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Case Report
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A Molecular Approach to *AGBL4* Gene Deletion in Autism: A Case Report

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Abstract

Genetic studies have recently identified several candidate genes that have been associated with autism. Genetic defects related to microtubule formation and functions have been implicated in autism. Microtubules and microtubule-associated proteins play a fundamental role in the regulation of neurodevelopmental processes. Tubulin is a component of microtubules that requires posttranslational modifications for normal development and several key enzymes for proper functioning. Cytosolic carboxypeptidases are among these enzymes. The CCP6 (Cytosolic Carboxypeptidase 6) subtype enzyme, encoded by the *AGBL4* gene, is involved in the posttranslational modification of tubulin.

Our case was a 6-year-old boy with autism. Chromosome analysis was determined as normal karyotype. Then array Comparative Genomic Hybridization (aCGH) analysis was performed. We detected a deletion in chromosome band 1p33, where the *AGBL4* gene is located. This gene encodes the CCP-6 enzyme, a cytosolic carboxypeptidase.

It has been discussed the potential impact of a deletion in this gene region on enzyme function and the effects of this modification on neuronal processes and brain development.

Keywords: Autism; Microtubul; Tubulin modification; *AGBL4* gene; CCP6

Introduction

Autism is a clinically heterogeneous group of neurodevelopmental disorders commonly referred to as Autism Spectrum Disorders (ASD). Twin studies in the late 1980s suggest that autism has a genetic basis. Genetic alterations responsible for autism can be classified as cytogenetically observable chromosomal anomalies (~5%), Copy Number Variations (CNVs) (~10–20%), and single-gene disorders (~5%). Approximately 75-80 % of cases are idiopathic. At the present time, the use of array Comparative Genomic Hybridization (aCGH), also known as chromosomal microarray analysis, has increased the number of diagnosed cases. ASD are not a single clinical entity and do not follow a specific inheritance pattern. Therefore, to fully understand the causes of this spectrum of disorders, it is necessary to consolidate the evidence provided by neuroscience. The genetic changes that occur may interrupt the formation of functional neuronal networks by altering neuronal apoptosis and protein synthesis and may thereby affect synaptic activity [1,2].

ASD are characterized by neuroanatomical abnormalities. Microtubule (MT)-related gene mutations and alterations have been associated with structural brain abnormalities in ASD. MT and Microtuble-Associated Proteins (MAPs) have key roles in the regulation of neurodevelopmental processes such as neuronal polarization and migration, neuronal branching, and synaptogenesis [3].

MTs are essential components of the cytoskeleton in all eukaryotic cells and are involved in numerous processes including maintenance of cell shape, intracellular transport, cell polarity, and cell division. Although structurally similar, they may have different properties depending on the combination of α-tubulin and β-tubulin isotypes (tubulin dimerization), posttranslational modifications of tubulin, interaction with various MAPs [4].

Another of these factors is that posttranslational modifications of tubulin are evolutionarily conserved. These modifications are crucial for the biogenesis and maintenance of complex microtubule arrays like those found in spindles, cilia, neuronal processes, and platelets [5]. Known posttranslational modifications of tubulin include detyrosination and delta modification, glutamylation, glycylation, acetylation, phosphorylation, and palmitoylation [6].

Tubulin modifications are catalyzed by enzymes related to Tubulin Tyrosine Ligase (TTL),

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called Tubulin Tyrosine Ligase-Like (TTLL) enzymes [4]. Mammals have 13 TTLL enzymes. TTLL1,-4,-5,-6,-7,-9,-11 and -13 are glutamylases and TTLL-3,-8 and -10 are glycylases. TTLL2 has yet to be biochemically characterized, while TTLL12 is believed to function as a pseudo-enzyme that alters tyrosination and DNA mutation levels [5].

