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Neurodevelopmental outcome in Angelman syndrome: Genotype–phenotype correlations

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ABSTRACT

Angelman syndrome (AS) is a neurogenetic disorder characterized by intellectual disability, developmental delay, lack of speech, and epileptic seizures. Previous studies have indicated that children with AS due to 15q11.2-q13 deletions have a more severe developmental delay and present more often autistic features than those with AS caused by other genetic etiologies. The present study investigated the neurodevelopmental profiles of the different genetic etiologies of AS, and examined the evolution of mental development and autistic features over a 12-year period in children with a 15q11.2-q13 deletion. This study included 42 children with AS. Twelve had a Class I deletion, 18 had Class II deletions, three showed atypical large deletions, five had paternal uniparental disomy (pUPD) and four had UBE3A mutations. Children with a deletion (Class I and Class II) showed significantly reduced developmental age in terms of visual perception, receptive language, and expressive language when compared to those with a UBE3A mutation and pUPD. Within all subgroups, expressive language performance was significantly reduced when compared to the receptive performance. A follow-up study of seven AS cases with 15q11.2-q13 deletions revealed that over 12 years, the level of autistic features did not change, but both receptive and expressive language skills improved.

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%), paternal uniparental disomy (pUPD) (2–7%), imprinting defects (3–5%), and mutations in the maternal copy of *UBE3A* (10%)

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(Tan et al., 2011). There are two common types of 15q11.2-q13 deletions, a 5.9-Mb (Class I) deletion, and a 5.0-Mb (Class II) deletion, differing only in the position of the proximal (centromeric) breakpoint (Tan et al., 2011; Mertz et al., 2013).

Clinical characteristics of AS include severe developmental delay, ataxia, a low threshold for laughter, and 80–90% of AS patients have epileptic seizures (Conant, Thibert, & Thiele, 2009). In addition, a high percentage of individuals with AS has been found to meet the formal diagnostic criteria of autism, defined as total score above autism cut off in ADOS, (autism diagnostic observation schedule) (Lord et al., 2000). Autism or autism spectrum disorder was previously reported in 50–80 per cent of patients with AS (Bonati et al., 2007; Peters, Horowitz, Barberi-Welge, Taylor, & Hundley, 2012; Sahoo et al., 2007; Trillingsgaard & Østergaard, 2004).

Previous studies have indicated that children with AS due to 15q11.2-q13 deletions have a more severe developmental delay and present more often autistic features than those with AS caused by other genetic etiologies (Lossie et al., 2001; Peters et al., 2012; Sahoo et al., 2006, 2007; Varela, Kok, Otto, & Koiffmann, 2004). However, not all studies have used validated standardized instruments, and the total number of investigated individuals remains small. Furthermore, virtually all previous studies have a cross-sectional design, and none have performed longitudinal consecutive assessments of neurodevelopmental parameters in children with AS.

The present study investigated the neurodevelopmental profiles of the different genetic etiologies of AS, including Class I and Class II deletions, and examined the evolution of mental development and autistic features over a 12-year period in children with a 15q11.2-q13 deletion.

2. Methods

2.1. Patient recruitment

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 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, Mellemkjaer, & Olsen, 1999). The 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 syndromes. 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 (Mertz et al., 2013). The deadline for identification was January, 1 2012. This study was approved by the National Ethic Commitee (M-20090028) and the Danish Data Protection Agency (2009-41-3133). The legal guardian of each participant provided verbal and written informed consent.

2.2. Genetic analysis

Patients who were previously diagnosed with a deletion were further investigated using a high resolution 1000-kb array CGH. DNA was extracted from peripheral blood with an automated Chemagic Magnetic Separation Module (PerkinElmer, Waltham, MA, USA) and was purified prior to array CGH analysis using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Deletion breakpoints were determined by microarray-based comparative genomic hybridization with the SurePrint G3 Human CGH microarray 1 M (Agilent Technologies, Santa Clara, CA, USA). Sample and reference genomic DNA (1500 ng) were labeled with Cy5 (reference) or Cy3 (patient) using the Genomic DNA ULS labeling kit (Agilent Technologies) and purified as per the manufacturer's protocol. Labeled sample and reference DNA were pooled, and mixed with 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 using Genomic Workbench version 6.5 (Agilent Technologies).

Copy number was determined with the adm-2 algorithm. Profile deviations consisting of six or more neighboring oligonucleotides were considered genomic aberrations, yielding an approximately 12 kb resolution. Deletion breakpoints were based on the positions of the first and last oligonucleotide probes within the region of the deletion that showed a copy number loss. We also identified copy number gains or losses on chromosomes other than 15q11-q13. Copy number variations (CNVs) in areas containing previously reported CNVs in healthy control samples from the database of genomic variants (DGV) were excluded. UCSC hg19 version of the human genome and public CNV databases were used as reference.

2.3. Mental development and autism

Mental age was assessed with Mullen Scales of Early Learning (Mullen, 1995) based on four sub-scales: visual perception, receptive language, expressive language and fine motor development. The Mullen Scales assess the cognitive and motor

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