

SHORT REPORT

Detection of copy-number variation in *AUTS2* gene by targeted exonic array CGH in patients with developmental delay and autistic spectrum disorders

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Small genomic rearrangements and copy-number variations (CNVs) involving a single gene have been associated recently with many neurocognitive phenotypes, including intellectual disability (ID), behavioral abnormalities, and autistic spectrum disorders (ASDs). Such small CNVs in the *Autism susceptibility candidate 2 (AUTS2)* gene have been shown to be associated with seizures, ID, and ASDs. We report four patients with small CNVs ranging in size between 133–319 kb that disrupt *AUTS2*. Two patients have duplications involving single exons, whereas two have deletions that removed multiple exons. All patients had developmental delay, whereas two patients had a diagnosis of ASDs. The CNVs were detected by an exon-targeted array CGH with dense oligonucleotide coverage in exons of genes known or hypothesized to be causative of multiple human phenotypes. Our report further shows that disruption of *AUTS2* results in a variety of neurobehavioral phenotypes. More importantly, it demonstrates the utility of targeted exon array as a highly sensitive clinical diagnostic tool for the detection of small genomic rearrangements in the clinically relevant regions of the human genome.

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INTRODUCTION

The availability of high-resolution genome analysis by array comparative genomic hybridization (aCGH) has led to the discovery of a wide range of benign and pathogenic copy-number variations (CNVs) in humans.^{1–4} Although some of the relatively larger CNVs have been associated with recognizable genomic disorders,^{5–12} others have been implicated in causation of clinically overlapping neuro-developmental phenotypes, including autism spectrum disorders (ASDs), developmental delay (DD), and intellectual disability (ID).^{13–19} More recently, smaller deletions or intragenic deletions disrupting a single gene have been associated with neurodevelopmental phenotypes.^{19–22} Increasing the number of array probes provides higher resolution and can hence detect such smaller CNVs. However, such an approach can be associated with increase in the costs of clinical tests and the number of clinically irrelevant CNVs detected. One way to overcome this difficulty would be with the use of a targeted exon array with a dense coverage of protein-coding genes that are known or suspected to be causative of human phenotypes.²⁰ Use of such targeted testing can detect CNVs in the genes that are clinically relevant.

In this report, as a proof of concept, we describe the detection of CNVs in exon(s) of the *Autism susceptibility candidate 2 (AUTS2, MIM 607270)* gene in patients evaluated for developmental delay. Detection of such small CNVs in specific genes can help identify the genetic causes for various human phenotypes and may also

help delineating the pivotal roles of specific protein domains in disease causation.

PATIENTS AND METHODS

The clinical information was obtained from the health-care provider by the use of a standardized checklist. The research was reviewed and approved by the Institutional Review Board of Baylor College of Medicine (BCM). DNA was extracted from the whole blood using the Puregene DNA Blood Kit (Gentra, Minneapolis, MN, USA), according to the manufacturer's instructions. We performed aCGH analysis on the clinical microarray platform routinely used in our institution. The microarrays were designed in the Medical Genetics Laboratory (MGL) at BCM. The patient samples were interrogated using a custom-designed array with ~180 000 interrogating oligonucleotides (60 mer), manufactured by Agilent Technologies, Inc. (Santa Clara, CA, USA). This array contains the 'best-performing' oligos selected from Agilent's online library (eArray; <https://earray.chem.agilent.com/earray/>) and has been further optimized using empirical data. This array is designed to provide interrogation of all known microdeletion and microduplication syndrome regions, pericentromeric and subtelomeric regions, and computationally predicted NAHR-mediated genomic instability regions flanked by low-copy repeats, as previously described.²³ In addition, ~1800 known or candidate disease genes have exonic coverage (101 644 probes in 24 319 exons; average of 4.2 probes/exon). The entire genome is covered with an average resolution of 30 kb, excluding low-copy repeats and other repetitive sequences. Further details are available at (<https://www.bcm.edu/geneticlabs/>). The procedures for DNA digestion, labeling, and hybridization, and data analysis, were performed as previously described.²⁴

