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Rare Inherited *A2BP1* Deletion In A Proband With Autism And Developmental Hemiparesis

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Introduction

Ataxin 2 binding protein 1 (*A2BP1* aka *FOX1, RBFOX1*) is an RNA binding protein found on chromosome 16p13.2 responsible for regulation of pre-mRNA splicing events in a number of critical developmental genes. It is expressed in muscle, heart and neuronal cells and is known to bind to the (U)GCAUG element found in mRNA precursors [Jin et al., 2003; Shibata et al., 2000; Underwood et al., 2005].

A2BP1 exists in many alternatively spliced isoforms that exhibit a tissue specific expression pattern [Nakahata et al., 2005]. It plays a significant role in alternative splicing of critical neuronal developmental genes and over expression has been shown to increase splicing activation of exons specific for neuronal activation [Jin et al., 2003; Nakahata and Kawamoto 2005; Underwood et al. 2005; Kuroyanagi et al., 2006; Li et al., 2007; Zhou et al., 2007]. For example, *A2BP1* is responsible for inclusion of exon five in the neuronal form of glutamate receptor NMDA receptor 1 (*GRIN1*) [Brudno et al., 2001; Minovitsky et al., 2005; Nakahata and Kawamoto 2005; Underwood et al., 2005; Zhang et al., 2008; Yeo et al., 2009].

Rare copy number abnormalities of *A2BP1* have been previously associated with cognitive impairment, attention deficit disorder and autism [Elia et al., 2010; Martin et al., 2007]. Additionally, *A2BP1* has been under investigation in autism due to its potential functional relevance as well as its candidate status in positional mapping [Barnby et al., 2005] and association studies [Martin et al., 2007]. Recently, *A2BP1* has been identified as a hub in the top expression module in the autistic brain [Voienagu et al., 2011]. Here we provide an in depth case study of a family in which a paternally transmitted *A2BP1* deletion was identified in a proband with autism and developmental left hemiparesis.

Molecular Methods

Illumina 1M-Duo DNA Analysis BeadChip

Whole blood DNA from the proband was genotyped for 1.2 million markers using the Human 1M-Duo DNA Analysis BeadChip [Illumina Inc., San Diego, CA, USA] according to the manufactures protocol. Briefly, 200ng of DNA (4uL at 50ng/uL) was amplified, biotin labeled, and hybridized to the microarray. The array was then scanned with default settings using the Illumina BeadArray Reader (BAR) at The Center for Applied Genomics genotyping core [Toronto, CA, http://www.tcag.ca/]. Genotype calls were generated using

the Illumina-provided genotype cluster definitions file (Human 1M Duo, generated using HapMap project DNA samples) with a Gencall cutoff of 0.15.

SYBR Green Quantitative Real-Time PCR

SYBR green qPCR was performed on the proband, mother and father to 1) validate the deletion detected by the microarray and 2) determine if the deletion was inherited or *de novo*. All qPCR primers were picked from hg18 build of genomic DNA sequence obtained from the UCSC Genome Browser (table 1). The primers were designed using Primer3 [Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html. Rozen and Skaletsky, 1998]. Specificity was evaluated in-silico using the UCSC PCR and BLAT tools, and determined empirically by evaluation of the melt curve data. The qPCR reactions contained 4.7μl of 2× Roche SYBR Green PCR Master Mix (Roche), 4.8μl genomic DNA (1ng/μl) and 0.25μl of each primer (10pmol/μl) in a total volume of 10μl. Realtime PCR was run using a Roche 480 Light Cycler Real-Time PCR System. Each sample was amplified in quadruplicate with primers designed to two assay control genes, *GUSB* and *HMBS* as well as the putative CNV. qPCR results were analyzed using the ΔΔCt method and the data was normalized by setting a pooled genomic DNA reference (Promega) to a fold change of 1.0.

Sequencing

An assay was developed to sequence the 2nd exon of the 4th isoform of *A2BP1*, which is located within the deletion region. Primers were selected using the hg18 assembly on the UCSC browser (table 1). The sequencing PCR was run using standard conditions at 58° in triplicate on an ABI 9700 thermocycler. The resultant product was sequenced using an ABI BigDye reaction, which was subsequently analyzed with an ABI 3730 Sequencer and visualized using the Sequencher program.

