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Original article

Neurological aspects of the Angelman syndrome☆

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Abstract

Angelman syndrome (AS) has emerged as an important neurogenetic syndrome due to its relatively high prevalence and easier confirmation of the diagnosis by improved genetic testing. In infancy, nonspecific clinical features of AS pose diagnostic challenges to the neurologist and these include any combination of microcephaly, seizure disorder, global developmental delay or an ataxic/hypotonic cerebral palsy-like picture. In later childhood, however, absent speech, excessively happy behavior, ataxia and jerky movements usually present as a recognizable clinical syndrome. Brain MRI shows nonspecific or normal findings but occasionally the characteristic EEG patterns alone can lead to the correct diagnosis. The physical, clinical and behavioral aspects appear to be attributable to localized CNS dysfunction of the ubiquitin ligase gene, *UBE3A*, located at 15q11.2. In certain brain regions, UBE3A normally has mono-allelic expression from the maternally derived chromosome 15. Several distinct genetic mechanisms can inactivate or disrupt the maternally derived UBE3A: chromosome microdeletions, paternal uniparental disomy, imprinting defects and intragenic UBE3A mutations. Those with the deletion type of AS are the most prevalent (about 70% of cases) and appear to have a more severe clinical phenotype. The unique epileptic patterns and distinct behavioral features may be related to multiple actions of UBE3A, possibly occurring during, as well as after, the time of neuronal development.

Keywords: Angelman syndrome; Seizures; Ataxia; UBE3A; E6AP; Ubiquitin; 15q11.2; Absent speech

1. Introduction

Initially described in 1965 [1], Angelman syndrome (AS) is familiar to most child neurologists as a recognizable syndrome associated with infantile seizures. Several general reviews have recently appeared in the genetic literature [2–4] and this article reviews the salient neurological and diagnostic aspects of the condition.

2. Incidence

It appears that AS occurs worldwide without geographic clustering. Studies on school age children, age 6–13 years, show a minimum prevalence of AS of 1/12,000 in Sweden [5] and 1/10,000 in Denmark [6]. Several reports address the prevalence among individuals with established developmental delay, showing rates of 0% [7], 1.3% [8], 1.4% [9], and 4.8% [10]. The latter study extrapolated data in order to compare it to the population of the Washington state (using 1997 US Census Bureau figures) and obtained an estimate of 1/20,000. It thus seems that AS has prevalence among children and young adults is between 1/10,000 and 1/20,000.

3. Clinical presentation

Clinical consensus criteria for the diagnosis have been published is illustrated in Table 1 [11]. Severe speech deficit (usually absent speech), severe mental retardation, behavioral abnormalities and movement problems are ubiquitous in AS. Other features, such as microcephaly or seizures may be absent. The AS clinical gestalt is heavily dependent on the combination of the behaviors of excessive laughter and apparent happiness combined with tremulous movements and gait ataxia.

The neurologist often first encounters AS while consulting on an infant with the problem of developmental delay, microcephaly or seizures [12]. The normal prenatal

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Table 1
Angelman syndrome: consensus criteria for clinical diagnosis

A	Consistent (100%)
	Developmental delay, functionally severe
	Speech impairment, none or minimal use of words; receptive and non-verbal communication skills higher than verbal ones
	Movement or balance disorder, usually ataxia of gait and/or tremulous movement of limbs
	Behavioral uniqueness: any combination of frequent laughter/smiling; apparent happy demeanor; easily excitable personality, often with hand flapping movements; hypermotoric behavior; short attention span
B	Frequent (more than 80%)
	Delayed, disproportionate growth in head circumference, usually resulting in microcephaly (absolute or relative) by age 2
	Seizures, onset usually <3 yr. of age
	Abnormal EEG, characteristic pattern with large amplitude slow-spike waves (usually 2–3/s), facilitated by eye closure
C	Associated (20–80%)
	Flat occiput
	Occipital groove
	Protruding tongue
	Tongue thrusting; suck/swallowing disorders
	Feeding problems during infancy
	Prognathia
	Wide mouth, wide-spaced teeth
	Frequent drooling
	Excessive chewing/mouthing behaviors
	Strabismus
	Hypopigmented skin, light hair and eye color (compared to family), seen only in deletion cases
	Hyperactive lower extremity deep tendon reflexes
	Uplifted, flexed arm position especially during ambulation
	Increased sensitivity to heat
	Sleep disturbance
	Attraction to/fascination with water