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RESULTS

Among 6054 DNA samples from subjects with ID, DD, or ASDs, we identified six patients with CNVs involving the *AUTS2* gene in 7q11.22. This CNV was not identified in 6214 patient samples analyzed during the same period, where the requisition for testing did not mention ID, DD, or ASD as an indication. From six patients with CNVs in *AUTS2*, we were able to obtain detailed clinical information on four patients. The clinical phenotypes of the patients are summarized in Table 1. Patient 1 was referred for evaluation of developmental delay. The prenatal and birth history were unremarkable. The developmental milestones were significantly delayed as evidenced by walking at 21 months of age and developing first words at 4 years. Physical examination was remarkable for camptodactyly and mild scoliosis of thoracic spine. The developmental evaluation by the individualized education program from the school revealed that her performance was at the level of a 5-year-old individual at the chronological age of 10 years. Patient 2 was referred for evaluation of global developmental delay. This subject had moderate to severe delay as evidenced by crawling at 14 months, standing at 17 months, and walking at two years. Her first words were at 2 years of age and at the age of 2.5 years, her vocabulary was limited to three words. She had no craniofacial dysmorphism or abnormalities on physical examination. Neuro-imaging and a metabolic work up, including plasma amino acids, urine organic acids and acyl-carnitine profile were normal. Patients 3 and 4 are siblings, who presented with developmental delay. The sister in the sibship had radio-ulnar synostosis, microcephaly, and dysmorphic features such as triangular facies, and mid-face hypoplasia. Her ID was graded as mild to moderate. Based on qualitative impairments in social interaction and communication that included avoidance of eye contact, inconsistent responses to questions, inability to maintain conversation, repetitive language, and lack of social reciprocity, she was diagnosed with pervasive developmental disorder (PDD). The brother aged 16 years had developmental delay, generalized tonic-clonic seizures, and behavioral abnormalities. He had significant language delay, significant impairment in social interaction, lack of emotional reciprocity, and stereotypic motor movements, and was diagnosed with PDD. The seizures in this subject began at age of 6 years, and have been well controlled by treatment with carbamazepine. In all these patients, exon-targeted

aCGH identified small CNVs, ranging in size between 133–319 kb (Table 1).

The deletions in patients 1 and 2 were intragenic, involving exons 6–14 (Figure 1). Patients 3 and 4 had a CNV inherited from the mother that duplicated exon 5. The mother of patients 3 and 4 has microcephaly and mild ID. Patient 4 had an additional duplication at 15q11.2 between BP1/BP2, which was inherited from the phenotypically normal father. The CNV in patients 1 was a *de novo* rearrangement. The deletion observed in patient 2 was not present in the phenotypically normal mother and the father was unavailable for testing. The CNVs in each patient were confirmed either with FISH or a repeat analysis using the exon-targeted platform.

DISCUSSION

The *AUTS2* gene spans 1.2 Mb of genomic DNA on chromosome 7q11.22, and consists of 19 exons encoding for a protein of yet undetermined function. By use of *in situ* hybridization, immunohistochemistry, and immunoblotting, Bedogni *et al*²⁵ showed high levels of *AUTS2* mRNA and protein in the developing murine cerebral cortex and cerebellum. Early embryonic expression of *AUTS2* was predominant in the cortical plate, and postnatal expression was observed in frontal cortex, hippocampus, cerebellum, and the basal ganglia nuclei.²⁵

Haploinsufficiency of *AUTS2* (three reports of apparently balanced translocation and two reports of inversion) has been associated with ASDs, ID, seizures, and attention deficit hyperactivity disorder.^{26–31,34} Sultana *et al*³⁰ reported monozygotic twins with ID and autism with an apparently balanced t(7;20) (q11.2; p11.2) that disrupted *AUTS2*. Kalscheuer *et al*³² described translocations involving *AUTS2* in three unrelated individuals with mild to moderate ID. Huang *et al*³³ reported autism in a patient with an apparently balanced t(6;7)(q14;q11.2). An inversion disrupting both the *AUTS2* and *contactin-associated protein-like 2 (CNTNAP2)* genes was reported in a child with ASD and ID.³¹ More recently, by sequencing patients with balanced chromosomal abnormalities, Talkowski *et al*³⁴ showed the disruption of *AUTS2* in a subject with ASD, mild learning disability, poor oral motor coordination, tongue protrusion, and flexion contractures. The disruption of *AUTS2* in this patient because of an inversion was in intron 4 with the other breakpoint disrupting