Copy Number Detection

We used CNVision, a program developed by Stephan Sanders and Christopher Mason at Yale University, to detect copy number variations in our Illumina 1M array data [http://www.softpedia.com/get/Science-CAD/CNVision.shtml]. CNVision utilizes PennCNV [Wang et al 2007], QuantiSNP [Collela et al., 2007] and Gnosis (unpublished) in a bundled program designed to identify and merge overlapping CNV calls for high confidence CNV detection.

Phenotypic Characterization

Behavioral Assessment

The family was recruited and assessed by the Autism Center of Excellence (ACE) at the University of Illinois at Chicago according to an approved IRB protocol. The proband, at the time of testing, was twelve years old and in fourth grade. He was in a general education classroom, receiving occupational, speech, and vision therapy while at school. His family indicated that he is right-hand dominant.

A full diagnostic evaluation was performed including the Autism Diagnostic Interview-Revised (ADI-R) [Le Couteur et al., 1989], completed by the proband's mother and module 3 of the Autism Diagnostic Observation Schedule (ADOS-WPS) [Lord, Rutter, et al., 1999]. Additional measures including the Repetitive Behavior Scale-Revised (RBS-R)[Lam and Aman, 2007], the Aberrant Behavior Checklist-Community Version (ABC-CV) [Aman, Singh, et al., 1985] and the Childhood Routines Inventory (CRI) [Evans, Leckman et al., 1997] were completed to gather information on repetitive behaviors. The Social Communication Questionnaire (SCQ) [Rutter, Bailey et al., 2003] and the Social Responsiveness Scale (SRS) [Constantino, Davis et al., 2003] assessed his current level and

quality of social interaction. The Clinical Evaluation of Language Fundamentals (CELF) [Semel, Wiig, et al., 2003] was administered to evaluate language ability and the Wechsler Abbreviated Scale of Intelligence (WASI) [Wechsler, 1999] was used to evaluate current levels of verbal and nonverbal abilities. Finally, the Vineland Adaptive Behavior Scales (VABS-II) [Sparrow, Cicchetti et al., 2005] were administered to assess adaptive functioning. Results from these tests are summarized and presented in table 2.

At evaluation, his language was fluent, and conversational, but could be repetitive. His use of gestures was limited and he primarily communicated verbally. Per his evaluation, the proband used overly formal words in conversation and his tone and prosody were atypical. He also showed impairments in nonverbal communication. The proband exhibited multiple sensory-seeking behaviors, and was also sensitive to certain stimuli. For example, he often touched objects to his lips, neck, and face to see how they felt. He was sensitive to loud noises, like train horns, and covered his ears in response to these sounds.

Notable behavior patterns included avoidance of eye contact, staring at hands, preference for certain foods and routines, and sensitivity to particular pieces of clothing. While he appeared to want to be around others and enjoyed physical contact, he indicated that other people irritated him. He primarily had conversations about a circumscribed range of topics, preferred solitary activities, and talked to himself loudly. He consistently engaged in reciprocal social smiling, but was described as often having a flat affect. During his evaluation, he exhibited limited insight into others' experiences and showed a narrow understanding of the nature of interpersonal relationships.

Developmental and Family History

Though the family is considered simplex, the pedigree is notable for anxiety (sister) according to maternal report (Figure 1) and social isolation (father) (Table 1). The father previously had a dermatofibrosarcoma protuberans removed.

The proband was the 7lb 15 oz product of a 38-week gestation. The mother reported six miscarriages and one ectopic pregnancy prior to conception. The parents participated in a recurrent miscarriage study between the fifth and twelfth weeks of pregnancy, which involved maternal subcutaneous injection of paternal white blood cells. No apparent complications arose as a result of participation in the study.

