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# Epilepsy and cataplexy in Angelman syndrome. Genotype-phenotype correlations

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ABSTRACT

*Background:* Angelman syndrome (AS) is a neurogenetic disorder characterized by intellectual disability, epilepsy, and low threshold for laughter.
*Aims:* We investigated the occurrence and severity of epilepsy and laughter-induced loss of postural muscle tone determined by the different genetic subtypes.
*Methods:* This study included 39 children with AS. Deletion breakpoints were determined by high resolution CGH microarray (1 × 1 M Agilent). Clinical data were based on a parent interview and medical record review.
*Results:* All patients with AS based on a deletion had epilepsy. Epilepsy was present in 3/4 children with UBE3A mutation, and 4/5 with pUPD. Onset of epilepsy occurred earlier in deletion cases compared to pUPD or UBE3A mutations cases. Laughter-induced postural muscle tone loss occurred only among deletion cases. We found no differences in severity of epilepsy between children with a larger Class I or a smaller Class II deletions, or between the total group with a deletion compared to children with pUPD or a UBE3A mutation. The drugs most frequently prescribed were benzodiazepines in monotherapy, or a combination of benzodiazepines and valproic acid.
*Conclusion:* Epilepsy is very common in patients with AS, especially in patients with a deletion. Postural muscle tone loss and collapsing during outbursts of laughter were seen in patients with a deletion only.



## 1. Introduction

Angelman syndrome (AS) is a neurogenetic disorder caused by loss of expression of the maternal imprinted gene *UBE3A*, which codes for the protein ubiquitin-protein ligase E3A. Four known molecular mechanisms lead to deficient maternal *UBE3A* expression and AS development: deletion of the AS critical region on the maternal chromosome 15q11.2–q13 (70–75%), paternal uniparental disomy (pUPD) (5–10%), imprinting defects (2–5%), and mutations in the maternal copy of *UBE3A* (10%) (Tan et al., 2011). Among individuals with a 15q11.2–q13 deletion, approximately one-third carry a 5.9 Mb (Class I) deletion, whereas the remainders have a smaller 5.0 Mb (Class II) deletion; these deletions differ in the location of

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the centromeric breakpoints of the deletion (Mertz et al., 2013; Tan et al., 2011). The deleted region between BP1 and BP2 contains 4 genes that are not imprinted: *TUBGCP5*, *NIPA1, NIPA2*, and *CYFIP1* (Burnside et al., 2011). Deletion of this region may by itself increase the susceptibility to neurodevelopmental problems, including delayed psychomotor development, speech delay, autism spectrum disorder, and possibly seizures (Burnside et al., 2011). The GABA receptor genes are deleted in both Class I and Class II.

Characteristics of AS include severe intellectual disability, lack of speech, laughter outbursts, ataxia, craniofacial dysmorphic features, autistic behavior, and 80–90% of AS patients are reported to have epilepsy (Conant, Thibert, & Thiele, 2009; Pelc, Boyd, Cheron, & Dan, 2008). The pathogenesis of epilepsy in AS remains to be fully understood. It has been hypothesized that deletion of the genes coding for three subunits of the $GABA_A$ receptor complex is an important factor (Fiumara, Pittalá, Cocuzza, & Sorge, 2010). In addition, it has been suggested that the functional absence of *UBE3A*, which occurs in all genetic subtypes, may impair the regulation of the $GABA_A$ receptor complex (Pelc et al., 2008). Epilepsy seems more prevalent in patients with a deletion compared to other genetic subtypes of AS (Thibert et al., 2009). So far, only a single study (Valente et al., 2013) has investigated the occurrence and severity of epilepsy determined by the breakpoints. In that study, including six children with a Class I deletion and ten children with a Class II deletion, no differences in electroencephalographic (EEG) features were found, but more frequent and disabling seizures occurred in patients with the larger deletion (Class I).

In the present study we investigated the different genetic etiologies of AS, including Class I and Class II deletions, and examined the occurrence and severity of epilepsy within the different genetic subtypes. Finally, we investigated whether the typical outburst of laughter in Angelman syndrome may result in loss of postural muscle tonus, mimicking a seizure.

## 2. Methods

### 2.1. Patients and samples

We identified patients with AS who were born in Denmark between 1991 and 2009 through the Danish National Patient Registry (NPR) and the Danish Cytogenetic Central Registry (DCCR), supplemented by personal contact with all Danish pediatric and clinical genetic departments and the Patient Organization of Angelman Syndrome in Denmark. NPR contains administrative and clinical data from all hospitalizations and out-patient clinics in Denmark. Reporting data to the NPR is mandatory for all Danish hospitals and is further encouraged by the government funding system. Thus, the data in this registry are known to be of very high validity (Andersen, Madsen, Jorgensen, Mellemkjoer, & Olsen, 1999). Discharge diagnoses are recorded according to the tenth revision of the International Classification of Diseases (ICD-10) from the World Health Organization. The DCCR contains data from every prenatal genetic test and every postnatal karyotype performed in Denmark since 1960. It also contains national postnatal genetic data on selected diseases such as 22q11.2 deletion, Prader Willi and Angelman syndrome. Data reporting is self-imposed by all clinical genetic departments that perform these genetic tests, and the registry is administered by representatives from these departments.

In total, we identified 51 patients with genetically verified AS. The deadline for identification was January 1, 2012. The National Ethic Committee (M-20090028) and the Danish Data Protection Agency (2009-41-3133) approved the study. The legal guardian of each participant provided verbal and written informed consent.

### 2.2. Genetic analysis

Patients previously diagnosed with a deletion were further investigated by high resolution comparative genome hybridization (CGH). DNA was extracted from peripheral blood with an automated Chemagic Magnetic Separation Module (PerkinElmer, Waltham, MA, USA). DNA was purified prior to an array CGH analysis with the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Deletion breakpoints were determined using microarray-based CGH with the SurePrint G3 Human CGH microarray 1 M (Agilent Technologies, Santa Clara, CA, USA). Patient and reference genomic DNA samples (1500 ng) were labeled with Cy5 (reference) or Cy3 (patient) with the Genomic DNA ULS labeling kit (Agilent Technologies) and purified as per the manufacturer's protocol. Labeled sample and reference DNA were pooled, and 50 μl of human COT-1 DNA (1 mg/ml), 10× blocking agent, and 2× hybridization buffer were added. Hybridization was performed for 40 h at 65 °C. Scanning and image acquisitions were carried out with an Agilent microarray scanner, and microarray image files were quantified with Agilent's Feature Extraction software version 10.7. Data analysis was performed with Genomic Workbench version 6.5 (Agilent Technologies).

Copy number was determined with the adm-2 algorithm. Profile deviations that consisted of six or more neighbouring oligonucleotides were considered genomic aberrations, which yielded a resolution of approximately 12 kb. Deletion breakpoints were based on the position of the first and last oligonucleotide probes within the region of the deletion that showed a loss in copy number. Identification of copy number gains or losses on chromosomes other than 15q11.2–q13 was performed. Copy number variations (CNVs) in areas containing previously reported CNVs from the Database of Genomic Variants (DGV) without known clinical significance were excluded. UCSC hg19 version of the human genome was used as reference.