Table 1 Clinical Phenotype of patients with CNVs in *AUTS2*

	Patient 1	Patient 2	Patient 3	Patient 4
Age	10	3	10	3
Gender	F	F	F	M
Height (centile)	25	17	90	NA
Weight (centile)	50	3	40	NA
Head circumference (centile)	90–97	11	<3rd	50
DD	+	+	+	+
Autistic spectrum disorders	–	–	+	+
Seizures	–	–	–	+
Brain MRI/CT	NI	NI	NA	NA
Dysmorphic features	+	–	+	+
Other features	FTT, scoliosis, atrial septal defect	None	Radioulnar synostosis	None
CNV	Deletion	Deletion	Duplication	Duplication
Inheritance	<i>De novo</i>	CNV not present in the mother	Maternal	Maternal
Size (kb)	133	319	179	179
Genomic coordinates	69 750 889–69 884 307	69 569 118–69 887 732	69 450 913–69 630 331	69 450 913–69 630 331

Abbreviations: *AUTS2*, Autism susceptibility candidate 2; CNV, copy-number variations; CT, computed tomography; DD, developmental delay; F, female; M, male; MRI, magnetic resonance imaging; NA, datum not available; NI, normal; DD, developmental delay.

+ denotes the presence of a characteristic whereas – denotes absence. The genomic coordinates are based on hg18 genome build.