and birth history typically provides no clues that AS is the diagnosis. Feeding problems and muscle hypotonia are often reported, however. Brain MRI or CT scans are normal but may show nonspecific changes such as mild cortical atrophy or delay in myelination. Laboratory tests of blood and urine are also normal including metabolic screening. If the child is less than 12 months age, tremulous movements, ataxia, or severe lack of speech may not be apparent. Likewise, seizures may not have occurred yet. The facial features and general physical examination are generally normal (Fig. 1), although protruding tongue, strabismus, brisk deep tendon reflexes, and an apparent happy demeanor may be present. For infants with AS due to a chromosome deletion, absolute or relative skin hypopigmentation may be present in infancy due to deletion of a pigment gene (the P gene) that resides within the deletion area [13]. This hypopigmentation is usually overlooked unless the physician is specifically thinking about the AS possibility.

As the child with AS develops, the correct diagnosis may become evident during follow-up neurology visits, especially when it becomes apparent that speech is essentially absent and attempts at walking are compromised because of severe jerkiness and ataxia. Additionally, onset of seizures, more common after 1 year of age, usually forces reassessment of the working diagnosis of such entities as cerebral palsy, static encephalopathy or idiopathic mental retardation. The EEG in AS is usually very abnormal and more abnormal than clinically expected. It usually has symmetrical high voltage slow wave activity (4–6 c/s) persisting for most of the record and unrelated to drowsiness; and very large amplitude slow activity at 2–3 c/s occurring in runs and more prominent anteriorly. In addition, spikes or sharp waves, mixed with large amplitude 3–4 c/s components, are seen posteriorly and usually provoked by passive eye closure [4,14,15]. The EEG findings alone can point strongly to the AS diagnosis but it can be normal at times in individuals genetically proven to have AS.

It is more likely to consider the clinical diagnosis of AS when the child is older than 3 years of age. Here the behavioral and movement characteristics predominate, often in the setting of microcephaly and an established seizure disorder. In these children there is no evidence of neurodeterioration as they are socially outgoing, quite hypermotoric, and are moving forward developmentally. They may be hyperexcitable with excessive laughing, grabbing and pulling so as to engage others, often constantly putting objects in their mouth. Drooling is frequent. However, mild expression can be present in cases where there is no microcephaly, seizures, and only mild ataxia or tremulousness. In these cases, the EEG may be the first suggestion that AS is the correct diagnosis. Often the parents may be the first to suggest the syndrome possibility. Once neurologists have had experience with a confirmed case, it is not uncommon for additional ones to be identified from their practices.

4. Genetic etiology

It was not until the 1980s that chromosome 15 was implicated in its causation. The first clue to this was the discovery that the majority of individuals with AS had microdeletion of 15q11.2–15q13. Initially confusing was the observation that the Prader-Willi syndrome (PWS) could also be caused by the same microdeletion. It soon became evident that deletions on the paternally derived 15 caused PWS and ones on the maternally derived 15 caused AS. The two syndromes are, however, caused by different genes but they lie in close proximity to one another.

The last decade led to the identification of UBE3A (encoding for a ubiquitin ligase enzyme) as the AS gene [16,17]. In certain regions of the normal brain, UBE3A is expressed only from the maternal chromosome and its expression in the AS brain with 15q11.2–15q13 deletion is only about 10% that of normal [18].

