From Genomic Imprinting to Developmental Physiology: Identifying Stepping Stones

Bernard Dan*,[a,b], Stewart G. Boyd[c], Guy Cheron[b,d]

[a]*Department of Neurology, University Children's Hospital Queen Fabiola, Free University of Brussels (ULB), 15 Avenue J. J. Crocq, 1020 Brussels, Belgium,* [b]*Laboratory of Physiology, ISEPK, Free University of Brussels, Belgium,* [c]*Department of Clinical Neurophysiology, Great Ormond Street Hospital for Children, London, United Kingdom,* [d]*Laboratory of Electrophysiology, University of Mons-Hainaut, Belgium*

Abstract: Genomic imprinting is a process that determines differential expression of genes according to their parental origin. Most imprinted genes play roles in growth, development and tumour suppression. Angelman syndrome is one of the most studied human diseases related to a gene that is expressed on the maternal chromosome only (at least in certain brain cells). It is caused by inactivation of the *UBE3A* gene in the brain due to various abnormalities of chromosome 15q11-q13 inherited from the mother. Its phenotype includes developmental delay, absent speech, motor impairment, a typical electroencephalogram, seizures and a peculiar behaviour. Lack of *UBE3A* expression may result from deletion of the 15q11-q13 region where this gene and *GABRB3* are located, paternal uniparental disomy, imprinting defect or *UBE3A* mutation. Animal models corresponding to the different molecular classes have been generated. An integrative hypothesis for the molecular pathophysiology of the syndrome suggests dysregulation of synaptic neurotransmission through *UBE3A*-related modulation of functional $GABA_A$ receptors and *GABRB3*-related amount of β3 sub-unit in these receptors. This would account for developmental changes as well as for the differences in severity between deletion and non-deletion cases. In addition to rehabilitation programmes adapted to the patients' individual needs, promising management approaches may include pharmacological agents interfering with $GABA_A$ receptors, increasing *GABRB3* expression or altering DNA methylation.

Key Words: Genomic imprinting, DNA methylation, Angelman syndrome, Chromosome 15, UBE3A, GABRB3, $GABA_A$.

INTRODUCTION

Genomic imprinting is defined as differential expression of genes according to their maternal or paternal origin. In humans, about 50 genes are imprinted (for an updated list, see www.otago.ac.nz/IGC). About half are expressed when they are inherited from the mother and half when inherited from the father. Most of these genes seem to play roles in growth, development and tumour suppression. For some of them, imprinted expression is partial [Chung *et al.* 1996], specific to a developmental stage [Ekstrom *et al.* 1995] or to a tissue [DeChiara *et al.* 1991]. Imprinting of one allele (maternal or paternal) is signalled at concerned loci by DNA methylation at cytosine sites and/or histone modification [Reik *et al.* 2001]. DNA methylation has been studied more extensively than histone modification. After DNA synthesis has been completed, addition of methyl groups to cytosine alters the major groove of DNA to which DNA binding proteins attach. This may result in either decreased or increased rate of transcription, according to the position of the methylation change with respect to the transcription initiation site [Jones & Takai, 2001]. Such epigenetic markers can be copied post-synthetically, resulting in heritable changes in chromatin structure.

*Address correspondence to this author at the Department of Neurology, University Children's Hospital Queen Fabiola, Free University of Brussels (ULB), 15 Avenue J. J. Crocq, 1020 Brussels, Belgium; Tel: +322 477 3174; Fax: +322 477 2176; E-mail bernard.dan@ulb.ac.be

DNA cytosine methylation has been implicated as an important epigenetic determinant in human disease, particularly in cancer and developmental disorders (e.g. Rett syndrome, OMIM#312750). DNA methylation associated with genomic imprinting is also implicated in human disease [Clayton-Smith, 2003], as in Angelman syndrome (OMIM# 105830), Prader-Willi syndrome (OMIM#176270), Silver-Russell syndrome (OMIM*180860), Huntington disease (OMIM*143100), Albright's hereditary osteodystrophy (OMIM#103580) and Beckwith-Wiedemann syndrome (OMIM#130650). Angelman syndrome is one of the most studied human diseases related to a gene that is expressed on the maternal chromosome only in at least some brain cells. It is caused by inactivation of the *UBE3A* gene in the brain due to various abnormalities of chromosome of 15q11-q13 inherited from the mother. It is characterised by severe developmental delay, seizures, virtual absence of speech, motor impairment and a peculiar behavioural phenotype. Abnormalities in the corresponding region of the chromosome 15q11-q13 of paternal origin give rise to Prader-Willi syndrome, a clinically distinct condition with hypotonia, learning difficulties, obesity and hypogonadism, the factor determining the phenotypic outcome being the parental origin of the chromosome defect. In addition, these two disorders provided the first example of 'imprinting mutations' in humans. This has offered much insight into genomic imprinting and its processes. In this review, we will focus on recent findings and current understanding of the processes that lead to the expression of Angelman syndrome, as this

1570-1603/04 $45.00+.00

condition seems to provide an archetype of neurodevelopmental disruption due to dysfunction of an imprinted gene that may occur through several distinct mechanisms.

ANGELMAN SYNDROME

This syndrome was originally described in three unrelated children with facial dysmorphism, cognitive impairment, inability to speak, easily provoked laughter, ataxia and seizures [Angelman, 1965]. Angelman syndrome has an estimated prevalence of 1:12000 [Steffenburg *et al.* 1996]. It has mostly been described in children, but awareness of the syndrome has been growing in the adult population, particularly in institutionalised patients [Therasse *et al.* 1997; Sandanam *et al.* 1997; Buckley *et al.* 1998], and the natural history has been increasingly documented [Buntinx *et al.* 1995; Laan *et al.* 1996; Clayton-Smith, 2001]. Beyond individual situations, Angelman syndrome can serve as a model opening broad questioning of genetic and epigenetic influences in neurology, as well as of several concepts such as psychomotor development, cerebral palsy, behavioural phenotypes and epileptic syndromes [Dan & Cheron 2003].

CLINICAL DIAGNOSIS

Clinical diagnosis of Angelman syndrome is based on a set of physical and behavioural features [Williams *et al.* 1995] (Table **1**). The main cranio-facial signs are illustrated in Fig. (**1**). Patients have developmental delay with severely impaired cognitive skills, though accurate assessment is often difficult. About one third of them speak no words at all, while it is very rare that patients use more than 5 words. This contrasts with better receptive verbal communication and relatively good communication skills based on spontaneous or learned signs. Behaviour is characteristically overactive, happy and sociable. Muscle tone abnormalities include axial hypotonia, present from birth, and spastic hypertonia of the limbs that becomes apparent during the first year of life. Despite varying degrees of ataxia, most patients develop independent walking. Gait is distinctive, with a wide base, lower limb extension and lateral rotation, and associated elbow flexion and wrist supination. More than 80 % of patients have epileptic seizures. The interictal electroencephalogram shows three typical rhythmic patterns that may be found independently, respond differently to drugs and show different developmental profiles [Boyd *et al.* 1988; Dan & Boyd, 2003]. These electroencephalographic patterns are not related to specific genotypes but seem to be caused by *UBE3A* dysfunction, possibly related to $GABA_A$ receptor dysfunction [Dan & Boyd, 2003]. However, they do not seem to be epileptic and should be differentiated from non-convulsive status epilepticus. In addition, less specific epileptic activity may occur, more marked in patients with a deletion involving the *GABRB3* gene.

Table 1. Clinical features of Angelman Syndrome (Adapted from [Williams *et al.* 1995]).

