

# Torticollis in 15q11.2 Microdeletion Syndrome: a Rare Association in Angelman-like Syndromes

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#### Abstract

15q11-13 chromosome region contains five breakpoints (BP1-BP5). Chromosomal rearrangements are common in this region. The microdeletion of BP1-BP2 region represents the 15q11.2 microdeletion syndrome associating with variable phenotype. We investigated a ten years old boy with hypotony. His motoric functions, speech and intellectual development were delayed. He suffered from epilepsy and showed dysmorphic features. Some of these dysmorphic features such us epicanthus and the clynodactyly of the fifth fingers can be observed in Angelman or Prader-Willi syndromes but have not been described in the 15q11.2 microdeletion syndrome so far. He has congenital torticollis that has been described earlier neither in this microdeletion syndrome nor in Prader-Willi - Angelman syndromes. Our aim is to find the possible mechanisms leading to the phenotype using Metilation Specific - Multi Ligand Probe Assay, Polimerase Chain Reaction and Array Comparative Genomic Hybridization. The 15q11.2 microdeletion syndrome represents an example for the incomplete penetrance and variable expressivity. Further genetic changes, such as other defective genes, further copy number variations, variability in non-coding regions, the mRNA quantity, environmental effects and epigenetic modification may also influence on the severity of the symptoms. We suggest to classify the symptoms into two groups (major and minor criteria). Depending on the existing minor criteria, this syndrome could be identified as Angelman-like or Prader-Willi-like syndromes.

**Keywords:** 15q11.2 microdeletion; Genotype-phenotype correlations; Torticollis; Major and minor criteria; Angelman-like syndrome

## Introduction

The proximal 15q11-13 chromosome region contains five breakpoints (BP1-BP5). Chromosomal rearrangements such as duplications, microdeletions are common changes in this region. The well known microdeletion syndromes related to this region are the Prader-Willi (PWS) and Angelman syndromes (AS), depending on the parental origin of the deleted allele [1]. The severity of the symptoms depends on the size of the deleted region [2-4]. BP1-BP2 region is composed of approximately 500 kb and contains four evolutionarily conserved, non-imprinted genes. The microdeletion of this smaller region has recently been considered as a separate entity, the 15q11.2 microdeletion syndrome [5]. The main phenotypic attributes of this microdeletion syndrome are as follows: developmental delay, idiopathic generalized epilepsy, behavioural abnormalities, motor apraxia and dysmorphic features. Since in some cases these rearrangements/microdeletions can be inherited from unaffected parents, while the majority of patients suffer from serious symptoms, the pathogenicity of 15q11.2 microdeletion remains unclear. Incomplete penetrance, high variability of expression, genetic and epigenetic changes provoked by environmental events may lead to these differences, but further investigations are needed to clarify the perfect genotype-phenotype correlations [6]. Herein, we report a male patient with BP1-BP2 microdeletion associated AS symptoms and his mother with the same genetic rearrangement but without any clinical symptom. By analysis of genotype-phenotype correlation of the family

presented here we try to find the explanation of this variance. As the symptoms of the boy are the same as characteristic of the AS, we concern the mechanisms presumably can cause AS or Angelman-like syndromes.

# Case and Methods

#### **Case presentation**

The ten years old boy was born at full term by vaginal delivery with normal birth weight (3050 gramm). The mother had vaginitis caused by fungus during the pregnancy treated locally. She did not use drugs, alcohol, tobacco or medications. Neither intrauterin retardation nor any other abnormalities were found by ultrasonography. After three months of breastfeeding, bottle-feeding was introduced. A generalized hypotony could be observed from the neonatal period. His movement, speech and intellectual development were delayed. He had epilepsy from three months of age treated for five years, but during the last five years no seizures can be detected without medication. On physical examination plagiocephaly and strabism were found. The skull was flat on the right, his ears were dysmorphic. A congenital torticollis, prognathy, hypertelorism and wide space between teeth could also be identified. He had puppet like posture, ataxia, scoliosis and clinodactyly on the fifth fingers (Figures 1 and 2).



Figure 1: Dysmorphic features of the patient.



Figure 2: Clynodactyly of the fifth finger.

He showed a happy behaviour, hyperactivity, inappropriate laughs with clapping and seemed to be unable to concentrate for a long time. His sensitivity of warmness was high along with an inordinate affinity to the water. He is bound to wheelchair. Brain MRI revealed a minimal temporal liquor space dilatation on the right side and frontopolar liquor space dilatation. The parents of the boy are with normal intellectual capacity, without any clinical symptoms by physical examinations.