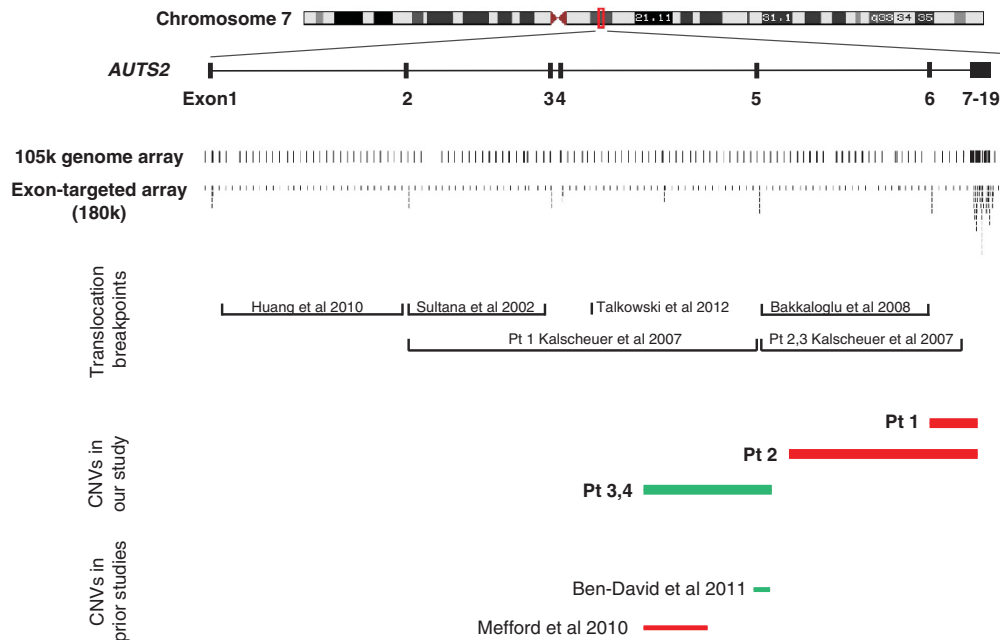


Figure 1 Results of targeted exon-specific aCGH showing CNVs in four patients with developmental delay and/or ASDs. The oligonucleotide coverage in the 180k exon-targeted array clearly shows more oligos per exon as compared to the 105k genome array. The previously published reports on either translocation breakpoints (Huang *et al*,³³ Sultana *et al*,³⁰ Kalscheuer *et al*³²) or inversion breakpoints (Talkowski *et al*,³⁴ Bakkaloglu *et al*³¹) disrupting *Autism susceptibility candidate 2* (*AUTS2*) have been depicted. Red bars depict loss and green bars depict gain of copy number. Patients 3 and 4 with ASDs, as well as three of the previously reported patients with ASDs, have alterations in the 5' region of the gene. Patients 1 and 2 with multiple-exon deletions have developmental delay but not ASDs.

PTPRN2. Whereas these reports point that haploinsufficiency in *AUTS2* may lead to neurobehavioral phenotypes, the interpretation of data in cases with translocation and inversion is complicated by the disruption of other genes.

As of yet, there have been only two reports of intragenic CNVs disrupting *AUTS2*.^{26,29} Ben-David *et al*²⁶ reported a duplication of 140 kb in size, involving exon 5 that resulted in monoallelic expression of *AUTS2* in a patient with ASDs. Mefford *et al*²⁹ used whole-genome oligonucleotide aCGH in a cohort of 517 individuals with idiopathic epilepsy and found two patients with small intragenic deletions. Recently, Girirajan *et al*³⁵ used a custom aCGH platform with high probe density for 107 genomic hotspots and found CNVs in *AUTS2* gene in two unrelated families with dyslexia and a subject with ID. Interestingly, these authors also found a statistically significant enrichment *AUTS2* CNVs in subjects with neurodevelopmental phenotypes as compared with control population. However all these studies were done on a research basis on a selected patient population. Our data constitute the first report on two intragenic deletions and two small duplications, involving *AUTS2* in a clinical setting by exon-targeted array CGH. The ID in patients 3 and 4 and their mother segregated with CNV that duplicated exon 5 of *AUTS2*. Our results support the existing literature regarding the pathogenicity of CNVs involving *AUTS2*.

CNVs involving one or more exons in a gene can affect gene expression dosage in multiple ways. Alteration in the reading frame may result in a premature stop codon followed by nonsense-mediated decay. Alternatively, an in-frame loss or gain may lead to an altered protein product that may be associated with either loss or gain of function.^{36,37} Thus, CNVs involving exons may lead to a null allele or a hypomorphic allele, leading to haploinsufficiency or alternatively, to antimorphic or neomorphic mutant alleles with toxic gain of

function. Hence CNVs in the same gene may expectedly confer various phenotypes, depending on its effects on particular protein domains and protein function. Detection of such small CNVs in the genes known or hypothesized to convey human phenotypes is best accomplished with the use of aCGH with targeted exon coverage as these typically escape detection with standard whole genomic untargeted arrays of similar probe number.²⁰ It is to be noted that very small rearrangements like those involving single exons may need confirmation by additional methods before reporting.

In the case of CNVs involving *AUTS2*, previous reports have shown that patients with ASDs have disruption of the gene proximal to exon 6.^{30,32,34,38} Our results are consistent with this observation as patients 3 and 4 with duplication of exon 5 have ASDs. Both these duplications are not expected to alter the reading frame, but can result in addition of amino-acid residues to the amino terminus of the protein. These results, although preliminary, may imply that a particular motif at the amino-terminus of *AUTS2* may be critical for the neurodevelopmental processes critical for behavior and social interaction.

In conclusion, our study adds to the existing literature supporting the role of *AUTS2* in normal neurological development and that its altered expression may result in ASDs and ID. In addition, our results highlight the utility of targeted exon aCGH in detecting small CNVs in genes associated with human phenotypes.

CONFLICT OF INTEREST

Drs Nagamani, Erez, Patel, Stankiewicz and Cheung are faculty members of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the chromosomal microarray analysis offered in the Medical Genetics Laboratory. The remaining authors declare no conflict of interest.

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