Constant features (100 %)
developmental delay, usually severe
severe language impairment (virtual absence of words, expressive language better than receptive language
movement and balance impairment (tremor, hypertonia, myoclonia, ataxia)
Common features (80%)
relative microcephaly
happy demeanour, frequent smiling and laughing
epileptic seizures
abnormal electroencephalogram
hyperactive behaviour
Other features (20-80 %)
brachycephaly
wide mouth, widely spaced teeth, protruded tongue
excessive mouthing behaviour
strabismus
skin, hair and eye hypopigmentation
poor heat tolerance
feeding difficulties in infancy

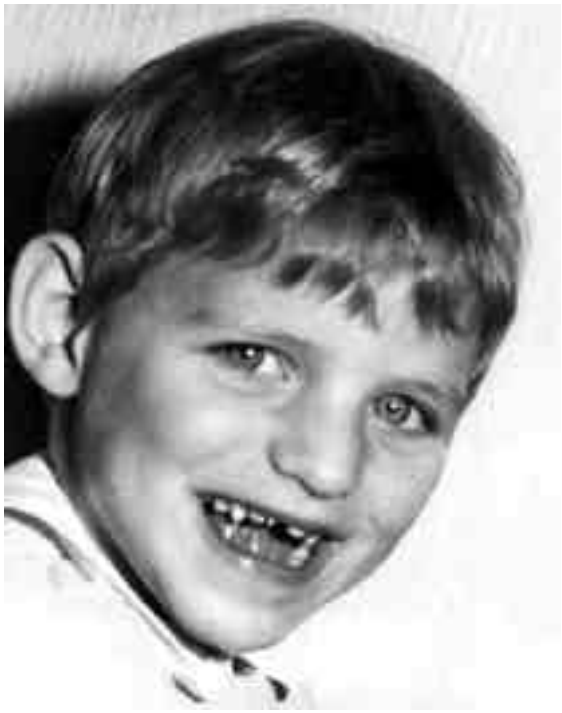

Fig. (1). Facial characteristics of a patient with Angelman syndrome. Note visual contact, fair eyes, pointed nose, midface hypoplasia, wide smiling mouth, prognathism and sialorrhoea.

MOLECULAR CLASSES

Genetic testing may confirm the diagnosis in around 85 % of cases, allowing categorisation into 6 molecular classes (Table **2**). Sub-classes have been defined on the basis of the mechanism giving rise to each molecular class [Clayton-Smith & Laan, 2003]. All patients with a molecular diagnosis of Angelman syndrome have a functional absence of the maternally inherited *UBE3A* gene. In about 70%, this is due to a ~4 Mb interstitial microdeletion of chromosome 15q11-q13 [Knoll *et al.* 1989]. The region concerned by such microdeletions (Fig. (**2**)) is remarkably consistent despite

Table 2. Molecular Classes of Angelman Syndrome (with Approximate Proportions).

I	15q11-q13 deletion	70%
II	Paternal uniparental disomy	2-3%
III	Imprinting defect	3-5%
IV	*UBE3A* gene mutation	5-10%
V	other 15q11-q13 abnormalities	<2%
VI	unidentified	<10%

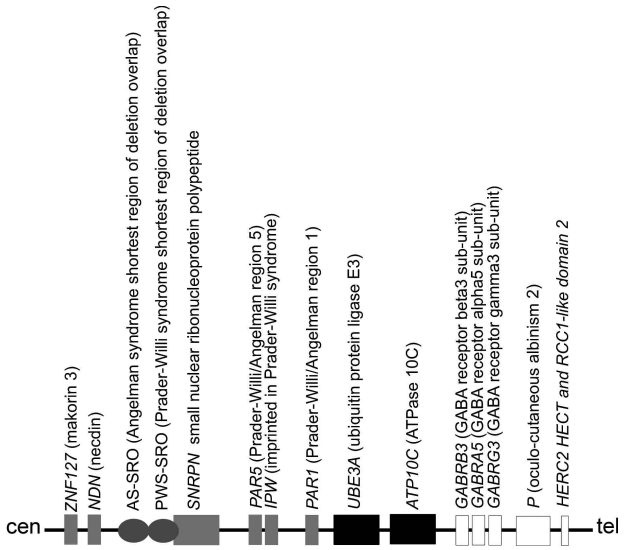

Fig. (2). Map of chromosome 15q11-q13 that is commonly involved in deletions causing Angelman syndrome. Grey and black boxes represent paternally and maternally imprinted genes, respectively. White boxes represent unimprinted genes.

some degree of variability in the breakpoint of the deletion closest to the centromere [Knoll *et al.* 1999]. This may be related to the presence of multiple incomplete copies of the *HERC2* gene, increasing the risk of recombination between the repeats during meiosis [Nicholls, 1994]. In addition to the *UBE3A* and *HERC2* genes, other genes are implicated in the deletion, possibly resulting in a contiguous gene syndrome. The *ATP10C* gene is expressed preferentially from the maternal chromosome only [Meguro *et al.* 2001]. However, other genes in the deletion region, such as *SNRPN*, *ZNF127*, *NDN* and *IPW*, are only active on the paternally-derived chromosome 15, so that maternal deletion should not affect the level of their product. However, absence of a copy of the *GABRB3*, *GABRG3* and *GABRA5* genes, which code for sub-units of the γ-aminobutyric acid receptor $GABA_A$, has tentatively been related to abnormalities in GABAergic neurotransmission [Olsen & Avoli, 1997].

There is a mutation in the maternal *UBE3A* gene [Kishino *et al.* 1997; Matsuura *et al.* 1997] in another 5 to 10% of patients. This gene comprises 16 exons with a coding region from exons 8 to 16. Point mutations have been described throughout the coding region [Malzac *et al.* 1998]. Most of them appear to be private mutations, with a high occurrence rate of *de novo* mutations. Most mutations are frameshift mutations in exon 9, which constitutes approximately half of the coding region. By contrast, exons 15 and 16 appear to constitute hot spots with approximately 3 times more mutations than expected from their size. Many reported missense and single amino acid mutations are located in the catalytic cleft between the two lobes of the (highly conserved) HECT domain in exon 16 [Huang *et al.* 1999].

A further 3 to 5% of patients have an imprinting defect resulting in a lack of the typical maternal pattern of DNA methylation [Buiting *et al.* 1995]. This defect may be related to an abnormality of another microregion in chromosome 15q11-q13 that contains a 880 bp sequence (Angelman syndrome shortest region of deletion overlap abbreviated AS-SRO) located 35 kb upstream of exon 1 of the *SNRPN* gene. AS-SRO or an element inside it is thought to be necessary for the establishment of maternal imprinting in the female germline. It contains one of several upstream *SNRPN* exons (u5), but it is unclear whether a 5' alternative *SNRPN* transcript or an element close to u5 is important for this activity. In this region an 'imprinting centre' regulates chromatin structure, DNA methylation and gene expression through *cis*-acting elements. Imprinting centre defect results in the incorrect setting of the methylation patterns within 15q11-q13 during gametogenesis, as well as the reversal of allele-specific expression of imprinted genes on the affected homologue in the offspring. It may be due to microdeletion [Buiting *et al.* 1995] or mutation [Ohta *et al.* 1999] of the imprinting centre. In most cases, imprinting centre mutations are inherited. However, the majority of imprinting defects are due to epimutations that occurred spontaneously in the absence of DNA sequence changes [Buiting *et al.* 2003].

Approximately 2 to 3% of patients have inherited both copies of chromosome 15 from the father and none from the mother, i.e. paternal uniparental disomy [Malcolm *et al.* 1991]. As a result, no functional copy of the *UBE3A* gene is inherited from the mother. The majority of cases of paternal uniparental disomy of chromosome 15 arise when an egg that has no copy of chromosome 15 as a result of a maternal meiotic error (nullisomic gamete) is fertilised by a sperm with a single copy of chromosome 15, which undergoes subsequent duplication [Robinson *et al.* 2000]. This leads to uniparental isodisomy. Rare instances of translocation or other rearrangements of chromosome 15 have also been reported.

These different mechanisms may result in phenotypes of varying severity [Bürger *et al.* 1996; Minassian *et al.* 1996; Minassian *et al.* 1998; Moncla *et al.* 1999; Lossie *et al.* 2001; Dan *et al.* 2000b], although some features such as rhythmic electroencephalographic patterns [Dan & Boyd 2003] and motor control strategies [Dan *et al.* 2000a; Dan *et al.* 2001; Dan & Cheron in press] appear to be broadly similar across the different genotypes. Communication, cognitive and motor impairment as well as seizure disorder are generally more severe in cases with chromosome 15q11-q13 deletion than in cases with imprinting defect and paternal uniparental disomy [Bürger *et al.* 1996; Minassian *et al.* 1998; Moncla *et al.* 1999; Lossie *et al.* 2001; Dan *et al.* 2000b]. This may be related to the presence of relatively

reduced silencing of the *UBE3A* gene when two paternal copies are present.