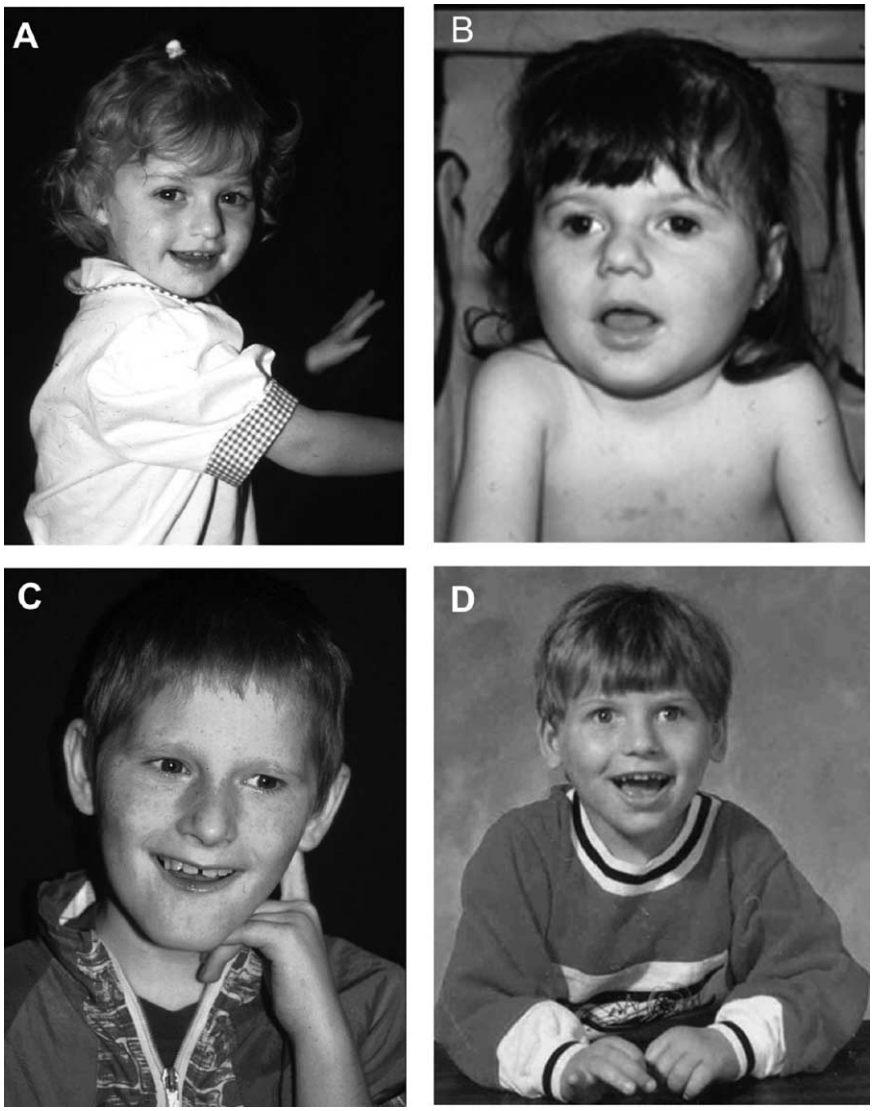

Fig. 1. Children with genetically proven diagnosis of Angelman syndrome. The common 15q11.2–15q13 deletion was present in A, C and D. A UBE3A mutation was detected in B.

This phenomenon of monoallelic or single chromosome regional expression is termed genomic imprinting and it is one of the hallmarks of AS. Mice lacking the maternal *UBE3A* gene have very low level of mRNA in hippocampus, cerebellar Purkinje cells, and olfactory bulb [19].

Imprinted genes like *UBE3A* often have novel as well as complex control mechanisms. This is illustrated in Fig. 2 where the current gene map of the 15q11.2–15q13 region illustrates how a distant imprinting control area (IC) can affect the transcription of the *UBE3A* gene even though the IC is spatially located several hundred thousand base pairs from the *UBE3A* gene. It appears that the IC accomplishes this control through regulation of another gene, *SNRPN*, that indirectly affects whether UBE3A is turned on or off. The details of this control are being rapidly dissected and are beyond the scope of this review [20–22]. However, knowledge that UBE3A is an imprinted gene is fundamental to understanding the genetic defects that cause AS.

