INTERACTION BETWEEN E6-AP & ARC

The E6-AP protein also influences ARC, an important neuro protein vital for synaptic plasticity, learning, and memory whose transcription is stimulated by the presence of the steroid hormone estradiol. Arc controls post-synaptic internalization of AMPA-type glutamate receptors through clathrin-mediated endocytosis [20]. In mouse models, Arc is expressed at high levels when E6-AP expression is absent [3]. Some data suggests that Arc can be ubiquitinated and targeted for degradation by E6-AP, although other scientists believe that the E6-AP serves as a repressor of Arc

transcription [3, 20]. A role for Arc and E6-AP has been demonstrated in mouse models for long-term effects on strengthening of synapses between nerve cells and neuronal synapse firing [4]. The mechanism for how E6-AP may negatively affect transcription of ARC and its role in the pathogenesis of E6-AP-related diseases has yet to be determined [3,24]. Many believe that the pathogenesis of Angelman syndrome in neural tissues is directly related to modification of transcription of *Arc* and other genes and the influence of E6-AP on steroid hormone function [20].

## **INFLUENCE ON E6-AP ON NEURAL FUNCTION**

### **1. E6-AP MUTATION EFFECT ON BRAIN STRUCTURE**

Reduced levels or function of E6-AP can alter neural architecture. Microcephaly is thought to be related to microdeletions of the genomic area of the UBE3A mutation [17]. Diffusion tensor imaging studies, utilizing MRI-based neuroimaging techniques, have demonstrated altered architecture within the white matter tracts, thinning of the corpus callosum, which connects the two hemispheres of the brain and delayed myelination in AS patients [11]. Myelination of nerve cells, a protein that coats the axons and enhance transmission of electrical impulses, is disrupted in AS mouse models. Astrocytes are star-shaped glial cells of the central nervous system located within the brain and spinal cord. These cells are the most abundant glial cell type in the brain play an essential role in maintenance of synaptic activity including control of signaling between neurons, guidance of axons to correct targets, and regulation of the blood brain barrier [28]. Oligodendrocytes, the glial cell that creates the myelin sheath,

is the one of the most susceptible cell types of the central nervous system due to the fact that the high production rate of myelination consumes massive amounts of oxygen and ATP, forming hydrogen peroxide and reactive oxygen species byproducts [29]. Some of the myelination enzymes require iron as a co-factor, which has the potential to accumulate leading to free radical formation and lipid peroxidation [29]. Both of which have the potential to be toxic and damaging to oligodendrocytes. In murine AS models, oligodendrocytes showed no reduction in expression of paternal E6-AP, suggesting that *UBE3A* is not imprinted in oligodendrocytes [11]. However, it is interesting that in primary astrocyte and oligodendrocyte cultures from murine models of AS, E6-AP expression occurs [11]. Although astrocytes from AS mice had reduced E6-AP expression as compared to the wild-type mice, this level of protein expression suggests that *UBE3A* is not imprinted in astrocytes [11]. In summary, data suggests that E6-AP mutations cause decreased expression in astrocytes, modify white matter tracts, diminish the size of the corpus callosum, and slow the process of myelination within the central nervous system thereby leading to disruption of normal synapse physiology.

## 2. ABSENCE OF E6-AP FUNCTION AT THE NEURON LEVEL

The absence the E6-AP function leads to dysfunction at both cellular and physiological levels that can lead to development of disease symptoms. Lack of E6-AP function elevates the concentration or continuous existence of substrates normally targeted for degradation within the proteasome [4], and can promote tumor formation. When E6-AP function is altered by an E6 oncoprotein, the p53 suppressor gene is

ubiquitinated and degraded thereby promoting neoplasia. Reports in the literature have linked E6-AP disruption to the occurrence of breast, prostate, and cervical cancers [5].

Lack of E6-AP activity can be detrimental on both a cellular and physiological level. Absence of E6-AP function contributes to reduced formation of excitatory synapses in the brain (probably through Ephexin5) and impacts dendrite structure during early neurogenesis causing reduced development of communications between neurons thereby leading to impaired learning, memory, motor deficits, and lack of cognitive development. Loss of function can impact transmission of electrical signals in the brain and alter brain structure. Lack of E6-AP function may also impact nervous tissues outside the central nervous system as a *Drosophila* model demonstrated a correlation between lack of E6-AP expression and decreased growth and branching of dendrites in peripheral nerves [11]. It has been hypothesized that decreased levels of E6-AP reduce the threshold for a seizure response [17].

AS is known to involve genetic mutations resulting in decreased expression or altered forms of E6-AP within neurons including mutated forms of E6-AP, decreased activity of E3, or lack of expression of E6-API [3]. However, little is known about the control of E6-AP at the post-translational level in neurons [14]. The amount of substrate proteins utilized by E6-AP within neurons may be important in development of AS [3].