ANIMAL MODELS

The molecular characterisation of Angelman syndrome has allowed the development of animal models of the various mechanisms underlying the syndrome. There are now several mouse models based on the genetic homology between human chromosome 15q11-q13 and murine chromosome 7C-D1. The human and mouse gene homologues are imprinted in the same way in the two species, located in the same order on the chromosome but in the opposite orientation.

An equivalent of human Angelman syndrome microdeletion has been constructed by transgene insertion in chromosome 7B-C of mice [Gabriel *et al.* 1999]. Although paternal transmission of the transgene resulted in a lethal phenotype within the first week of life, maternal transmission did not result in obvious phenotypical abnormalities but has not been studied neurophysiologically. Mice with maternal inheritance of the transgene had virtually absent *Ube3a* expression in the cerebellum compared to normal levels in wild-type littermates and mice with paternal inheritance of the transgene (which are a deletion mouse model for Prader-Willi syndrome).

Mice with partial paternal uniparental disomy of chromosome 7A-C, showing paternal duplication and maternal deficiency for ~30 cM of the central chromosome 7, had a high incidence of failure to thrive as well as spontaneous postnatal death in the first month compared to normal siblings [Cattanach *et al.* 1997]. However, survivors were grossly obese by 6 months despite shorter tail and femur lengths than normal siblings. They had mild gait ataxia with slight aversion of the hind limbs, increased startle and hyperactive behaviour. Electrocorticographic recordings showed bilateral prolonged runs of high amplitude delta rhythmic activity in marked contrast to the normal low amplitude irregular fast activity seen in the wild type. Neuropathological examination disclosed no abnormality except for thinning of the cerebral cortex, though no cell loss was observed.

Surviving homozygotes with null mutation of the *Ube3a* gene tended to show failure to thrive, hypoactive behaviour and abnormal posture when held by the tail [Jiang *et al.* 1998]. There was no obvious phenotypic abnormality in heterozygous mice with either maternal or paternal deficiency. However, specific motor testing showed impaired coordination in mice with inactivated maternally inherited *Ube3a* gene. They also showed learning impairment and deficits in hippocampal long-term potentiation that have been related to diminished calcium/calmodulin-dependent protein kinase II activity [Weeber *et al.* 2003]. Electrocorticographic recordings showed almost continuous runs of rhythmic 3/s activity while they were awake and active [Jiang *et al.* 1998]. This activity was mixed with polyspikes and slow waves. Audiogenic seizures were induced in 20-30% of these mice and in none of the wild-type or paternal *Ube3a*-deficient mice. The brain weight of maternally deficient mice was lower than that of the wild-type or paternally deficient mice but no morphological differences were noted. Another mouse model with targeted inactivation of the maternally inherited *Ube3a* gene was constructed by inserting a cassette containing an internal ribosome entry site and a *LacZ-neo*R fusion gene to permit detection of allele knockout expression by LacZ staining [Miura *et al.* 2002]. These mice also showed no obvious phenotypic abnormalities, but fine testing revealed impaired motor coordination and learning compared to paternal *Ube3a* deficient siblings. Hippocampal electrocorticographic recordings showed runs of high amplitude 4-5/s spike-waves in maternally deficient mice. These mice also had low brain weight but no morphological abnormality in the hippocampus, cerebellum and olivary bulbs compared to paternally deficient siblings. In maternally deficient mice, LacZ staining was found in the Purkinje cell layer of the cerebellum, the hippocampus, dentate gyrus, ventricular ependyma and scattered cells in the frontal cortex.

Of the transgenic models implicating other genes in the murine chromosomal region homologous for Angelman syndrome, the most relevant appears to be a mouse that is deficient in the *GABRB3* gene [Homanics *et al.* 1997]. Half of the homozygous knockout mice had cleft palate and neonatal mortality was very high. Survivors had seizures, hyperactive behaviour, coordination and learning impairment [Homanics *et al.* 1997; DeLorey *et al.* 1998]. These mice showed developmental changes in their electrocorticographic recordings consisting of progressive slowing and the subsequent appearance of high amplitude irregular slow and sharp waves, and generalised seizures associated with spiking [DeLorey *et al.* 1998]. Heterozygotes tended to show behaviours intermediate between wild-type and homozygous null mutants, with significant abnormalities in electrocorticogram, seizures and rest-activity patterns [DeLorey *et al.* 1998].

$GABA_A$ RECEPTOR DYSFUNCTION

Many features of Angelman syndrome have been hypothesised to be related to dysfunction of the $GABA_A$ receptor complex. This heteropentameric complex constitutes a family of membrane chloride channel proteins produced from a number of genes encoding for about 19 different sub-units that produce about 20 different $GABA_A$ receptors with different localisations, biological and pharmacological properties [Olsen *et al.* 1999]. $GABA_A$ receptors play an important role in fast inhibitory synaptic transmission [Olsen & Avoli, 1997], as well as an important developmental role in neuronal survival, migration, and synaptogenesis [Belhage *et al.* 1998]. Deficits in these receptors have been related to neurodevelopmental impairment and seizure disorder [Olsen & Avoli, 1997; Olsen *et al.* 1999; Perez Velazquez, 2003]. In particular, the importance of $GABA_A$ receptors in synaptic transmission related to motor control, cognitive processing and seizure disorder has focussed attention on their possible alterations in the pathophysiology of Angelman syndrome [DeLorey *et al.* 1998; Lalande *et al.* 1999]. Such considerations have been increasingly supported by observation of the modulating effect of some drugs on particular electroencephalographic findings and have influenced the use of pharmacological agents in patients with Angelman syndrome.

In both patients and animal models of Angelman syndrome electroencephalography shows distinctive rhythmic patterns [Dan & Boyd, 2003]. Such cortical rhythms are thought to result from thalamo-cortical interactions [Kandel & Buzsaki, 1997]. In both physiological and pathological phenomena the generation of thalamo-cortical rhythms is dependent on $GABA_A$ receptor-mediated synaptic inhibition [Blumenfeld & McCormick, 2000], which may be significantly altered by changes in the sub-unit composition [Browne *et al.* 2001]. Mutations in different genes coding for $GABA_A$ receptor sub-units have been shown to result in altered inhibition through different mechanisms, including reduced surface expression of functional receptors and altered gating [Bianchi *et al.* 2002].

$GABA_A$ receptors also play a major role in cerebellar physiology. Except for granule cells and unipolar brush cells, all neurone populations in the cerebellar cortex produce synaptic γ-aminobutyric acid. In Angelman syndrome, cerebellar dysfunction contributes to the motor impairment [Dan *et al.* 2001; Dan & Cheron in press], as already suspected by Angelman [1965], and possibly to communication and learning impairment. Neuroradiological and neuropathological studies of this syndrome have shown no abnormalities except for some reports of mild to moderate non-specific cerebral atrophy and one documented case of cerebellar atrophy that may be secondary to anticonvulsant therapy [Jay *et al.* 1991]. However, isotopic imaging using ligands binding onto the benzodiazepine site of the $GABA_A$ receptor complex showed decreased binding in several regions including the cerebellum [Holopainen *et al*. 2001]. *In situ* hybridisation studies showed lack of *Ube3a* expression in Purkinje cells of a mouse model of Angelman syndrome with partial uniparental disomy [Albrecht *et al*., 1997]. Another mouse model with targeted inactivation of maternally-inherited *Ube3a* showed increased levels of cytoplasmic p53 in Purkinje cells [Jiang *et al*. 1998]. More recently, succedaneous activity to *Ube3a* was found in the Purkinje cell layer in genotypically and phenotypically similar mice in which a *Lac-z* cassette was inserted in the maternally-inherited *Ube3a* gene [Miura *et al*., 2002]. These converging evidences suggest cerebellar dysfunction involving Purkinje cells. The latter occupy a final integrating position in the cortical cerebellar network. Oscillatory local field potentials were recently found in mice with maternal inactivation of the *Ube3a* gene, generated by Purkinje cells with high levels of synchrony of Purkinje cell activity along the parallel fibre beam [Dan *et al*. 2003]. The implication of cerebellar $GABA_A$ circuitry in this oscillation (demonstrated by gabazine injection) points to the involvement of the cerebellar inhibitory network linking the Purkinje cells and the coupled (by mutual GABAergic synapses and gap junctions) molecular interneurones.