Fig. 3 shows the four genetic mechanisms known to cause AS. Chromosome microdeletions are clearly the most common type and almost always involve consistent breakpoints in flanking cassettes of repetitive genes (sometimes called duplicons) [22–24]. These repetitive gene cassettes have accumulated from ancestral duplication events. Unequal or misaligned crossing over between these chromosome 15 repetitive elements causes the AS deletion. A recent study indicated that normal mothers, in some families having an AS deletion children, had rearrangements or inversions related to these duplicated gene cassettes [25]. Failure of the zygote to incorporate the normal maternal and paternal chromosome 15s can lead to only two paternal 15s, a term called paternal uniparental disomy (UPD). In such cases, UBE3A is not expressed in critical brain areas because only the two paternal

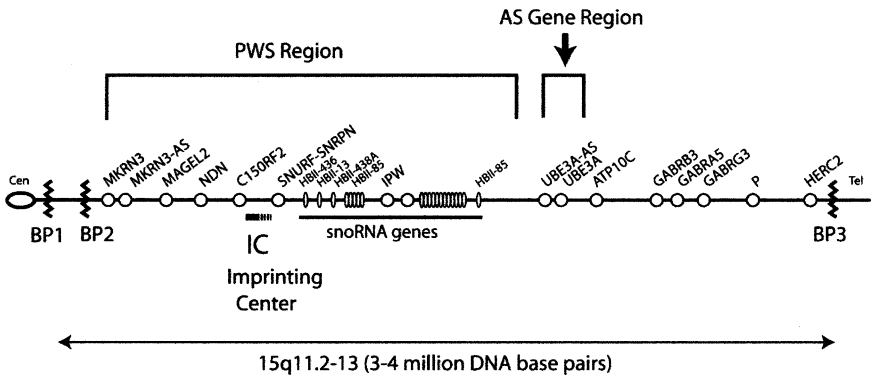

Fig. 2. Illustration of the complexity of the genetic map in the 15q11.2–15q13 region. BP 1, 2 and 3 represent common breakpoint related to the presence of repetitive genes (duplicons). The impinting center (IC) is depicted adjacent to the promoter region of the *SNRPN* gene. See text for details of regulation of UBE3A.

chromosomes 15 (with inactive UBE3As) are present. The mechanism leading to the uniparental disomy appears to be mainly post-zygotic, perhaps representing a mitotic 'correction' event in response to the normal fertilization of an abnormal egg that is nullisomic for chromosome 15 [26]. Imprinting center (IC) defects can involve small molecular deletions that can be detected by molecular or cytomolecular FISH methods. More likely, however, no actual DNA deletion is found but DNA methylation abnormalities are still detected in the IC region [27]. Intragenic UBE3A mutations can also cause AS by creating an abnormal protein that is either degraded or functions abnormally. Finally, about 10–15% of individuals with the appropriate clinical diagnosis of AS have negative testing for all four of these above mechanisms. For them, the diagnosis could be incorrect or they could still have AS due to yet-to-be identified genetic mechanisms.

There is some correlation between the clinical severity of AS and its type of genetic mechanism [4,28]. Individuals with the large chromosome deletions are more likely to have seizures and microcephaly and are more likely to have skin, eye, and hair hypopigmentation. These features are probably due to additional deleted genes in the 3–4 Mb deleted region. Those with uniparental disomy are more likely to have no seizures, normal head circumferences, and better cognitive functioning although severe to profound impairment is still present. Those with UBE3A and IC defects are more likely to have clinical severity between that seen in the large deletion and the UPD mechanisms. Presence of somatic mosaicism can result in milder clinical features in those with IC defects [27] and has been noted in a case of 15q11.2–15q13 deletion [29]. Overall, however, regardless of the mechanism, individuals with AS are more alike in their clinical features than they are different.

5. Genetic diagnostic testing

DNA methylation testing of blood is a sensitive and specific screening for three of the four known genetic mechanisms. There are several methods available for this testing and all rely on the observation that the AS DNA methylation pattern in the IC control region is easily distinguishable from normal when AS is caused by chromosome deletions, UPD or IC defects. The diagnosis of AS is thus confirmed if this methylation result is abnormal but it does not distinguish which of the three above mechanisms is operative. To determine this, the next step is to perform chromosome 15 FISH analysis (to detect 15q11.2–15q13 deletions that will be present in the majority of cases). If this FISH test is normal, additional molecular genetic testing is necessary to determine if either UPD or IC defects are present.

If the initial DNA methylation test is normal, the child with AS could still have an intragenic UBE3A mutation since these mutations have no effect on the DNA methylation patterns in the 15q11.2–15q13 region. If UBE3A mutation testing is normal, it could then be that these patients still have AS but they would be one of the 10–15% in whom genetic test confirmation is not possible.