During infancy the proband demonstrated feeding difficulties, generalized hypotonia and weakness of the left leg and arm. At approximately 19 months his mother noticed loss of previously normal language development, including eight different words. Language loss persisted for 11 months. At the age of 22 months, the proband underwent a neurological examination, which was notable for macrocephaly. Tests included EEG, MRI of the brain, serum amino acids, and Fragile X testing, all of which were negative or normal. At the age of 43 months, the proband was evaluated and given a diagnosis of autistic disorder. Repeat EEG at 5 years showed left temporal and frontal epileptiform waves, but to date there has been no report of clinical seizure.

Neuropsychiatric Assessment

During a neurological examination, his parents reported that he had achieved gross motor milestones within normal limits, but that he dragged his left leg while learning to crawl. At present, he shows a pattern of asymmetric physical development consistent with mild left hemiparesis. His left arm is shorter (56.5 vs 60 cm) and has reduced circumference relative to his right arm. Stretch reflexes were 2+ and symmetrical throughout. Additionally there were no asymmetries evident in his legs. The left side of his face was less mobile than the

right side and his head circumference was in the macrocephalic range (56.5 cm, 98th percentile).

Neuropsychological testing was repeated after one year to ensure that results that appeared much poorer than his functional level were not confounded by inattention or fatigue (Table 3). He was right-hand dominant for the majority of tasks (16/23) but also was ambidexterous for 7 tasks [Oldfield, 1971]. The proband's performance was in the average range on tests of visuomotor planning (Trails A) [Spreen and Gaddes, 1969], cognitive flexibility (Trails B) and bilateral gross motor speed (finger tapping) [Baron, 2003]. Overall grip strength was greater with his dominant relative to his non-dominant hand (32 vs. 23 kg). During a test of fine-motor planning and speed (grooved pegboard), his performance was relatively impaired for his dominant compared to his non-dominant hand (dominant hand standard score (SS); time 1: <50; time 2: 80; non-dominant hand SS; time 1: 87; time 2; 107) [Knights and Norwood, 1980; Heaton, Grant, et al., 1986]. An isometric grip force test was administered in which the proband was instructed to sustain a steady force contraction while a line moved vertically to provide online visual feedback about his performance [Vaillancourt, Mayka, et al., 2006]. He showed a 38% increase in error variability for his dominant (root mean squared error = 10.45 (7.50)) compared to his non-dominant hand (RMSE=7.57 (5.56)) across different force levels.

Molecular Characterization of *A2BP1* Deletion

The 1.3 kb deletion was detected in the proband by all three CNV algorithms in CNVision based on a loss of signal intensity for 16 consecutive probes on the 1M array. The deletion (chr16:6356187-6369513, hg19) removes the second exon (63 bps) of the fourth (i.e., the longest) isoform of *A2BP1* along with flanking intronic sequence. The deletion was validated by real-time PCR in the proband and found to be paternally inherited (Figure 2). The exon residing within the deleted region was sequenced to rule out the possibility of a compound heterozygous mutation. The sequencing was negative for any variation within the deleted region.

Discussion

A2BP1 is one of the largest genes in the genome (1.7 Mb) and plays an important role as a master regulator of splicing activity in the brain and muscle. Interestingly, the protein serves, in a tissue specific manner, to *repress* splicing when bound to an intron located upstream of the target alternative exon and as a splicing *enhancer* when bound to a downstream element of the alternative exon [Black, 1992; Huh and Hynes, 1994; Modafferi and Black, 1997; Jin et al., 2003; Underwood et al., 2005; Zhang et al., 2008; Yeo et al., 2009]. For example, muscle specific isoforms of *A2BP1* have been shown to repress inclusion of muscle specific exons in nonmuscle tissues in mice while neuronal isoforms of A2BP1 are responsible for inclusion of neuron-specific exons in brain [Nakahata and Kawamoto, 2005; Underwood et al., 2005]. While the mRNA recognition element for A2BP1 has been known for some time, the target genes are less well characterized. Zhang et al. [2008] attempted to identify and characterize all targets of A2BP1. Eleven thousand and three target transcripts were predicted and significant enrichment of neuromuscular GO terms and disease annotations were found in this target set. Notable within the list of targets were *NLGN3* and *NLGN4X*, which have previously been implicated in autism [Jamain et al., 2003].