MOLECULAR PATHOPHYSIOLOGY

The 15q11-q13 region contains a cluster of genes for three sub-units (α5, β3, and γ3) of the $GABA_A$ receptor that are expressed in several regions of the adult brain and are even more abundant in many regions of the embryonic and neonatal brain [Laurie *et al*., 1992]. The discovery of the *GABRB3* gene made it a strong candidate for explaining the molecular pathogenesis of Angelman syndrome, as all $GABA_A$ receptor subtypes are believed to contain a β sub-unit and the β3 is the only one present early in life [Laurie *et al*. 1992]. However, it has become clear that this cannot account for all the features of Angelman syndrome. Indeed, patients with 15q11-q13 cytogenetic alterations, such as deletions (Prader-Willi syndrome), pericentromeric inversions and duplications (inv-dup(15) syndrome) or other types of duplications (in some cases of autism), have distinct clinical phenotypes. Furthermore, about 30 % of patients with features of Angelman syndrome do not have a deletion involving the *GABRB3* gene, i.e. patients with imprinting defect, uniparental disomy, *UBE3A* mutation or no detectable 15q11-q13 abnormality. Finally, a patient with a deletion involving the *GABRB3* gene but not the critical region for Angelman syndrome did not show the clinical characteristics of Angelman syndrome although he had seizures [Minassian *et* a*l*. 1996].

Other genes located in the 15q11-q13 region have been implicated in specific features of Angelman syndrome, such as the *P* gene in hypopigmentation in patients with a deletion [Bürger *et al.*1996] and the *ATP10C* gene in eventual obesity in patients with a deletion or an imprinting defect [Meguro *et al.* 2001]. The latter has been tentatively linked to deficient membrane phospholipid transport that depends on an aminophospholipid translocase [Meguro *et al.* 2001]. However, it is now evident that all patients with a molecular diagnosis of Angelman syndrome have a functional absence of the maternally inherited *UBE3A* gene. As in humans with Angelman syndrome [Rougeulle *et al.* 1997], brain Ube3a protein levels are reduced in mouse models which do not knock out the *GABRB3* gene, due either to lack of imprinting [Albrecht et al. 1997] or to *UBE3A* knockout [Jiang *et al.* 1998, Miura *et al.* 2002].

Research has therefore concentrated on the role of the *UBE3A* gene, though the mechanism by which its inactivation results in Angelman syndrome is still unclear. This gene is specifically imprinted in the brain, with different region-specific levels of silencing of the paternal allele. In different mouse models, evidence of expression of *UBE3A* has been found predominantly in the hippocampus, olfactory bulbs and cerebellar Purkinje cells (or in the Purkinje cell layer). The *UBE3A* gene encodes a sense transcript maternally in neurones but biallelically in glial cells, and an anti-sense transcript only in neurones and only from the paternal allele [Yamasaki *et al.* 2003]. The product of the *UBE3A* gene, ubiquitin-protein ligase E3A abbreviated UBE3A, exists in at least three isoforms of UBE3A that are determined by alternative splicing of the gene [Yamamoto *et al.,* 1997]. A major advance in our understanding of *UBE3A* structure and insights into the effects of mutations on *UBE3A* function were provided by a fine description of the crystal structure of the complex between HECT domain of the *UBE3A* gene and an E2 protein [Huang *et al.*, 1999]. E2 interacts with E3 through the N lobe of the HECT domain, then transfers ubiquitin to C820 of UBE3A. C820 is located in the catalytic cleft between the two lobes of the HECT domain, where many mutations found in patients with Angelman syndrome have been located. UBE3A plays a role in ubiquitin-mediated

protein catabolism. This pathway leads to selective proteolysis implicating covalent attachment of ubiquitin, a highly conserved polypeptide, on to lysine residues of target proteins by the sequential action of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3), which appear to be implicated in substrate recognition [Glickman & Ciechanover, 2002]. E3 enzymes have been classified into three groups on the basis of their primary structures: the HECT, RING-finger and U-box families. UBE3A is a HECT-domain ubiquitin E3 ligase. The best-characterised function of ubiquitination is to mark target proteins for proteolysis by proteasomes. The ubiquitin-proteasome system has been regarded as a quality-control system for intracellular proteins that is particularly active in the degradation of misfolded or unfolded proteins [Hatakeyama & Nakayama, 2003]. UBE3A alias E6-AP, was originally identified by its ability to promote the degradation of the p53 oncoprotein in association with the E6 protein of the human papilloma virus [Huibregtse *et al.* 1991]. The p53 protein interacts with a large number of intracellular proteins and may transactivate many genes, thereby modulating cell proliferation or apoptosis [Chêne, 2001]. In addition to p53, UBE3A ubiquitinates human homologues of yeast Rad23 protein [van der Spek *et al.* 1996], implicated in nucleotide repair processes, and B lymphocyte kinase or Blk [Oda *et al.* 1999]. UBE3A can also ubiquitinate itself in a reaction that implicates E6 [Kao *et al.* 2000].

Ubiquitination pathways have been implicated in the pathophysiology of other neurological disorders [Chung *et al.* 2001]. For example, early-onset autosomal recessive Parkinson disease (OMIM#600116) is due to abnormalities of the gene coding for parkin, another E3 ubiquitin-protein ligase [Shimura *et al.* 2001].

Although the specificity of ubiquitin ligases is yet far from being clarified, it may be speculated that the effect of functional absence of the *UBE3A* gene gives rise to Angelman syndrome through several mechanisms. It has been proposed that disruption of a step in the ubiquitin pathway of proteolysis results in accumulation of intracellular proteins [Lalande *et al.* 1999]. These proteins could then interfere with the function of different tissues more or less markedly in function of their biochemical activities et perhaps more specifically with the expression of other genes. The brain and in particular certain brain regions might be more affected than other tissues, notably at crucial stages of neurological maturation. This phenomenon might be enhanced in the brain because of a lack of compensation by other proteolysis mechanisms that do not include the specific functional aspects of UBE3A and also because neurones do not divide after differentiation. Increased abundance of cytoplasmic p53, whose interaction with UBE3A/E6-AP is thought to require the presence of E6, has been found in Purkinje cells of a young adult with Angelman syndrome (hippocampus was not analysed) [Jay *et al.* 1991, Jiang *et al.* 1998] and Purkinje cells and hippocampal neurones in a mouse model of Angelman with maternal inactivation of the *Ube3a* gene [Jiang *et al.* 1998] but not in another, similar model [Miura *et al.* 2002].

However, ubiquitination has other roles in specific protein labelling than targeting for proteolysis. It has been implicated in specific activation of certain enzymes [Wang *et al.* 2001]. This would provide another possible mechanism for the role of *UBE3A* dysfunction in producing the expression of Angelman syndrome, though no putative enzymatic substrate has been proposed to date. Moreover, none of these mechanisms would account for the apparent role of the β3 sub-unit of the $GABA_A$ receptor as outlined above.

The ubiquitin pathway may also play a role in the regulation of the abundance of postsynaptic receptors [Burbea *et al.* 2002]. Kleijnen *et al.* [2000] recently demonstrated the interaction of UBE3A with ubiquitin-like Plic proteins. This is of particular interest since Plic-1 selectively binds to $GABA_A$ receptors containing the β3 sub-unit and regulates the number of these receptors in the cell membrane [Bedford *et al.* 2001]. This process appears critical for regulating GABAergic synaptic strength [Kneussel, 2002]. The molecular mechanism by which Plic-1 affects $GABA_A$ receptor function remains to be determined, but a direct role for proteasome function seems plausible, as suggested by recent characterisation of hPLIC-2–proteasome binding [Kleijnen *et al.* 2003]. This would provide a more complete mechanism for Angelman syndrome whereby functional absence of UBE3A would impair the regulation of $GABA_A$ receptors, likely leading to stoichiometric re-arrangements. In addition to the β sub-unit, the differential effect of benzodiazepines on the typical electroencephalographic patterns of Angelman syndrome suggests that $GABA_A$ receptor sub-unit re-arrangements also involve α and γ sub-units, as their action does not depend on the β sub-unit subtype [Bianchi *et al.* 2002]. Considering the biallelic expression of the genes encoding these sub-units, such rearrangements would depend on post-translational factors, even in deletion cases. Elevated plasma levels of γ-aminobutyric acid might reflect a compensatory process [Ebert *et al.* 1997].