### 3. EFFECTS OF E6-AP MUTATION ON SYNAPTIC TRANSMISSION

AMPA receptors are ionotropic glutamate receptors that control sodium channels, producing depolarization of the cell during neurotransmission [18]. These

receptors are normally present in high numbers within the cortex, sensory pathways, and basal ganglia. However, mutations in E6-AP decrease the numbers of synaptic AMPA receptors in these areas [18, 4] leading to a negative effect on synaptic plasticity and long-term potentiation, functions important for learning and memory functions (LTP) [4,19]. Long-term potentiation is a process in which synaptic connections between neurons become strengthened with frequent activation due to addition of more AMPA receptors resulting in the cell becoming more sensitive to glutamate. [4,19]. Addition of new AMPA receptors is lacking when E6-AP is absent [4,19]. This may be influenced by the relationship between the loss of E6-AP function and Arc, as the Arc protein is believed to contribute to reduced synaptic activity during AS [3]. Overall, E6-AP activity plays a critical role in the development, organization, and maintenance of neuronal connections in the brain, including experience-dependent plasticity within the cerebral cortex [23].

#### 4. E6-AP MUTATIONS IMPACT DENDRITIC SPINES AND ACTIN FILAMENTS

Lack of E6-AP reduces the functionality of actin filaments [4], cytoskeletal filaments that serve an important role in cell adhesion, muscle contraction, cell motility, cytoskeletal remodeling, and durability of dendritic spines [19]. Lack of E6-AP function has negative effects on long term potentiation and decreased density of dendritic protrusions (spines) [4]. Reduced density of dendritic correlates with lower neuronal excitability and slower excitatory synaptic transmission [26]. These effects could impact memory, learning, and impair intellectual development. Individuals are particularly

vulnerable during early neuronal development and impaired neural activity can impact the hippocampus functions [26].

## **CLINICAL SYNDROMES OF E6-AP**

### **1. AUTISM SPECTRUM DISORDERS**

Although many genes and environmental aspects are thought to also play a role, duplication of the *UBE3A* gene is one of the most prevalent genetic mutations in Autism Spectrum Disorders (ASD) [7, 20]. This group of heterogenous neurodevelopmental conditions predominately affects males and is defined by social interaction complications, communication difficulties, and the presence of repetitive actions [20]. Experimental studies using mouse and fruit fly models have suggested that autistic phenotypes are associated with the presence of increased E6-AP function after duplication or triplication of the *UBE3A* gene (similar to those that give rise to ASD), leading to overexpression of E6-AP [12, 20]. Elevated E6-AP levels leads to ubiquitination and degradation of X-linked inhibitor of apoptosis protein (XIAP) resulting in impairment of growth and branching in dendrites [25]. As a result, there is increased caspase activity, lessening of the size and branching ability of the dendrites, leading to local deterioration and loss of neural function [25]. These autistic patient's neurons have difficulty receiving information from neighboring neurons, thereby impacting overall brain function.

### **2. DUP15Q SYNDROME**

Duplication of the chromosomal region containing *UBE3A* also occurs in Dup15q syndrome, a developmental disorder related to autism [12]. This neurodevelopmental disorder arises from duplications of portions of the 15q11,2-13.1 chromosome that lead to approximately twice as much expression of *UBE3A* [4]. The duplication events predominately originate from the maternal copy of *UBE3A* during the course of oogenesis [24] and can be further specified into two categories based on whether the duplication events are interstitial or isodicentric 15q duplications [24]. The isodicentric 15q duplication contain three copies of the maternal allele and one copy of the paternal allele, whereas individuals with interstitial duplications have an additional copy of the 15q11.2-q13.3 region within the q arm of the maternal copy of chromosome 15 [24]. Symptoms of this disorder are similar to AS and may include developmental disabilities, speech impairments, developmental hypotonia, excessive drooling, seizures, and mild ambulatory problems, although most Dup15q patients have the ability to walk without assistance [24].

### 3. PRADER-WILLI SYNDROME

The imprinting properties of *UBE3A* and the genes that surround it are regulated by a control center, the Prader-Willi syndrome-AS imprinting center, within an area of chromosome 15q11-q13 in humans. This center is positioned upstream of the SNURF (SNRPN upstream reading frame)-SNRPN (small nuclear ribonucleoprotein-associated protein N) gene [23]. Prader-Willi Syndrome is caused by deletion or imprinting mistakes in the imprinting region of chromosome 15, causing cognitive and sexual deficiencies as well as obesity and excessive hunger. The maternal copy of

chromosome 15q is silenced in this disorder whereas in AS, the paternal copy of the chromosome is deleted [13]. The mechanism for inactivation of chromosome 15 is thought to be similar for both disorders and both could be the result of uniparental disomy, depending on the parent of origin for the chromosome [13].