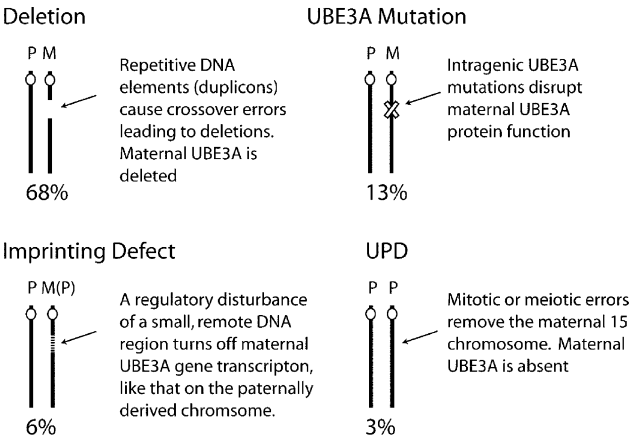

Fig. 3. Summary of the four known genetic mechanisms that causes Angelman syndrome. Percentages below each mechanism indicate the proportion of all clinically diagnosed individuals in whom that mechanism is detected. About 10% of all clinically diagnosed individuals will have normal genetic testing. Percentages are approximated and may vary in reported surveys.

It is also possible that this latter group is incorrectly diagnosed, as mimicking conditions, including other chromosome defects, have been reported [30]. According, all children with AS-like features not diagnosed by the above genetic tests should have at least a routine chromosome study performed. Families with AS should be offered genetic counseling since UBE3A mutations and IC defects can carry up to a 50% recurrence risk. The common deletion cases typically have less than 1% recurrence risk but exceptions to this can occur [31].

6. UBE3A and neuronal development in AS

The *UBE3A* gene has at least 16 exons that span about 100 kb and produces an mRNA of 5–8 kb size, spliced into five different mRNA types [32,33]. UBE3A produces a protein called the E6-associated protein (E6AP) which acts as a cellular ubiquitin ligase enzyme. It is termed 'E6-associated' because it was first discovered as the protein able to associate with p53 in the presence of the E6 oncoprotein of the human papilloma virus, type 16 [34]. The E6AP enzyme's function is to create a covalent linkage (e.g. the 'ligase' function) between the small ~76 amino acid ubiquitin molecule and its target protein [35]. After initial ubiquitin attachment, for example onto p53, E6AP can then add ubiquitins onto the first ubiquitin to create a polyubiquitylated substrate. Proteins modified in this way can then be targeted for degradation though the 26S proteasome complex [36,37]. The E6AP is the prototype of what is termed the E3 component of the ubiquitin cycle; E1 and E2 proteins, respectively, activate and transfer the ubiquitin molecule to E3. The E3 is then able to bind to a target protein and transfer and ligate ubiquitin to the target. This ligation reaction occurs mainly in a catalytic region of the E3 enzyme, called the homologous to E6AP C terminus (HECT) domain [38]. Most Angelman UBE3A mutations disrupt function of this region of the protein [39] (Fig. 4).

Ubiquitin-dependent proteolysis has been implicated in many cellular events and degradation of targeted proteins by the proteosome is the best studied. More recently, it has been appreciated that ubiquitylation, like phosphorylation, methylation, and acetylation, can regulate protein function or gene expression [40,41]. Indeed, many E3 proteins (and their specific genes) have now been discovered and they have distinct ways of mono or polyubiquitylation. These proteins play a role in diverse cellular events such as DNA repair, cell cycle control, antigen presentation,