Extensive application of this hypothesis to the evolution of the syndrome expression with age is currently difficult because of limited data on maturation of GABA receptors. However, the absence of the distinctive electroencephalographic abnormalities in the first months of life may be explained by the known change of GABA function related to *KCC2* gene expression [Rivera *et al.* 1999]. Similarly, the diminished $GABA_A$ receptor expression with increasing age reported in epileptic children [Chugani *et al.* 2001] may partly account for the decreased amplitude and prevalence of distinctive electroencephalographic patterns in Angelman syndrome in later childhood and perhaps the occasional development of alpha rhythm and the enhancement of anterior sharp slow-waves in adolescence.

PERSPECTIVES FOR MANAGEMENT

Management of Angelman syndrome rests very largely on re-habilitation programmes aimed at optimising the motor, cognitive and communication development. Such programmes are tailored individually, based on the specific needs of patients rather than on the aetiological diagnosis. It is unlikely that postnatal "attempts to modify anatomic destiny" [Philippart, 2001] will be effective in fundamentally altering the natural history of the syndrome given the importance of the $GABA_A$ receptor complex in early develop-

ment, including the foetal period. In contrast, recognition and management of shortcomings and special risks remains a high priority, including effective control of epilepsy.

Pharmacological agents that interact with the $GABA_A$ receptor complex, such as benzodiazepines, barbiturates and corticosteroids, and other drugs that increase the level of γ-aminobutyric acid at GABAergic synapses have been abundantly used to control the seizure disorder associated with Angelman syndrome. However, several cases of clinical deterioration have been reported following treatment of epilepsy with vigabatrin [Kuenzle *et al.* 1998; Østergaard & Balslev, 2001]. As this medication potentiates GABAergic neurotransmission by inhibiting GABA-transaminase, it seems paradoxical that it would aggravate a condition thought to be due to deficiency in $GABA_A$ receptors [Olsen & Avoli, 1997]. Nevertheless, $GABA_A$-ergic drugs have not been proposed to alter other aspects of the syndrome expression.

The heterogeneity of $GABA_A$ receptor sub-units determines not only functional differences between subtypes but also differential interactions with drugs. In the case of Angelman syndrome, it might be interesting to develop a pharmacological agent that would specifically augment $GABA_A$ receptors containing a β3 sub-unit. In this context, it is of interest that lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) has been recognised as an effective anticonvulsant in Angelman syndrome [Ruggieri & McShane, 1998; Østergaard & Balslev, 2001]. Although this drug has no direct effect on $GABA_A$ receptors [Gibbs *et al.* 2002], it was recently shown that chronic treatment with lamotrigine increases *GABRB3* gene expression in primary cultured rat hippocampal cells (CA1, CA3 and dentate gyrus) [Wang *et al.* 2002].

The effect of methylation of the *UBE3A* gene on its activity has also fostered hopes that epigenetic modulation might lead to restoration of its function. The larger part of the paternal chromosome 15q11-q13 is unmethylated and most of the genes located in this region are expressed. DNA methylation can be affected by dietary levels of methyl-donor components, such as folic acid [Van den Veyver, 2002]. Empirical oral supplementation of folinic acid normalised 5-methyltetrahydrofolate levels in the cerebro-spinal fluid and led to partial clinical improvement in four patients with Rett syndrome [Ramaekers *et al.* 2003], a condition that shows a clinical overlap with Angelman syndrome [Ellaway *et al.* 1998]. Given the possible phenotypic similarities between Angelman syndrome and methylenetetrahydrofolate reductase (*MTHFR*) deficiency (OMIM#607093) [Williams *et al.* 2001], Beaudet hypothesised that low folic acid might decrease *UBE3A* gene expression and high folic acid might increase it [Beaudet & Bacino, 2003]. Formulating the hypotheses that dietary manipulation might increase DNA methylation, that this eventual increase would favour expression of the paternal *UBE3A* gene and that eventual, even late expression of this gene would improve the patients' condition, this team proposed to treat patients with Angelman syndrome with a diet enriched in high doses of folic acid and betaine. Folic acid was expected to increase DNA methylation and betaine to enhance the effect of folic acid. Early clinical results of a double-blind trial conducted in children have not shown clear changes, though the possibility of moderate benefits has not been analysed [Beaudet & Bacino, 2003]. In addition, this line of thought might benefit from the growing body of clinical research aimed at altering DNA methylation patterns in oncology both through dietary manipulation and pharmacology, though many of the drugs currently developed in this field are directed at inhibiting DNA methylation in order to re-activate methylation-silenced genes.

Another avenue to alter *UBE3A* gene methylation relates to epigenetic mechanisms that contribute to the regulation of imprinting, such as modification of the chromatin structure which constitutes the functional mark distinguishing the two alleles at imprinted domains. Developmentally, this may be generated through a number of different molecular pathways, including differential DNA methylation or asynchronous replication timing [Simon *et al.* 1999]. Histone modifications constitute a crucial level in epigenetic regulation. Tails of histone amino acids protruding from the nucleosome are sites of post-translational enzyme-catalysed modifications including acetylation, methylation, phosphorylation, and ubiquitination. These modifications affect histone interactions with the DNA and other proteins, especially during chromatin formation. This represents a complex set of epigenetic information with combinatorial potential forming a 'histone code' that specifies patterns of gene expression. The role of histone methylation in the maintenance of parent-specific DNA methylation [Xin *et al.* 2003] may point to a role of histone methylation in establishing DNA methylation patterns and have direct relevance to *UBE3A* gene expression. The AS-SRO has been shown to be packaged with acetylated histone H4 and methylated histone H3(K4) only on the maternal allele, and this imprinted epigenetic structure is maintained through cell divisions despite the absence of clear differential DNA methylation [Perk *et al.* 2002]. The molecular steps controlling the imprinting process have recently been characterised in the mouse, which shows remarkable similarity with man in the sequence of the imprinting centre [Kantor *et al.* 2004]. Pharmacological approaches which could induce histone modification or more generally interfere with chromatin packaging, might promote demethylation or remethylation of imprinted genes or of imprinting control regions. Nucleosome remodelling induced by specifically designed complexes, covalent histone modification within the nucleosome or replacement of core histones by variants might be of particular interest in this regard.

CONCLUSION

Almost all manifestations of Angelman syndrome seem to be related to lack of *UBE3A* expression in the brain. In physiological conditions, only the maternal allele is expressed in some brain regions. Lack of *UBE3A* expression may result from several mechanisms including deletion of the 15q11-q13 region where this gene and *GABRB3* are located, which may be isolated or rarely be due to chromosome re-arrangement, paternal uniparental disomy, which may occur postzygotically or less often arise through meiotic non-disjunction, imprinting defect, which may

occasionally be due to imprinting centre mutation, or *UBE3A* mutation. It may be hypothesised that the phenotypic features are caused by dysregulation of synaptic neurotransmission through *UBE3A*-related modulation of functional $GABA_A$ receptors in all cases and additional *GABRB3*-related deficit in β3 sub-unit in these receptors in deletion cases. Promising therapeutic avenues include pharmacological agents interfering with $GABA_A$ receptors, increasing *GABRB3* expression or altering DNA methylation.

REFERENCES

Albrecht, U.; Sutcliffe, J. S.; Cattanach, B. M.; Beechey, C. V.; Armstrong, D.; Eichele, G. and Beaudet, A.L. (1997) Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat. Genet.* **17**, 75-78.

Angelman, H. (1965) 'Puppet' Children: a report on three cases. *Dev. Med. Child Neurol.* **7**, 681-688.