Glutamylation involves the addition of a variable number of glutamate residues onto glutamate residues in the C-Terminal Tails (CTTs) of both alpha and beta tubulin, and is common in neurons, centrioles, axonemes, and mitotic spindles [6]. Glutamates can be added in different ways to specific tubulin isotypes or at different sites. Some TTLL enzymes (TTLL4,5 and 7) add a single glutamate, while others (TTLL1,6,11 and 13) add glutamate side chains through standard peptide bonds to form polyglutamate side chains [7,8].

The enzymes that execute these evolutionarily conserved modifications are essential for normal development. Increased modification levels are indicative of cancers and various neurodegenerative and neurodevelopmental diseases, and these conditions have been associated with mutations in the tubulin genes that can inhibit enzyme function. When tubulin dimers are polymerized into MTs, the tubulin CTTs remain exposed on the outer surface to provide binding sites for several MAPs and molecular motors [5]. Polyglutamylation can regulate the interaction between MAPs and MTs because it increases the negative charge of MT tails. Therefore, as polyglutamylation can further increase these charges by adding glutamate side chains, it has been shown to act as a molecular potentiometer to modulate MAP binding as a function of polyglutamyl chain length. Polyglutamylation can also control the targeted binding of a particular MAP to microtubules at a given level of glutamylation, without altering the binding of other MAPs [9,10].

Tubulin glutamylation levels are determined by the balance between modification enzymes in the TTLL family and the enzymes that reverse these modifications. Deglutamylation modulation is conducted by a novel family of carboxypeptidases from the M14D subfamily of Metallocarboxypeptidases (MCPs) [5]. MCPs are zinc-dependent enzymes that cleave individual amino acids from the C-termini of peptides and proteins [11]. Carboxypeptidases hydrolyze peptide bonds to release the C-terminal amino acids of target proteins and are classified into different families based on sequence similarity, mechanism, and functions. One of the largest groups is the M14 family, which carry single catalytic zinc in the active site. This family is divided into four subfamilies: M14A, M14B, M14C, and M14D. The recently described M14D family was named Cytosolic Carboxypeptidases (CCPs) to reflect their cellular location [12]. CCPs are specifically responsible for the cleavage of polyglutamate chains [13]. CCP enzymes show functional diversity. Mammals typically produce six different CCP enzymes (CCP1-6), each with unique substrate specificities. CCP1,4 and 6 catalyze polyglutamate chain truncation and generate delta-2 tubulin, while CCP5 exclusively removes the branching-point glutamate [5,14].

In short, CCP6 is a member of the CCP family that generates Δ2-tubulin and acts as a long-chain deglutamylase during tubulin processing [15].

In this report, we examine the relationship between autism and a deletion in the *AGBL4* gene, which encodes the deglutamylating enzyme CCP6, that was detected by aCGH analysis.

Case Presentation

A 6-year-old boy with autism was referred to the genetics unit for assessment. He had no history of abnormalities in prenatal or postnatal evaluations and no parental consanguinity. There was not any individual with ASD in the family. Clinical examination was unremarkable. The consent belong to the case was obtained from family.

It was made conventional cytogenetic analysis. The result of karyotype analysis was normal (46.XY). After that CGH analysis was performed to determine possible genomic loss or gain. Genomic DNA of patient was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). Extracted DNA was performed by Agilent Oligonucleotide Microarray (8x60k microarray system), and visualization of the data was performed with Agilent Cytogenomic Edition 2.5.8.1 (GRCh37/hg19) software. CGH analysis revealed a deletion of 280 base pairs (bp) in the 1p33 band. Thus, genomic loss was determined.

This region contains the *AGBL4* (ATP/GTP-Binding Protein-Like 4) (OMIM*616476) gene, which encodes CCP6 protein (Figure 1).

Discussion

In the literature, there were studies supporting the relationship between AGBL4 gene and autism.