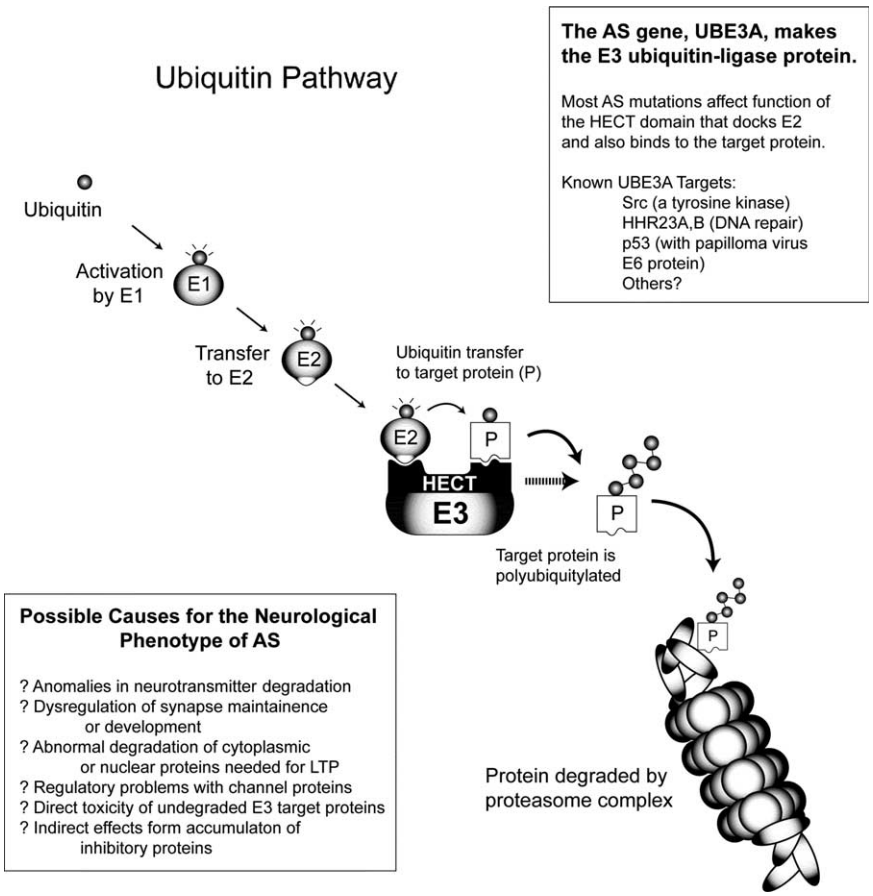

Fig. 4. Illustration of the ubiquitin pathway and the role of the UBE3A (E6-AP) protein. It functions as a ubiquitin E3 ligase and transfers activated ubiquitin to target proteins resulting in their degradation by the 26S proteasome. Many types of E3 ligase proteins function to regulate to complex process of cellular protein homeostasis. Refer to text for details.

chromosome organization, intracellular translocation of proteins, intracellular signaling and apoptosis [42].

What then are the protein targets for the E6AP/UBE3A protein? Unfortunately, no clearly pathogenic target protein has yet been identified. The cell cycle control protein p53, a target in the presence of the E6 protein, was first to be indentified but its role in AS is unclear [43]. The activated form of Src family tyrosine kinase Blk, and HHR23A and HHR23B (homologues of RAD23, an excision repair protein in yeast) appear to be targets [44,45]. However, these targets do not yet give insight into the neuronal pathophysiology of AS.

The UBE3A deficient mouse model provides some insight into regional brain dysfunction with recent work focused on the well-studied phenomenon of long-term potentiation (LTP). Learning in the context of LTP is abnormal in the AS mouse [43,46]. In recent LTP studies involving mouse hippocampus, abnormal ratios of phospho-calcium/calmodulin-dependent protein kinase II (CaMKII) have been found. Other downstream effectors of the LPT process such as protein kinase C (PKC), and cAMP-dependent protein kinase A (PKA) appeared to function normally. It appears, however, that CaMKII is not an actual target for ubiquitylation by UBE3A. Presumably there is some indirect connection to this protein's phosphorylation status [43].

Theoretically, UBE3A disruption could cause the mental retardation and seizures of AS at many cellular sites. Ubiquitin processes have been implicated in axonal guidance [47] and synapse development [48] although UBE3A per se has not yet been implicated in such events. It is intriguing to speculate that neuronal channel proteins, be they voltage-gated (e.g. the alpha subunit of sodium channels) or ligand gated (e.g. glutamate receptors) are in some perturbed by disruption in UBE3A function.