There is a growing body of evidence to suggest that *A2BP1* may play a role in the etiology of developmental delay and autism. Previous studies of autism, ADHD, intellectual disability and epilepsy have identified rare copy number variants in *A2BP1* [Elia et al.,

2010; Martin et al, 2007; Bhalla et al., 2004]. One such study found translocations in two unrelated patients with cognitive impairment and epilepsy, both of which disrupted the *A2BP1* gene [Bhalla et al., 2004]. Martin et al. [2007] identified a *de novo* translocation with a subsequent loss of *A2BP1* at one of the breakpoints. Additionally Elia et al., [2010] reported three copy number variants in their sample of children with ADHD including one duplication and two deletions, all of which were inherited and two of which were inherited from affected parents. The deletion presented in this paper along with previously reported translocations and CNVs associated with psychiatric phenotypes are presented in Figure 3. More recently, Voingeau et al. [2011] also identified differential alternative splicing events in the brain tissue of donors with autism and reduced expression of *A2BP1*, when compared to controls. *GRIN1*, and *MEF2C* are notable genes in this list of *A2BP1* mediated splicing events unique to the autism transcriptome.

The first three exons of *A2BP1* (isoform 4) are not translated and contain extensive regulatory elements. The deletion found in the proband and his father encompasses the second exon and flanking intronic regions of the fourth isoform (chr16:6296188-6309514). The case presented here is remarkable not only for his autism but also for his global hypotonia, his left side developmental hemiparesis and an additional lateralized deficit in motor planning and coordination affecting his dominant hand. There was no central nervous system abnormality found on MRI during a prior evaluation, and no complications were experienced during the pregnancy or during delivery. During early development, the hemiparesis did not interfere with daily functions using his non-dominant (left) hand or arm. He is right hand dominant and he and his parents had not previously noted the physical asymmetry in the size and circumference of his arms but were aware of early developmental left side weakness. At the time of his clinical evaluation, the heimparesis was largely resolved with no difference between lower extremities and only size asymmetry remaining apparent in upper extremities as well as reduced mobility on the left side of the face. Interestingly, the patient described in Martin et al., [2007] suffered from a similar phenotype of hypotonia in the lower extremities. The left-sided developmental hemiparesis and contrasting right-sided dyspraxia may reflect an alteration in the maturation of hemispheric specialization or independent pathologies that conferred distinct abnormalities on his physical and motor development. In order to confirm our original neuropsychological findings of dyspraxia affecting the hand (right) opposite his hemiparetic limb (left), the proband returned for a second testing session. His performance improved during the second testing session, judged clinically to most likely result from increased attentiveness. The relative deficit was still present in his right hand. Similarly, the proband demonstrated decreased force stability with his right compared to his left hand during a test mediated by cortico-cerebellar circuitry [Coombes et al., 2011]. These findings are consistent with previously documented disruptions of motor lateralization in autism suggesting an alteration in the maturation of hemispheric specialization or an abnormality that differentially affects left motor cortices and/or right cerebellum [Escalante-Mead et al., 2003; Mosconi et al., 2010]. It is not clear whether this distinct neurological phenotype is unique to the *A2BP1* deletion or more generally associated with autism. We were not able to collect neuropsychological and sensorimotor data on the father, so it remains unclear whether the motor atypicalities observed in the proband are also present in his father. Heterogeneity among individuals carrying the same risk associated structural variant has been well documented in autism [Sanders et al., 2011].

Copy number variants in *A2BP1* have been noted in the Database of Genomic Variants (DGV). Intronic losses and gains are not uncommon among unscreened control populations, such as those residing in the DGV. This phenomenon is not surprising considering that *A2BP1* is one of the largest genes in the genome (1.7 Mb). Exonic CNVs, while also present, are much less common. One CNV in the DGV overlaps the CNV region presented

in this paper. We present in this study an in depth phenotypic analysis of a confirmed deletion in the *A2BP1* gene. As with all rare findings, we cannot conclusively state that the *A2BP1* deletion in this proband results directly in his autism. However, taken together with previously published studies, our data provides further evidence for a role of *A2BP1* in the development of autism and associated motor asymmetries.