#### 4. RETT SYNDROME

Rett syndrome is brain disorder, most commonly affecting females, that is X-linked dominant to abnormalities of the *MECP2* (Methyl CpG binding protein 2) gene on chromosome Xp28. Mutations at this locus disrupt gene expression through the binding of methylated DNA [20, 21]. Symptoms include severe developmental problems after approximately 18 months of age, stunted growth, language and learning impairments, coordination problems, and loss of hand function [21]. Other common symptoms include seizures, scoliosis, and respiratory difficulties [21]. The *MECP2* gene is thought to regulate synapses between neurons thereby influencing communication between neurons [21]. The influence of E6-AP on *MECP2* function has been suggested, but is not currently proven. Some scientists believe that *MECP2* activates *UBE3A* transcription [20]. This syndrome is similar to AS in that mutations that result in the absence of *MECP2* function induce lower expression of E6-AP [20]. Although both AS and Rett syndrome have similar clinical symptoms and neurodevelopmental impairments, AS is often diagnosed at an earlier age than Rett syndrome.

#### 5. MECP2 DUPLICATION SYNDROME

In contrast to Rett syndrome, MECP2 duplication syndrome occurs almost exclusively in males. In this syndrome, two copies of the *MECP2* gene arise due to a gene duplication event on the q arm of the X chromosome [22]. These patients have increased MECP2 protein production which disrupts gene regulation and neuron activity, resulting in moderate to severe intellectual disability and a broad autism phenotype [22, 20]. As with Rett syndrome, these patients have difficulties with motor coordination, breathing, speech, muscle tone, and many individuals also experience seizures [22]. In contrast to Rett syndrome, individuals with MECP2 duplication syndrome are more likely to learn to walk, but roughly one third of these individuals will require assistance [22]. As these patients are prone to respiratory symptoms, half of affected individuals will not live to see 25 years of age due to susceptibility to recurrent respiratory infections [22]. It is unknown if E6-AP is affected in this disease. However, the establishment of autistic attributes is correlated to the duplication of the *UBE3A* gene and both genes are important for neurodevelopment [20].

## **OTHER CLINICAL EFFECTS ASSOCIATED WITH E6-AP**

### **1. E6-AP INFLUENCES REPRODUCTIVE DEVELOPMENT AND STEROID HORMONE RECEPTORS**

E6-AP is involved in reproductive organ development and puberty, and also functions in cellular signaling pathways and serves as a transcriptional co-activator of steroid hormone receptors [5,4]. As a steroid hormone receptor co-activator, E6-AP can enhance activation of androgen, estrogen, and progesterone receptors, as well as ubiquitinating and targeting the receptors for degradation [5, 20]. Within the prostate

gland, E6-AP can influence steroid hormone receptor cells by blocking apoptosis, enhancing cell division, and increasing cell growth thereby promoting tumorigenesis [5].

## 2. RELATIONSHIP BETWEEN AS MUTATIONS & OBESITY

Cases of Angelman syndrome that do not have extensive deletions within the 15q11-q13.3 locus tend to have an abnormal appetite and are prone to morbid obesity. Symptoms resulting in an excess of fat mass are linked to Prader-Willi syndrome and some cases of Dup15q syndrome [24]. In fact, Prader-Willi syndrome is presently considered to be the most prevalent genetic cause of morbid obesity in adolescents [38]. Obesity affects 600 million people worldwide and is also the leading cause of morbidity and mortality in individuals with Prader-Willi syndrome [34, 37]. It has been proposed that uneven amounts of paternal and maternal genetic transcripts in this region of chromosome 15 could cause problems with maintenance of metabolism [24]. The 15q11-q13.3 region that contains the *UBE3A* gene also contains several GABA (gamma aminobutyric acid) receptor subunits, which are associated with the paternal allele [38]. When the paternal allele is absent, the amount of GABA, an important inhibitory neurotransmitter associated with control of appetite, metabolism, compulsive behavior, and memory is decreased [38]. The cellular mechanisms relating obesity to AS is not known. In general, the lack of motor coordination associated with AS makes it difficult for individuals with the disease to live an active lifestyle, likely increasing the risk for development of obesity.

## 3. E6-AP & CERVICAL CANCER

Malignant neoplasms of the cervix or inferior portion of the uterus is often brought on by a sexually transmitted, human papillomavirus (HPV) infection [20]. All cervical neoplasms contain a minimum of one integrated copy of the virus' DNA within the genome resulting in constitutive expression of early viral genes and production of E6 and E7 oncoproteins [20]. On a molecular level, the presence of HPV E6 oncoprotein can overpower E6-AP during formation of the E6/E6AP complex. This occurs because two zinc binding domains located at the N-terminus of the E6 oncoprotein overwhelms the activity of the LXXLL motif of E6-AP [20] and leads to polyubiquitination and degradation of p53. It should be noted that p53 is not normally a substrate of E6-AP. In the absence of p53 activity, proliferation of cancerous cells and prevention of apoptosis is promoted. There are differences between HPV viruses as high-risk HPV virions promote the degradation of p53, whereas low-risk forms of the virus produce E6 proteins that can bind to E6-AP but do not degrade p53 [20]. As high activity of E6-AP is hypothesized to contribute to neoplastic growth, cancer proliferation is suppressed and apoptosis is promoted when the activity of E6-AP is knocked out [5]. Therefore, E6-AP could potentially be a good target for cervical cancer treatment [5]. Data suggests that overexpression of E6-AP is also involved in the growth, proliferation, invasion, and spread of prostate cancer [5].

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