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References

[1] Angelman H. Puppet children: a report on three cases. Dev Med Child Neurol 1965;7:681–8.
[2] Jiang Y, Lev-Lehman E, Bressler J, Tsai TF, Beaudet AL. Genetics of Angelman syndrome. Am J Hum Genet 1999;65:1–6.
[3] Mann MR, Bartolomei MS. Towards a molecular understanding of Prader-Willi and Angelman syndromes. Hum Mol Genet 1999;8: 1867–73.
[4] Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. J Med Genet 2003;40:87–95.
[5] Steffenburg S, Gillberg CL, Steffenburg U, Kyllerman M. Autism in Angelman syndrome: a population-based study. Pediatr Neurol 1996; 14:131–6.
[6] Petersen MB, Brondum-Nielsen K, Hansen LK, Wulff K. Clinical, cytogenetic, and molecular diagnosis of Angelman syndrome: estimated prevalence rate in a Danish county. Am J Med Genet 1995;60:261–2.
[7] Vercesi AM, Carvalho MR, Aguiar MJ, Pena SD. Prevalence of Prader-Willi and Angelman syndromes among mentally retarded boys in Brazil. J Med Genet 1999;36:498.
[8] Aquino NH, Bastos E, Fonseca LC, Llerena Jr JC. Angelman syndrome methylation screening of 15q11–q13 in institutionalized individuals with severe mental retardation. Genet Test 2002;6: 129–31.
[9] Jacobsen J, King BH, Leventhal BL, Christian SL, Ledbetter DH, Cook Jr EH. Molecular screening for proximal 15q abnormalities in a mentally retarded population. J Med Genet 1998;35:534–8.
[10] Buckley RH, Dinno N, Weber P. Angelman syndrome: are the estimates too low? Am J Med Genet 1998;80:385–90.
[11] Williams CA, Angelman H, Clayton-Smith J, Driscoll DJ, Hendrickson JE, Knoll JH, et al. Angelman syndrome: consensus for diagnostic criteria. Angelman syndrome foundation. Am J Med Genet 1995;56: 237–8.
[12] Fryburg JS, Breg WR, Lindgren V. Diagnosis of Angelman syndrome in infants. Am J Med Genet 1991;38:58–64.
[13] King RA, Wiesner GL, Townsend D, White JG. Hypopigmentation in Angelman syndrome. Am J Med Genet 1993;46:40–4.
[14] Boyd SG, Harden A, Patton MA. The EEG in early diagnosis of the Angelman (happy puppet) syndrome. Eur J Pediatr 1988;147: 508–13.
[15] Laan LA, Renier WO, Arts WF, Buntinx IM, v.d. Burgt IJ, Stroink H, et al. Evolution of epilepsy and EEG findings in Angelman syndrome. Epilepsia 1997;38:195–9.
[16] Kishino T, Lalande M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome [published erratum appears in Nat Genet, 1997 Apr;15(4):411]. Nat Genet 1997;15:70–3.
[17] Matsuura T, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS, et al. De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 1997;15:74–7.
[18] Rougeulle C, Glatt H, Lalande M. The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain [letter]. Nat Genet 1997; 17:14–15.
[19] Albrecht U, Sutcliffe JS, Cattanach BM, Beechey CV, Armstrong D, Eichele G, et al. Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. Nat Genet 1997;17:75–8.
[20] Runte M, Huttenhofer A, Gross S, Kiefmann M, Horsthemke B, Buiting K. The IC-SNURF-SNRPN transcript serves as a host for multiple small nucleolar RNA species and as an antisense RNA for UBE3A. Hum Mol Genet 2001;10:2687–700.
[21] Perk J, Makedonski K, Lande L, Cedar H, Razin A, Shemer R. The imprinting mechanism of the Prader-Willi/Angelman regional control center. Eur Med Biol Org J 2002;21:5807–14.
[22] Nicholls RD, Knepper JL. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. Annu Rev Genomics Hum Genet 2001;2:153–75.
[23] Pujana MA, Nadal M, Guitart M, Armengol L, Gratacos M, Estivill X. Human chromosome 15q11–q14 regions of rearrangements contain clusters of LCR15 duplicons. Eur J Hum Genet 2002;10:26–35.
[24] Pujana MA, Nadal M, Gratacos M, Peral B, Csiszar K, Gonzalez-Sarmiento R, et al. Additional complexity on human chromosome 15q: identification of a set of newly recognized duplicons (LCR15) on 15q11-q13, 15q24, and 15q26. Genome Res 2001;11:98–111.
[25] Gimelli G, Pujana MA, Patricelli MG, Russo S, Giardino D, Larizza L. Genomic inversions of human chromosome 15q11–q13 in mothers of Angelman syndrome patients with class II (BP2/3) deletions. Hum Mol Genet 2003;12:849–58.