# Conventional G-banding karyotyping

Chromosome studies were performed on cultured peripheral blood lymphocytes using phytohemagglutinin as T-cell mitogen. The

preparation of chromosomes was performed according to standard laboratory protocols. The analysis of G-banded metaphases was carried out using Lucia software. The karyotype was given according to ISCN 2009.

## Methylation-Specific multiplex ligation-dependent probe amplification assay (MS-MLPA)

MS-MLPA analysis was performed with SALSA MLPA ME028-B2 Prader-Willi/Angelman probemix and EK1-FAM reagent kit (MRC-Holland, Amsterdam, The Netherlands). The probemix contains 32 probes specific for sequences in or near to the PWS/AS critical region. Five of these probes are specific for an imprinted sequence and can be used for the detection of uniparental disomy or imprinting defects. The MS-MLPA assay and the analysis of the results were performed according to manufacturer's protocol.

## Mutation testing of the UBE3A gene

Sequence analysis of the whole coding region of the *UBE3A* gene was performed by Sanger sequencing. After amplification of the 10 coding exons of *UBE3A* with previously published primer pairs and conditions [7] PCR products were sequenced on the ABI 310 sequencer with the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Electropherograms of the sequenced products were compared to the reference sequence of *UBE3A* (NG\_009268).

# Array CGH

Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). Detection of copy number variations was performed using Affymetrix CytoScan<sup>\*</sup> 750K Array (Affymetrix, USA). The DNA (250 ng) was amplified, labelled and hybridized to the array platform according to the manufacturer's protocols. The analysis was performed by Chromosome Analysis Suite (ChAS) software using the annotations of the genome version GRCH37 (hg19). Gains and losses that affected a minimum of a 100 kb length were initially considered.

# Results

By the physical examination, we found the symptoms specific of the Angelman syndrome. The four major criteria (developmental delay, speech impairment, movement or balance disorder, behavioural characteristics) and three from the six minor criteria (postnatal deceleration of head growth, seizures, attraction to water) could be observed [8]. After G-banding chromosome analysis showing normal male karyotype, MS-MLPA analysis was performed. Neither copy number changes distally from BP2, nor abnormal methylation of the *SNRPN* and *NDN* genes could be identified; however, a heterozygous deletion of the *TUBGCP5* and *NIPA1* genes located between BP1 and BP2 was detected (Figure 3).





**Figure 3:** Dosage quotients (DQ) of the MLPA probes (ME028-B2 probe kit).Lower than 0.7 DQ values of probes specific for TUBGCP5 and NIPA1 genes indicate heterozygous deletion.

Analyses of the parental samples revealed the maternal origin of the BP1-BP2 microdeletion. The mother did not have any symptoms of the BP1-BP2 microdeletion or those of Angelman syndrome. The maternal grandparents were not investigated. As about 10% of the Angelman

syndrome patients have a mutation in the *UBE3A* gene [9], mutation analysis of the whole coding region of *UBE3A* was also performed, but no mutation was identified. Array CGH analysis confirmed a 855 Kb deletion at 15q11.2, ranging from 22,770,421- 23,625,785 (Figure 4).



Figure 4: The four non-imprinted genes (TUBGCP5, CYFIP1, NIPA1, NIPA2) in the deleted BP1-BP2 region of chromosome 15.

Based on the SNP data, loss of homozygosity was not detected on chromosome 15 excluding the paternal uniparental disomy (patUPD). Genome-wide analysis of the genomic DNA did not revealed any other copy number variation which might have caused Angelman-like syndrome [10].

## Discussion

We performed a detailed genetic examination of a patient with clinical symptoms of Angelman syndrome. Even though the patient had the major and the minor criteria of the Angelman syndrome [8], the MS-MLPA and array CGH investigation revealed a deletion of the 15q11.2 BP1-BP2 chromosome region, suggesting that the patient's disease corresponds to the 15q11.2 microdeletion syndrome [5]. After a thorough investigation of the symptoms, we concluded that the phenotype of our patient was in accordance with the 15q11.2 microdeletion syndrome: delayed psychomotor development and speech, mental retardation, abnormal behaviour including attention deficit-hyperactivity disorder (ADHD), hypotony, happy appearance, ataxia, seizures. The plurality of dysmorphic features of this microdeletion syndrome was perceptible on our patient as well. He has