Beaudet, A.L. and Bacino, C.A. (2003) A double blind trial of folic acid and betaine in Angelman syndrome. Angelman Syndrome Foundation 8th Biennial Conference, International Angelman Syndrome Organisation 2nd World Conference, Washington D.C., USA, July 1-5.

Bedford, F. K.; Kittler, J. T.; Muller, E.; Thomas, P.; Uren, J. M.; Merlo, D.; Wisden, W.; Triller, A.; Smart, T. G. and Moss, S. J. (2001) GABA(A) receptor cell surface number and subunit stability are regulated by the ubiquitin-like protein Plic-1. *Nat. Neurosci.* **4,** 908-916.

Belhage, B.; Hansen, G. H.; Elster, L. and Schousboe, A. (1998) Effects of gamma-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect. Dev. Neurobiol.* **5**, 235-246.

Bianchi, M. T.; Song, L.; Zhang, H. and Macdonald, R. L. (2000) Two different mechanisms of disinhibition produced by GABA-A receptor mutations linked to epilepsy in humans. *J. Neurosci.* **22**, 5321-5327.

Blumenfeld, H. and McCormick, D.A. (2000) Corticothalamic inputs control the pattern of activity generated in thalamocortical networks. *J. Neurosci.* **20,** 5153-5162.

Browne, S. H.; Kang, J.; Akk, G.; Chiang, L. W.; Schulman, H.; Huguenard, J. R. and Prince, D. A. (2001) Kinetic and pharmacological properties of GABA(A) receptors in single thalamic neurons and GABA(A) subunit expression. *J. Neurophysiol.* **86**, 2312-2322.

Boyd, S. G.; Harden, A. and Patton, M. A. (1988) The EEG in early diagnosis of the Angelman (happy puppet) syndrome. *Eur. J. Pediatr.* **147**, 508-513.

Buckley, R. H.; Dinno, N. and Weber, P. (1998) Angelman syndrome: are the estimates too low? *Am. J. Med. Genet.* **80**, 385-390.

Buiting, K.; Saitoh, S.; Groß, S.; Dittrich, B.; Schwartz, S.; Nicholls, R. D. and Horsthemke, B. (1995) Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. *Nat. Genet.* **9**, 395-400.

Buiting, K.; Groß, S.; Lich, C.; Gillessen-Kaesbach, G.; El-Maari, O. and Horsthemke, B. (2003) Epimutations in Prader-Willi and Angelman syndrome: a moleculkar study of 136 patients with an imprinting defect. *Am. J. Hum. Genet.* **72**, 571-577.

Buntinx, I. M.; Hennekam, R. C.; Brouwer, O. F.; Stroink, H.; Beuten, J.; Mangelschots, K. and Fryns, J.P. (1995) Clinical profile of Angelman syndrome at different ages. *Am. J. Med. Genet.* **56**, 176-183.

Burbea, M.; Drier, L.; Dittman, J. S.; Grunwald, M. E. and Kaplan J. M. (2002) Ubiquitin and AP180 regulate the abundance of GLR-1 Glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron* **33**, 107-120.

Bürger, J.; Kunze, J.; Sperling, K. and Reis, A. (1996) Phenotypic differences in Angelman syndrome patients: imprinting mutations show less frequently microcephaly and hypopigmentation than deletions. *Am. J. Med. Genet.* **66**, 221-226.

Cattanach, B. M.; Barr, J. A.; Beechey, C. V.; Martin, J.; Noebels, J. and Jones, J. (1997) A candidate model for Angelman syndrome in the mouse. *Mamm. Genome* **8**, 472-478.

Chêne, P. (1996) The role of tetramerization in p53 function. *Oncogene* **20**, 2611-2617.

Chugani, D. C.; Muzik, O.; Juhasz, C.; Janisse, J. J.; Ager, J. and Chugani, H. T. (2001) Postnatal maturation of human GABA receptors measured with positron emission tomography. *Ann. Neurol.* **49**, 618-626.

Chung, W. Y.; Yuan, L.; Feng, L.; Hensle, T. and Tycko, B. (1996) Chromosome 11p15.5 regional imprinting : comparative analysis of *KIP2* and *H19* in human tissues and Wilms' tumors. *Hum. Mol. Genet.* **5**, 1101-1108.

Chung, K. K. K.; Dawson, V. L. and Dawson TM (2001) The role of the ubiquitin-proteasomal pathway in Parkinson's disease and other neurodegenerative disorders. *Trends Neurosci.* **24**, S7-S14.

Clayton-Smith, J. (2001) Angelman syndrome : Evolution of the phenotype in adolescents and adults. *Dev. Med. Child Neurol.* **43**, 476-480.

Clayton-Smith, J. (2003) Genomic imprinting as a cause of disease. *BMJ* **327**, 1121-1122.

Clayton-Smith, J. and Laan, L. (2003) Angelman syndrome: a review of the clinical and genetic aspects. *J. Med. Genet.* **40**, 87-95.

Dan, B.; Bouillot, E.; Bengoetxea, A. and Cheron, G. (2000a) Lower limb coordination patterns in locomotion in Angelman syndrome. *Mov. Disord.* **15** (suppl. 3), 234-235.

Dan, B.; Boyd, S. G.; Christiaens, F.; Courtens, W.; Van Maldergem, L. and Kahn, A. (2000b) Atypical features in Angelman syndrome due to imprinting defect or uniparental disomy of chromosome 15. *Neuropediatrics* **31**, 109-110.

Dan, B.; Bouillot, E.; Bengoetxea, A.; Boyd, S. G. and Cheron, G. (2001) Distinctive multijoint control strategies in spastic diplegia associated with prematurity or Angelman syndrome. *Clin. Neurophysiol.* **112**, 1618-1625.

Dan, B. and Boyd, S. G. (2003) Angelman syndrome reviewed from a neurophysiological perspective. The *UBE3A-GABRB3* hypothesis. *Neuropediatrics*, **34**, 169-176.

Dan, B. and Cheron, G. (2003) Le syndrome d'Angelman: un modèle clinique et génétique. *Rev. Neurol. (Paris)* **159**, 499-510.

Dan, B.; Servais, L.; Wagstaff, J. and Cheron, G. (2003) Neurophysiological behaviour of Purkinje cells in mice with inactivated maternally inherited *ube3a* gene. *Eur. J. Paediatr. Neurol.* **7**, 295-296.

Dan, B. and Cheron, G. (in press) Postural rhythmic muscle bursting activity in Angelman syndrome. *Brain Dev.*

DeChiara, T. M.; Robertson, E. J.; Efstratiadis, A. (1991) Parental imprinting of the mouse insulin-like growth factor II gene. *Cell,* **64**, 849-859.

DeLorey, T. M.; Handforth, A.; Anagnostaras, S. G.; Homanics, G. E.; Minassian, B. A.; Asatourian, A.; Fanselow, M. S.; Delgado-Escueta, A.; Ellison, G. D. and Olsen, R. W. (1998) Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J. Neurosci.* **18**, 8505-8514.

Ebert, M. H.; Schmidt, D. E.; Thompson, T. and Buttler M. G. (1997) Elevated plasma gamma-aminobutyric acid (GABA) levels in individuals with either Prader-Willi syndrome or Angelman syndrome. *J. Neuropsychiatry Clin. Neuropsy.* **9,** 75-80.

Ellaway, C.; Buchholtz, T.; Smith, A.; Leonard, H. and Christodoulou, J. (1998) Rett syndrome : significant clinical overlap with Angelman syndrome but not with methylation status. *J. Child Neurol.* **13**, 448-451.

Ekstrom, T. J.; Cui H, Li X.;and Ohlsson, R. (1995) Promoter-specific *IGF2* imprinting status and its plasticity during human liver development. *Development* **121**, 309-316.

Gabriel, J. M.; Merchant, M.; Ohta, T.; Ji, Y.; Caldwell, R. G.; Ramsey, M. J.; Tiucker, J. D.; Longnecker, R. and Nicholls, R. D. (1999) A transgene insertion creating a heritable chromosome deletion mouse model of Prader-Willi and Angelman syndromes. *Proc. Natl. Acad. Sci.* **96**, 9258-9263.

