Autism - Open Access
ISSN: 2165-7890

Szczaluba et al., Autism Open Access 2016, 6:3
DOI: 10.4172/2165-7890.1000182

Research Article | Open Access Journal

# Paternally Inherited *GABRB3* Intragenic Deletion in a Boy with Autistic Features and Angelman Syndrome Phenotype–Case Report and Literature Review

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**Received date:** 29 March 2016; **Accepted date:** 18 June 2016; **Published date:** 25 June, 2016



**Abstract**

We report on a 4 year old patient with a unique paternally inherited single-exon *GABRB3* gene deletion and clinical findings of severe speech delay, intellectual disability, autistic features, unusual behavior, tremor, and history of seizures and gait abnormalities. Similarities and significant differences with other cases involving rearrangements of 15q11-q13 are discussed. Further on, we provide literature review of the clinical picture of *GABRB3* mutations.

**Keywords:** *GABRB3* gene; Copy-number variants; Single nucleotide variants; Angelman syndrome; Intellectual disability; Autism

## Introduction

Recurrent rearrangements in particular chromosomal regions have been deemed causative for number of neurodevelopmental disorders. Microdeletions or microduplications (or so called copy number variants-CNVs) in 15q11-q13 region lead to a variety of phenotypes, including Angelman (AS) and Prader-Willi (PWS) syndromes as well as autism spectrum disorders [1]. Specifically, two subtypes of Angelman syndrome with different breakpoints result from approximately 4-5 Mb maternal allele deletions of 15q11-q13. Reciprocal microduplications lead to a clinical picture of autism, mental retardation, seizures, ataxia and behavioural problems [2,3]. Of the number of genes residing in the imprinted region, *GABRB3* encodes one of the subunits of GABA A receptor [4]. Mechanistically, the proper function and/or the number of GABA receptors may be affected by the mutations of *GABRB3* gene (Figure 1).

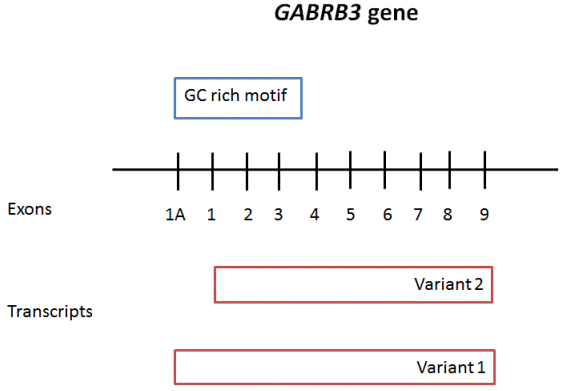


**Figure 1:** Schematic representation of *GABRB3* gene showing all the exons, transcript variants and a GC-rich motif within the gene.

Single nucleotide variants (SNVs) in *GABRB3* account for childhood absence epilepsy and, just recently, have been observed in cases of encephalopathy with intractable seizures or non-syndromic intellectual disability [5,6]. Genetic variation in *GABRB3* is also associated with Asperger syndrome and autism endophenotypes [7]. Gross deletions of the 15q11-q13 region involving *GABRB3* gene leading to neurological deficits of Angelman syndrome has been reported only once [8]. However, the presence of variants restricted to *GABRB3* gene, including intragenic deletions, has not been noted in Angelman syndrome phenotypes.

Herein, we describe a case of the paternally inherited single-exon deletion of *GABRB3* gene. We hypothesize that in our patients the deletion leads to the phenotype of intellectual disability, behavioural abnormalities, autistic features, and seizures that are consistent with Angelman syndrome phenotype. We also discuss the available literature on *GABRB3* function and the role of pathogenic variants within this gene.

## Case Report

The 4 year old proband boy was born to a non-consanguineous 30 year old mother and a 29 year old father. Family history revealed that a distant fourth degree relative of the mother was suffering from a possible autism spectrum disorder. No known exposure to teratogens was reported. The proband was born after cesarean section (cc in previous pregnancy) at 39 weeks of gestation. Apgar scores were 10 and 10 after 1 and 5 min, respectively. Birth weight was 4160 g, length 56 cm and occipito-frontal circumference (OFC) 36 cm. During the first couple of days after birth the episodes of body stiffening and clonic seizures limited to the limbs were observed. On further observation these symptoms subsided. Trans fontanel ultrasound in the neonatal period was normal. The EEG was normal as well. Tandem Mass Spectrometry (MS/MS) analysis from dried blood spot and Gas Chromatography–Mass Spectrometry (GC-MS) study in urine sample were performed and the results were normal.