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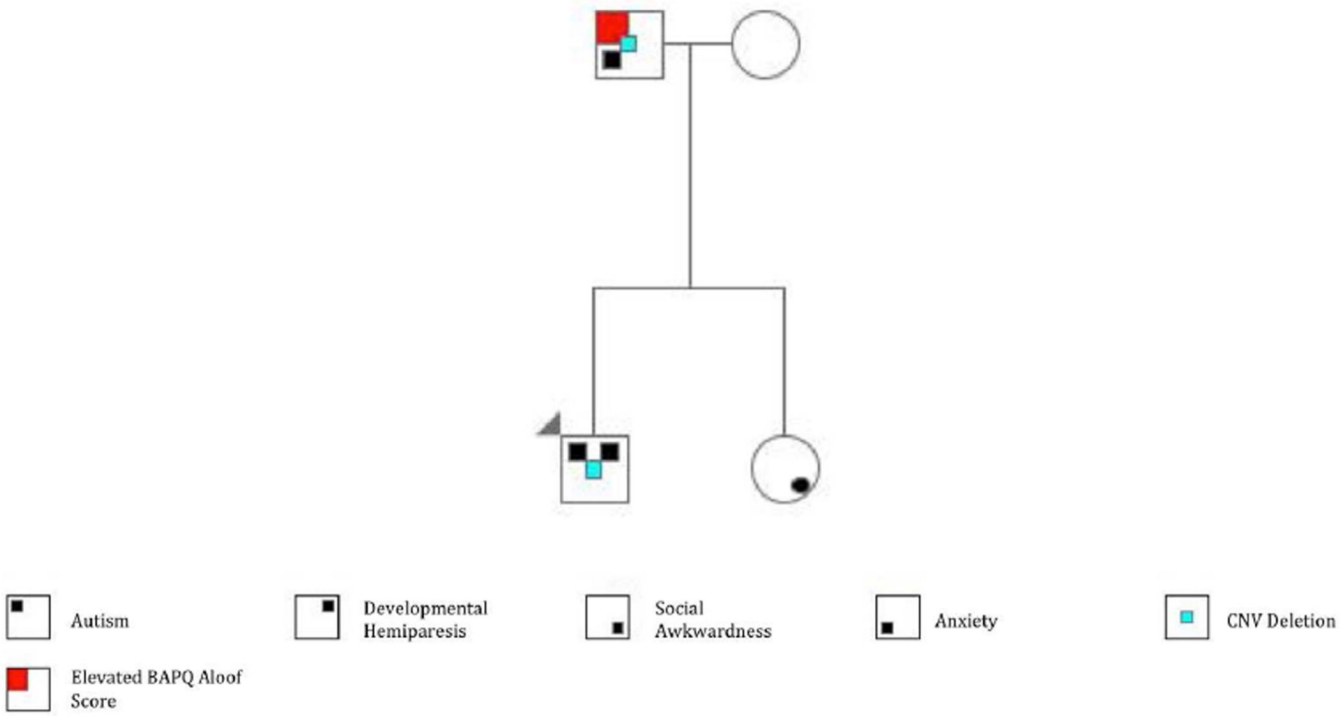

Figure 1. Pedigree

The proband presented with autism and mild paresis on his left side. The father was diagnosed with anxiety and had an elevated BAPQ aloof score. Both the proband and the father carried a deletion in the A2BP1 gene (chr16:6356187-6369513). The proband's sibling was unaffected, but was described as socially awkward. The arrowhead denotes the proband.