Gibbs, J. W. 3rd; Zhang, Y. F.; Ahmmed, H. S. and Coulter, D. A. (2002) Anticonvulsant actions of lamotrigine on spontaneous thalamocortical rhythms. Epilepsia; **43**, 342-349.

Glickman, M. H. and Ciechanover, A. (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* **82,** 373-428.

Hatakeyama, S. and Nakayama, K. I. (2003) Ubiquitylation as a quality control system for intracellular proteins. *J. Biochem. (Tokyo)* **134**, 1-8.

Holopainen, I. E.; Metsahonkala, E. L.; Kokkonen, H.; Parkkola, R. K.; Manner, T. E.; Nagren K. and Korpi, E. R. (2001) Decreased binding of C]flumazenil in Angelman syndrome patients with

GABA(A) receptor beta3 subunit deletions. *Ann. Neurol*. **49**, 110-113.

Homanics, G. E.; DeLorey, T. M.; Firestone, L. L.; Quinlan, J. J.; Handforth, A.; Harrison, N. L.; Krasowski, M. D.; Rick, C. E. M.; Korpi, E. M.; Makela, R.; Brilliant, M.; Hagiwara, N.; Ferguson, C.; Snyder, K. and Olsen, R. W. (1997) Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc. Natl. Acad. Sci.* **94**, 4143-4148.

Huang, L.; Kinnucan, E.; Wang, G.; Beaudenon, S.; Howley, P. M.; Huibregtse, J. M. and Pavletich, N. P. (1999) Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade. *Science* **286**, 1321-1326.

Huibregtse, J. M.; Scheffner, M. and Howley, P. M. (1991) A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. *EMBO J.* **10,** 4129-4135.

Jay, V.; Becker, L. E.; Chan, F. W.; and Perry, Sr. T. L. (1991) Puppet-like syndrome of Angelman: a pathological and neurochemical study. *Neurology* **41**, 416-422.

Jiang, Y.; Armstrong, D.; Albrecht, U.; Atkins, C. M.; Noebels, J. L.; Eichele, G.; Sweatt, J. D. and Beaudet, A. L. (1998) Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. *Neuron* **21**, 799-811.

Jones, P.A. and Takai, D. (2001) The role of DNA methylation in mammalian epigenetics. *Science* **293**,1068-1070.

Kao, W. H.; Beaudenon, S. L.; Talis, A. L.; Huibregtse, J. M. and Howley, P. M. (2000) Human papillomavirus type 16 E6 induces self-ubiquitination of the E6AP ubiquitin-protein ligase. *J. Virol.* **74,** 6408-6417.

Kandel, A. and Buzsaki, G. (1997) Cellular-synaptic generation of sleep spindles, spike-and-wave discharges and evoked thalamocortical responses in the neocortex of the rat. *J. Neurosci*. **17**, 6783-6797.

Kantor, B.; Makedonski, K.; Green-Finberg, Y.; Shemer, R. and Razin, A. (2004) Control elements within the PWS/AS imprinting box and their function in the imprinting process. *Hum. Mol. Genet.* Feb 12 Epub ahead of print]

Kishino, T.; Lalande, M. and Wagstaff, J. (1997) *UBE3A*/E6-AP mutations cause Angelman syndrome. *Nat. Genet.* **15**, 70-73.

Kleijnen, M. F.; Shih, A. H.; Zhou, P.; Kumar, S.; Soccio, R. E.; Kedersha, N. L.; Gill, G. and Howley, P. M. (2000) The hPLIC proteins may provide a link between the ubiquitination machinery and the proteasome. *Mol. Cell*, **6,** 409-419.

Kleijnen, M. F.; Alarcon, R. M. and Howley, P. M. (2003) The ubiquitin-associated domain of hPLIC-2 interacts with the proteasome. *Mol. Biol. Cell*. **14**, 3868-3875.

Kneussel, M. (2000) Dynamic regulation of $GABA_A$ receptors at synaptic sites. *Brain Res. Rev*.**39**, 74-83.

Knoll, J. H. M.; Nicholls, R. D.; Magenis, R. E.; Graham, J. M. Jr.; Lalande, M. and Latt, S. A. (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *Am. J. Med. Genet.* **32**, 285-290.

Knoll, J. H. M.; Nicholls, R. D.; Magenis, R. E.; Glatt, K.; Graham, J. M. Jr.; Kaplan, L. and Laan, L. A. E. M.; Haeringen, A. and Brouwer, O. F. (1999) Angelman syndrome: a review of clinical and genetic aspects. *Clin. Neurol. Neurosurg.* **101**, 161-170.

Kuenzle, C.; Steinlin, M.; Wohlrab, G.; Boltshauser, E. and Schmitt, B. (1998) Adverse effects of vigabatrin in Angelman syndrome. *Epilepsia*, **39**, 1213-1215.

Laan, L. A. E. M.; den Boer, A. T.; Hennekam, R. C.; Renier, W. O. and Brouwer O. F. (1996) Angelman syndrome in adulthood. *Am. J. Med. Genet.* **66**, 356-360.

Lalande M, Minassian BA, DeLorey TM, Olsen RW Parental imprinting and Angelman syndrome. (1999) In *Jasper's Basic Mechanisms of the Epilepsies*, Delgado-Escueta, A. V.; Wilson, W. A.; Olsen, R. W. and Porter, R. J.; Eds. ; 3rd edn., Adv. Neurol. 79. Philadelphia: Lippincott Williams and Wilkins, pp. 421-429.

Laurie, D. J.; Wisden, W. and Seeburg, P. H. (1992)The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J. Neurosci.* **12**, 4151-4172.

Lossie, A. C.; Whitney, M. M.; Amidon, D.; Dong, H. J.; Chen, P.; Theriaque, D.; Hutson, A.; Nicholls, R. D.; Zori, R. T.; Williams, C. A. and Driscoll, D. J. (2001) Distinct phenotypes distinguish the molecular classes of Angelman syndrome. *J. Med. Genet.* **38**, 834-845.

Malcolm, S.; Clayton-Smith, J.; Nichols, M.; Robb, S.; Webb, T.; Armour, J.A.L.; Jeffreys, A.J. and Pembrey, M.E. (1991) Uniparental paternal disomy in Angelman's syndrome. *Lancet* **337**, 694-697.

Malzac, P.; Webber, H.; Moncla, A.; Graham Jr, J. M.; Kukolich, M.; Williams, C.; Pagon, R. A.; Ramsdell, L. A.; Kishino, T. and Wagstaff, J. (1998) Mutation analysis in *UBE3A* in Angelman syndrome patients. *Am. J. Hum. Genet.* **62**, 1353-1360.

Matsuura, T.; Sutcliffe, J. S.; Fang, P.; Galjaard, R. J.; Jiang, Y.; Benton, C. S.; Rommens, J. M. and Beaudet, A. L. (1997) *De novo* truncating mutations in E6-AP ubiquitin-protein ligase gene (*UBE3A*) in Angelman syndrome. *Nat. Genet.* **15**, 74-77.

Meguro, M.; Kashiwagi, A.; Mitsuya, K.; Nakao, M.; Kondo, I.; Saitoh, S. and Oshimura, M. (2001) A novel maternally expressed gene, *ATP10C*, encodes a putative aminophospholipid translocase associated with Angelman syndrome. *Nat. Genet.* **28**, 19-20.

Minassian BA, DeLorey TM, Olsen RW et al. (1996) *GABRB3* gene : a gene involved in the epilepsy of Angelman syndrome. *Abs. Soc. Hum. Genet.* **59**, A273

Minassian, B. A.; DeLorey, T. M.; Olsen, R. W.; Philippart, M.; Bronstein, Y.; Zhang, Q.; Guerrini, R.; van Ness, P.; Livet, M. O. and Delagado-Escueta, A. V. (1998) Angelman syndrome: correlations between epilepsy phenotypes and genotypes. *Ann. Neurol*. **43**, 485-493.