The child's gross motor development during the first and second year was delayed. He could sit unsupported at 10 months and walk by the hand at 22 months. Since then, his gait has been unstable and out of balance with frequent falls leading to trauma when walking unassisted.

The proband was treated for febrile seizures by Clonazepam and Tegretol in an inpatient care at age 10 months and at age 16 months he was admitted to hospital with generalized tonic-clonic seizures. On both occasions the EEG was normal.

On examination at 4 years his weight was 19 kg, height 109 cm and OFC 52.5 cm. He had no malformations or major anomalies. No dysmorphism was observed. He could speak only single words. He was hyperactive and alert. He was rarely responsive to the parents' commands. Visuo-spatial coordination and hand skills were affected. He was not fully toilet-trained. At times, he showed aggressive and self-aggressive behavior. Tremor of upper and lower limbs was observed. The latter, together with the aggression, were attributed by the parents to the side effects of antiepileptic medication (Convulex since age 12 months and Vetira since age16 months). The parents also mentioned the boy's longstanding fascination with water and mirrors. At this time, the most recent epileptic episode he experienced was at age 2.5 years (generalized tonic-clonic seizures after a sudden fall from bed).

Magnetic resonance imaging (MRI) of the head at 2 months and again at 19 months did not show any abnormalities. The echocardiogram at 14 months was normal as well. The EEG results done at age 2 years revealed no abnormalities.

## Cytogenetic-molecular analyses

Array CGH was performed on DNA extracted from peripheral blood (Chemagic Prepito, Prepito DNA Cyto Pure Kit, Perkin Elmer) of the patient by using commercially available technology (CytoSure, ISCA 8x60K v2.0, Oxford Gene Technology, Oxfordshire, UK). In order to search for smaller copy-number variants in chromosomal regions well-known to be associated with autism, MLPA P343-C2 kit (MRC-Holland) was applied on a sample of patient's DNA. The P343-C2 probe mix contains MLPA probes for three of the chromosomal regions that are pathogenetically linked with autism: the 15q11-q13 (including UBE3A, *GABRB3* and the 15q13 micro deletion region with CHRNA7), the 16p11 micro deletion region and the SHANK3 gene at 22q13. The array study detected no abnormalities, while MLPA identified an exon 9 deletion of *GABRB3* gene. A single 148nt GABRB3_1 probe (24376510–24376570) was deleted (Figure 2). The presence of the deletion was later confirmed in DNA extracted from blood redrawn from the patient. The healthy father of the boy has been confirmed as a carrier of the identical *GABRB3* deletion.

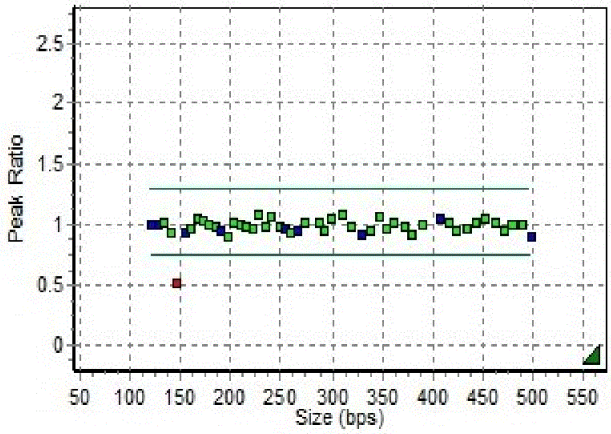


**Figure 2:** MLPA plot of the patient showing a single-exon *GABRB3* deletion.

## Discussion

Gamma-aminobutyric acid (GABA) receptors are a family of proteins involved in the GABAergic neurotransmission of the mammalian central nervous system. GABA-A receptors mediate excitatory signaling during development and play a significant role in neuronal growth and differentiation. Thus, they have been implicated in the pathogenesis of neurodevelopmental disorders, including autism spectrum disorders and epilepsy [9]. Functionally, a defect in any subunit of the GABA-A receptor, which is the principal inhibitory neurotransmitter in vertebrate brain, could account for the clinical manifestations of Angelman syndrome (AS) which include seizures, jerky arm movements, severe mental retardation and uncontrollable bouts of laughter. Neuronal *GABRB3* is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels through which inhibitory actions of GABA take place [10]. *GABRB3* occupies 15q12.1 locus, together with two other GABA receptor genes: GABRA5 and GABRG3.