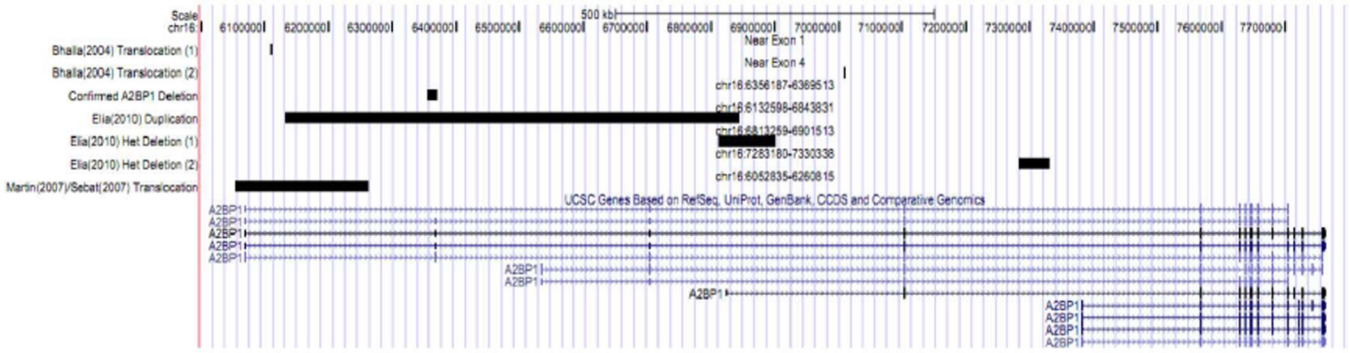

Figure 2. Validated Deletion of A2BP1

A)The circled block highlights the confirmed deletion found in the A2BP1 gene of the proband and father. This deletion consist of a loss of signal across 16 consecutive probes covering chr16:6296188-6309514. B) Quantitative PCR performed to confirm the deletion and establish inheritance in the proband utilizing HMBS as the reference gene. C) Quantitative PCR performed to confirm the deletion and establish inheritance in the proband utilizing GUSB as the reference gene (SD shown).