Miura, K.; Kishino, T.; Li, E.; Webber, H.; Dikkes, P.; Holmes, G. L. and Wagstaff, J. (2002) Neurobehavioral and electroencephalographic abnormalities in *Ube3a* maternal-deficient mice. *Neurobiol. Dis.* **9**, 149-159.

Moncla, A.; Malzac, P.; Voelckel, M. A.; Auquier, P.; Girardot, L.; Mattei, M. G.; Philip, N.; Mattei, J. F.; Lalande, M. and Livet, M. O. (1999) Phenotype-genotype correlation in 20 deletion and 20 non-deletion Angelman syndrome patients. *Eur. J. Hum. Genet.* **7**, 131-139.

Nicholls, R. D. (1994) Recombination model for generation of a submicroscopic deletion in familial Angelman syndrome. *Hum. Mol. Genet.* **3**, 9-11.

Oda, H.; Kumar, S. and Howley, P. M. (1999) Regulation of the Src family tyrosine kinase Blk through E6AP-mediated ubiquitination. *Proc. Natl. Acad. Sci. U S A.* **96,** 9557-9562.

Ohta, T.; Buiting, K.; Kokkonen, H.; McCandless, S.; Heeger, S.; Leisti, H.; Driscoll, D. J.; Cassidy, S. B.; Horsthemke, B. and Nicholls, R. D. (1999) Molecular mechanism of Angelman syndrome in two large families involves an imrpinting mutation. *Am. J. Hum. Genet.* **64**, 385-396.

Olsen, R. W. and Avoli, M. (1997) GABA and epileptogenesis. *Epilepsia*, **38**, 399-407.

Olsen, R. W.; DeLorey, T. M.; Gordey, M. and Kang, M. H. (1999) GABA receptor function and epilepsy. *Adv. Neurol.* **79,** 499-510.

Østergaard, J. R. and Balslev, T. (2001) Efficacy of different antiepileptic drugs in children with Angelman syndrome associated with 15q11-13 deletion: the Danish experience. *Dev. Med. Child Neurol.* **43**, 718-719.

Perez Velazquez, J. L. (2003) Bicarbonate-dependent depolarizing potentials in pyramidal cells and interneurons during epileptiform activity. *Eur. J. Neurosci.* **18,** 1337-1342.

Perk, J.; Makedonski, K.; Lande, L.; Cedar, H.; Razin, A. and Shemer, R. (2002)The imprinting mechanism of the Prader-Willi/Angelman regional control center. *EMBO J.* **21**, 5807-5814.

Philippart, M. (2001) Rett and Angelman's syndromes: models of arrested development. *Pediatr. Neurol.* **25**, 288-294.

Ramaekers, V. T.; Hansen, S. I.; Holm, J.; Opladen, T.; Senderek, J.; Hausler, M.; Heimann, G.; Fowler, B.; Maiwald, R. and Blau, N. (2003) Reduced folate transport to the CNS in female Rett patients. *Neurology* **26**; 506-515.

Reik, W.; Dean, W. and Walter, J. (2001) Epigenetic reprogramming in mammalian development. *Science* **293**, 1089-1093.

Rivera, C.; Voipio, J.; Payne, J. A.; Ruusuvuori, E.; Lahtinen, H.; Lamsa, K.; Pirvola, U.; Saarma, M. and Kaila, K. (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* **397**, 251-255.

Robinson, W. P.; Christian, S. L.; Kuchinka, B. D.; Penaherrera, M. S.; Das, S.; Schuffenhauer, S.; Malcolm, S.; Schinzel, A. A.; Hassold, T. J. and Ledbetter, D. H. (2000) Somatic segregation errors predominantly contribute to the gain or loss of a paternal chromosome leading to uniparental disomy for chromosome 15. *Clin. Genet.* **57,** 349-358.

Rougeulle, C.; Glatt, H. and Lalande, M. (1997) The Angelman syndrome candidate gene, *UBE3A/E6-AP*, is imprinted in brain. *Nat. Genet.* **17**, 14-15.

Ruggieri, M. and McShane, M. A. (1998) Parental view of epilepsy in Angelman syndrome: a questionnaire study. *Arch. Dis. Child.*; **79**, 423-426.

Sandanam, T.; Beange, H.; Robson, L.; Woolnough, H.; Buchholtz, T. and Smith, A. (1997) Manifestations in institutionalized adults with Angelman syndrome due to deletion. *Am. J. Med. Genet.* **70**, 415-420.

Shimura, H.; Schlossmacher, M. G.; Hattori, N.; Frosch, M. P.; Trockenbacher, A.; Schneider, R.; Mizuno, Y.; Kosik, K. S. and Selkoe, D. J. (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293**, 263-269.

Simon, I.; Tenzen, T.; Reubinoff, B. E.; Hillman, D.; McCarrey, J. R. and Cedar, H. (1999) Asynchronous replication of imprinted genes is established in the gametes and maintained during development. *Nature* **401**, 929-932.

Steffenburg, S.; Gillberg, C. L.; Steffenburg, U. and Kyllerman, M. (1996) Autism in Angelman syndrome: a population-based study. *Pediatr. Neurol.* **14**, 131-136.

Therasse, F.; Dan, B.; Hilbert, P. and Van Maldergem, L. (1997) Institutionalized adults with Angelman syndrome. *Eur. J. Paediatr. Neurol.* **1**, A4.

van der Spek, P. J.; Eker, A.; Rademakers, S.; Visser, C.; Sugasawa, K.; Masutani, C.; Hanaoka, F.; Bootsma, D. and Hoeijmakers, J. H. (1996) XPC and human homologs of RAD23: intracellular localization and relationship to other nucleotide excision repair complexes. *Nucleic Acids Res.* **24**, 2551-2559.

Van den Veyver, I. B. (2002) Genetic effects of methylation diets. *Annu. Rev. Nutr.* **22**, 255-282.

Wang, C.; Deng, L.; Hong, L.; Akkaraju, G. R.; Inoue, J. and Chen, Z. J. (2001) TAK1 is a ubiquitin-dependent kinase of MKK an IKK. *Nature* **412**, 346-351.

Wang, J. F.; Sun, X.; Chen, B. and Young, L. T. (2002) Lamotrigine increases gene expression of GABA-A receptor β3 subunit in primary cultured rat hippocampal cells. *Neuropsychopharmacol.* **26**, 415-421.

Weeber, E. J.; Jiang, Y. H.; Elgersma, Y.; Varga, A. W.; Carrasquillo, Y.; Brown, S. E.; Christian, J. M.; Mirnikjoo, B.; Silva, A. Beaudet, A. L. and Sweatt, J. D. (2003) Derangements of hippocampal calcium/calmodulin-dependent protein kinase II in a mouse model for Angelman mental retardation syndrome. *J. Neurosci.* **23**, 2634-2644.

Williams, C. A.; Angelman, H.; Clayton-Smith, J.; Driscoll, D. J.; Hendrickson, J. E.; Knoll, J. H.; Magenis, R. E.; Schinzel, A.; Wagstaff, J.; Whidden, E. M. and Zori, R. (1995) Angelman syndrome: consensus for diagnostic criteria. *Am. J. Med. Genet.* **56**, 237-238.

Williams, C. A.; Lossie, A. and Driscoll, D. (2001) Angelman syndrome: Mimicking conditions and phenotypes. *Am. J. Med. Genet.* **101**, 59-64.

Xin, Z.; Tachibana, M.; Guggiari, M.; Heard, E.; Shinkai, Y. and Wagstaff, J. (2003) Role of histone methyltransferase G9a in CpG methylation of the Prader-Willi syndrome imprinting center. *J. Biol. Chem.* **278**, 14996-5000.

Yamamoto, Y.; Huibregtse, J. M. and Howley, P. M. (1997) The human E6-AP gene (*UBE3A*) encodes three potential protein isoforms generated by differential splicing. *Genomics* **41**, 263-266.

Yamasaki, K.; Joh, K.; Ohta, T.; Masuzaki, H.; Ishimaru, T.; Mukai, T.; Niikawa, N.; Ogawa, M.; Wagstaff, J. and Kishino, T. (2003) Neurons but not glial cells show reciprocal imprinting of sense and antisense transcripts of *Ube3a*. *Hum. Mol. Genet.* **12,** 837-847.