[26] Robinson WP, Christian SL, Kuchinka BD, Penaherrera MS, Das S, Schuffenhauer S, et al. Somatic segregation errors predominantly contribute to the gain or loss of a paternal chromosome leading to uniparental disomy for chromosome 15. Clin Genet 2000;57:349–58.
[27] Buiting K, Gross S, Lich C, Gillessen-Kaesbach G, El-Maarri O, Horsthemke B. Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. Am J Hum Genet 2003;72:571–7.
[28] Lossie AC, Whitney MM, Amidon D, Dong HJ, Chen P, Theriaque D, et al. Distinct phenotypes distinguish the molecular classes of Angelman syndrome. J Med Genet 2001;38:834–45.
[29] Tekin M, Jackson-Cook C, Buller A, Ferreira-Gonzalez A, Pandya A, Garrett CT, et al. Fluorescence in situ hybridization detectable mosaicism for Angelman syndrome with biparental methylation. Am J Med Genet 2000;95:145–9.
[30] Williams CA, Lossie A, Driscoll D. Angelman syndrome: mimicking conditions and phenotypes. Am J Med Genet 2001;101:59–64.
[31] Stalker HJ, Williams CA. Genetic counseling in Angelman syndrome: the challenges of multiple causes [see comments]. Am J Med Genet 1998;77:54–9.
[32] Yamamoto Y, Huibregtse JM, Howley PM. The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. Genomics 1997;41:263–6.
[33] Kishino T, Wagstaff J. Genomic organization of the UBE3A/E6-AP gene and related pseudogenes. Genomics 1998;47:101–7.
[34] Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 1993;75:495–505.
[35] Huibregtse JM, Scheffner M, Beaudenon S, Howley PM. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc Natl Acad Sci USA 1995;92:2563–7.
[36] Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1–E2–E3 enzyme ubiquitin thioester cascade. Nature 1995;373:81–3.
[37] Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell 1994;79:13–21.
[38] Verdecia MA, Joazeiro CA, Wells NJ, Ferrer JL, Bowman ME, Hunter T, et al. Conformational flexibility underlies ubiquitin ligation mediated by the WWP1 HECT domain E3 ligase. Mol Cell 2003;11:249–59.
[39] Malzac P, Webber H, Moncla A, Graham JM, Kukolich M, Williams C, et al. Mutation analysis of UBE3A in Angelman syndrome patients. Am J Hum Genet 1998;62:1353–60.
[40] Weissman AM. Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol 2001;2:169–78.
[41] Hicke L. Protein regulation by monoubiquitin. Nat Rev Mol Cell Biol 2001;2:195–201.
[42] Conaway RC, Brower CS, Conaway JW. Emerging roles of ubiquitin in transcription regulation. Science 2002;296:1254–8.
[43] Miura K, Kishino T, Li E, Webber H, Dikkes P, Holmes GL, Wagstaff J. Neurobehavioral and electroencephalographic abnormalities in Ube3a maternal-deficient mice. Neurobiol Dis 2002;9:149–59.
[44] Oda H, Kumar S, Howley PM. Regulation of the Src family tyrosine kinase Blk through E6AP-mediated ubiquitination. Proc Natl Acad Sci USA 1999;96:9557–62.
[45] Kumar S, Talis AL, Howley PM. Identification of HHR23A as a substrate for E6-associated protein-mediated ubiquitination. J Biol Chem 1999;274:18785–92.
[46] Jiang YH, Armstrong D, Albrecht U, Atkins CM, Noebels JL, Eichele G, et al. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation [see comments]. Neuron 1998;21:799–811.
[47] Murphey RK, Godenschwege TA. New roles for ubiquitin in the assembly and function of neuronal circuits. Neuron 2002;36:5–8.
[48] Hegde AN, DiAntonio A. Ubiquitin and the synapse. Nat Rev Neurosci 2002;3:854–61.