plagiocephaly, hypertelorism, abnormal shape of ears, strabism and irregular teeth [5,6,11-16]. He has recurrent upper airway infections that are known to be frequent in 15q11.2 microdeletion syndrome [5]. The deleted region flanked by BP1 and BP2 of the Prader-Willi/ Angelman syndrome critical region contains four non-imprinted genes: TUBGCP5, NIPA1, NIPA2 and CYFIP1. As it was described in previous articles TUBGCP5 plays an important role in establishing of the behavioural phenotype of these patients [2,5,17]. The highest expression of this gene is detected in the subthalamic nuclei of the brain, that are involved in ADHD and obsessive-compulsive behaviour [5,6,18,19]. The deletion of the NIPA1 and CYFIP1 genes seem to have an important role in the motoric and speech delays [5]. NIPA1 is highly expressed in neuronal tissue and encodes a putative membrane transporter involved in intracellular magnesium transport. CYFIP1 interacts with FMRP and GTPase RAC1, which are involved in the development and maintenance of neuronal structures [5]. It is in synaptosomal extracts indicating co-localisation with FMRP and RAC1 in dendritic fine structures [20,21]. NIPA2 encodes a membrane transport protein that is important in renal magnesium metabolism [22]. Nevertheless, TUBGCP5 and NIPA1 are expressed in foetal heart that can account for the congenital heart disease, which is one of the symptoms of 15q11.2 microdeletion syndrome [23]. The patient presented here does not have any heart defect. Moreover, an association was described between the 15q11.2 (BP1-BP2) microdeletion and schizophrenia but not with ASD [24-26]. Our patient does not show any symptoms of this psychiatric disorder, but he is still too young for this disease. He does not have any sleeping problems, feeding difficulties and some of the dysmorphic features like cleft and narrow palate either [5,14]. There are some further symptoms that have been described only in few cases, such as congenital arthrogryposis [16], esophageal atresia (EA) and tracheoesophageal fistula (TEF) [15]. These symptoms could not been identified in our patient either. These latter disorders can be due to a gene disturbance or mutation present in the intact alleles in this region, as coincidental findings or due to an unrelated defective gene elsewhere in the genome [16]. Our patient has some dysmorphic features that were described in Angelman or Prader-Willi syndromes earlier, but they have not been mentioned so far as the symptoms of the 15q11.2 (BP1-BP2) microdeletion syndrome like the epicanthus and the clynodactyly of the fifth fingers. At the same time in our patient a congenital torticollis could be observed as additional symptom that has not been described earlier in this microdeletion syndrome or in PW - AS either. It can be a coincidental symptom, or the consequence of the failed neurological development. In the latter case it is a rare symptom of the 15q11.2 microdeletion syndrome.

## Conclusion

The 15q11.2 microdeletion syndrome seems to be a good example for the incomplete penetrance and variability of expression. As we found in our patient and in her mother, many cases were published where the microdeletion was inherited from a healthy, unaffected parent [5,6,13,16]. Further genetic alterations, such as other defective genes, further Copy Number Variations (CNVs), variability in noncoding regions, the mRNA quantity, environmental effects and epigenetic modification can also have effects on the phenotype, on the severity of symptoms [5,6]. To clarify which of these mechanisms or what kind of further genetic alterations are necessary for manifestation of the various symptoms of the syndrome, further studies are required. It is sure that like other pathogenic CNVs in the human genome, the 15q11.2 microdeletion also contributes to a specific phenotype and

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predisposes the deletion-carriers for the disease [5,27,28]. Based on our experience we may confirm the hypothesis that NIPA1 and CYFIP1 genes have an important role in the neurological development, and that the deletion of TUBGCP5 contributes to the behavioural problems [5]. We suggest to classify the symptoms of the 15q11.2 microdeletion syndrome into two groups: major and minor criteria. The major criteria are the following: delayed psychomotor development, speech delay and behavioural problems (ASD, ADHD and obsessive-compulsive disorder (OCD). The minor criteria include: seizures, congenital heart defect, happy appearance, dyspraxia, dyscalculia, dyslexia, sleep problems and dysmorphic features: different deformities of the skull, broad forehead, hypertelorism, cleft and narrow palate, dysmorphic ears and slender fingers, strabism, micrognathia, irregular teeth and bulbous nose. Those symptoms that are described in the literature only in one case (congenital arthrogryposis, EA and TEF) and the some symptoms of our patient, such as epicanthus, torticollis and clynodactyly of the fifth fingers can be classified as minor criteria. Since the BP1-BP2 microdeletion is associated with all typical symptoms only in a part of the cases, the knowledge of them is of paramount importance because they are very helpful in setting up the diagnosis and provide a basis for adequate genetic tests. Based on the minor criteria, this syndrome could be a member of the Angelman- or Prader-Willi -like syndromes. Further investigations of the genetic background of the 15q11.2 microdeletion syndrome are required to reveal the variability of the symptoms using modern molecular genetic methods. In the possession of more knowledge we can give adequate genetic advice to the affected families, offering them prevention as well as develop diagnostic guideline.

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## **Conflict of Interest**

The authors declare no conflict of interest.

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