Pinto *et al.*, identified many new candidate genes and their loci in their CNV analysis of ASD patients. *AGBL4* is one of these candidate genes. Deletions in the *AGBL4* gene region have been documented in 4 ASD patients (3 male and 1 female). Deletions of approximately 82392 bp (49688435-49770826) were detected in 3 patients, and a deletion of approximately 85180 bp (49605647-49770826) was detected in 1 patient [16].

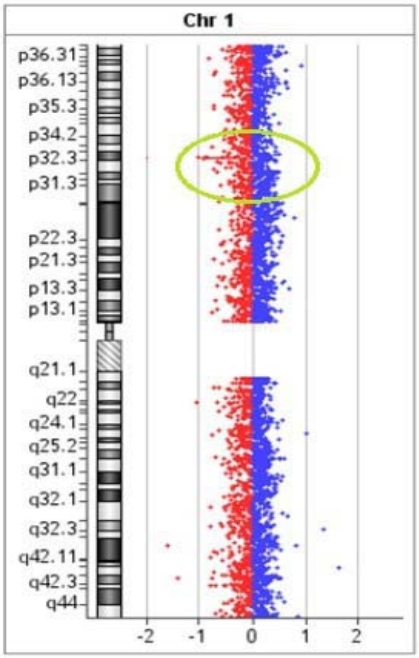

Figure 1: aCGH results of chromosome 1 in case. Arr hg [19] 1p33 (49 251 692-49 531 300) x1. It has been determined deletion of 280kb at 1p33 (green marked area).

Gombin *et al*., conducted a chromosomal microarray analysis of 63127 patients with various clinical conditions including ASD, and described the *AGBL4* gene as a disease-associated gene candidate [17]. Retinström detected a Single Nucleotide Polymorphism (SNP) at nucleotide position 48822262 in the *AGBL4* gene (rs7539694) in their genome-wide association study of ASD patients [18].

As normal brain development depends on proper MT function, defects in the MT cytoskeleton can have detrimental effects on neural proliferation, migration, and connections. Several recent studies have identified mutations in genes coding for proteins that directly modulate the structure and function of the MT cytoskeleton. The adverse effects of mutations in the AUTS2 (autism susceptibility candidate 2), ADNP (activity-dependent neuroprotective protein), JAKMIP1 (Janus kinase and MT interacting protein 1), MARK1 (MT affinity regulating kinase 1) on neuronal processes and the association of these genes with ASD has drawn attention to microtubule formation and dynamics in recent years [19].

Kimura *et al.,* isolated CCP6 as an in vivo negative regulator of tubulin polyglutamylation in *C.elegans (Caenorhabditis elegans)*. This supports the view that CCP6 may help prevent degeneration by balancing posttranslational modification [20].

Rogowski *et al.,* detected an abnormally high level of polyglutamylation in the cerebellar neurons of mice that carried a mutation in the gene encoding CCP1, another member of the CCP family, and exhibited Purkinje cell degeneration [15]. The Purkinje cell degeneration observed in these mice was eliminated by TTL1 glutamylase knockdown, indicating that neuronal death was mediated by tubulin hyperglutamylation. Other CCP members compensated for the CCP1 loss in spared tissues, while they were expressed at lower levels in severely affected neurons. These were the first observations demonstrating the molecular link between varying levels of tubulin glutamylation and neurodegeneration [7,21].

MTs have a multipurpose role in brain development. Structural and functional defects in the MT cytoskeleton negatively impact brain development. The deletion in the *AGBL4* gene region in our patient with autistic behavior caused structural and functional abnormalities in the CCP6 enzyme, which may bring about alterations in the posttranslational modification of tubulin. Disruption of glutamate homeostasis can lead to glutamate accumulation in the brain or defects in the interaction between neural MTs and MAPs. These changes may be associated with defects in neuronal development and deficits in synaptic functioning.

In conclusion, we believe that the *AGBL4* deletion detected in our patient is associated with autism. The findings in the present case show that *AGBL4* remains one of the candidate genes in autism.

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