Class I and II maternally inherited deletions of 15q11-q13 leading to Angelman syndrome are relatively large and involve GABA receptor genes as well as other genes like UBE3A, MAGEL2 or NDN. Typically, Angelman syndrome can be confirmed by finding the deletion of the critical region with the application of array CGH or targeted MLPA [11]. In rarer instances, single nucleotide variants in UBE3A gene must be sought. Mutations within the imprinting centre (IC), which has two critical regions, the AS-SRO (shortest region of deletion overlap) and

the PWS-SRO, are found in about 1% of patients [11]. However, by analyzing a very large series of PWS and AS patients with an imprinting defect, it has been shown that the vast majority of imprinting defects are primary epimutations that have occurred spontaneously in the absence of DNA sequence changes [12]. GABA-A receptor genes residing in the region are subject to epigenetic dysregulation in autism spectrum disorders [13]. One of them, *GABRB3* gene, lies outside of the imprinting center or an imprinted domain. Until now, single nucleotide variants or copy number variants restricted to this gene have not been confirmed in cases of Angelman syndrome or Angelman-like phenotypes.

In our patient we observed features that are typical of Angelman syndrome (AS): severe speech delay, intellectual disability, unusual behavior, autistic features, history of seizures and gait abnormalities. There were no abnormal EEG findings, dysmorphic features or behavioural findings typical of AS but these signs may not be seen in most individuals with the condition. Thus, the patient fulfills the criteria for Angelman syndrome diagnosis.

Addressing individual phenotypic features to a single gene variant may become a significant challenge. *GABRB3* variants have been reported as pathogenic in both animal models and humans.

In animal model, mice lacking only the maternal copy of *GABRB3* (m-/p+), show many of the phenotypes that are typical of Angelman syndrome and seen in Ube3a deficient mice as well. Regarding cerebellar function, adult *GABRB3* m-/p+ mice display deficits in motor learning as measured by the accelerating rotarod task [14].

In humans, one explanation for the possible role of *GABRB3* mutations in neurodevelopmental conditions is the finding of missense mutations and regulatory element single nucleotide variants in remitting childhood absence epilepsy cases. Tanaka et al. concluded that the functional abnormality resulting from these mutations causes reduced expression of *GABRB3*, and a concurrent reduction in inhibition, leading to an increase in susceptibility to absence seizures [5]. In another paper, a likely pathogenic in-frame insertion within *GABRB3* leads to the phenotype of non-syndromic intellectual disability [6]. The central role of *GABRB3* and the possible link between UBE3A and *GABRB3* genes in Angelman syndrome was first put forward by Dan and Boyd [15]. The list of currently known types of variants within *GABRB3* together with their clinical associations is presented in Table 1.

| Type of *GABRB3* variant | Phenotype | References |
|---|---|---|
| Missense mutations | Infantile spasms, Lennox-Gastaut syndrome, Autism spectrum disorder, Intellectual disability with seizures, Epileptic encephalopathy | [16-19] |
| Regulatory mutations | Autism spectrum disorder, Epilepsy | [10,17,20] |
| Gross deletions | Angelman syndrome | [8] |
| Gross insertions | Epileptic encephalopathy, Autism spectrum disorder | [21-23] |
| Small insertions | Intellectual disability with seizures | [16,17,24] |
| Splicing | Autism spectrum disorder | [16] |

**Table 1:** List of reported types of variants within *GABRB3* gene and their possible neurodevelopmental outcomes.

At this time, the inheritance of the deletion from the patient's healthy father has no explanation based on imprinting pattern seen in Angelman syndrome. However, *GABRB3* may escape paternal germ cell inactivation as it is located outside of the imprinting center. Concurrently, there can be another unknown single nucleotide variant in our patient, but not the father, that by itself or in addition to *GABRB3* gene deletion may lead to the clinical phenotype observed in the boy.

In summary, our patient is the first known case of a single-exon paternally inherited intragenic *GABRB3* deletion possibly responsible for the Angelman syndrome phenotype. Identification of the deletion in this patient may have significant effect on the therapeutic management as variety of drugs target the GABA A receptors. Of note, medium resolution 60K array was unable to pick up this small copy number variation. Only the application of the specific probes for genes associated with autism in 15q11-q13 autism critical region allowed the proper diagnosis.

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