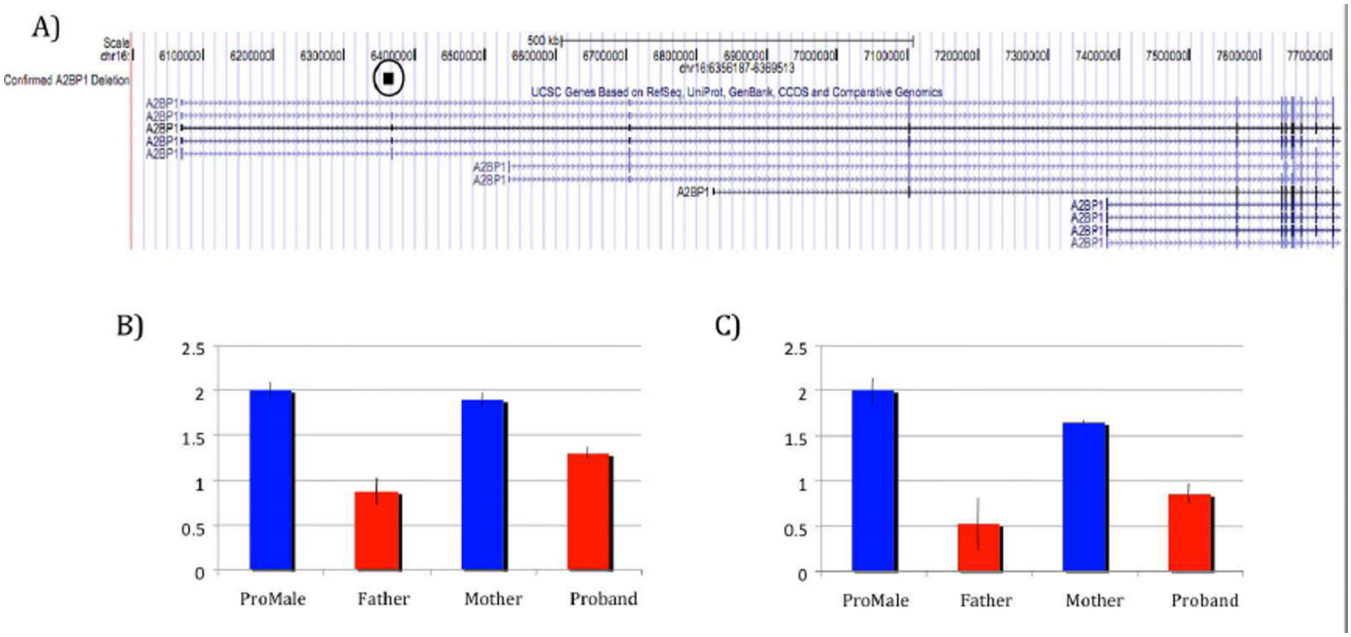

Figure 3. Confirmed A2BP1 Deletion and Previously Confirmed Deletions in A2BP1

A) Displays the validated CNV found in both the proband and father covering chr16:6296188-6309514, A2BP1 duplication and deletions detected via beadchip and found to be associated with ADHD and autism [Elia et al., 2010], and deletion detected through FISH from Martin et al. [2007] found in an autistic female with severe developmental disability, epilepsy, delayed walking with mild residual ataxia, behavioral regression, fluctuating liver function tests and mild cerebellar atrophy. B) Graphs display two deletion endpoints from Bhalla et al. [2004] determined by FISH. The deletions were detected in two patients, one with epilepsy, the second with intellectual impairment. The exact length of the deletions could not be determined. (Het, heterozygous deletion)

Table 1

Primers used in SYBR green assays and exon sequencing assay. All SYBR green assays were run in quadruplicate at an annealing temperature of 55 degrees on the Roche 480 Light Cycler. The exon sequencing reaction was run using standard conditions at an annealing temperature of 58 degrees.

Primer ID	Application	Gene Name	Amplicon Size	Primer Sequence
A2BP1_1F	SYBR target gene	A2BP1	115	TGTTTCTTACACAGCACAGG
A2BP1_1R	SYBR target gene	A2BP1	115	CTTGTTTCCTGATTACACAGC
A2BP1_2F	SYBR target gene	A2BP1	125	AATACATCCATGTCTTTGATCC
A2BP1_2R	SYBR target gene	A2BP1	125	TTCTCATCTCTGATACAATAGGG
GUSB_1F	SYBR reference gene	GUSB	90	CTGAATGCAGCCTTGACCTA
GUSB_1R	SYBR reference gene	GUSB	90	GGGCATGGTTATGAGTGCTT
HMBS_1F	SYBR reference gene	HMBS	90	GCAGCTCATAGGTGGGTTTT
HMBS_1R	SYBR reference gene	HMBS	90	CCCAGCCATTCTTGCAGT
A2BP1_set1f	Exon Sequencing	A2BP1	334	CTTTGCCAGGATCTGAGAGG
A2BP1_set1r	Exon Sequencing	A2BP1	334	GAGAACAGGATCAAAGACATGG

Table 2

Scores on clinical measures administered as a part of the UIC ACE diagnostic and clinical battery of tests

	Proband	Father	Mother
Autism Diagnostic Interview - Revised			
Impairments in Reciprocal Social Interaction	23	--	--
Qualitative Abnormalities in Communication	20	--	--
Restricted And Repetitive Patterns of Behavior	6	--	--
Abnormality Of Development Evident At Or Before 36 Months	3	--	--
Autism Diagnostic Observation Schedule			
Communication	4	--	--
Social	7	--	--
Social Affect	7	--	--
Restricted Repetitive Behaviors	3	--	--
Calibrated Severity Score	6	--	--
Additional Clinical Measures			
Vineland Adaptive Behavior Scale	98 (45)	--	--
Clinical Evaluation of Language Fundamentals (CELF-4)	109 (73)	--	--
Broader Autism Phenotype Questionnaire			
Rigid	--	2.92	3.08
Aloof	--	3.75	1.58
Pragmatic Language	--	2.67	2.00
Additional Research Measures			
Social Communication Questionnaire	26	--	--
Repetitive Behavior Scale - Revised	6	--	--
Aberrant Behavior Checklist-Community	27	--	--
Childhood Routines Inventory, version 1.2	44	--	--

Table 3

Standard scores and percentile ranks (in parentheses) for the proband, father and mother on a battery of neuropsychological tests (mean=100, sd=15). The trails tasks, the grooved pegboard task and the finger tapping task were administered to the proband at two different time points, one year apart in order to confirm a persistent asymmetric pattern of motor performance. The Edinburgh Handedness (Oldfield, 1971) was administered to quantitatively assess the proband's lateralization of functions of his hands, feet, and eyes. Scores reflect number of behaviors for which the patient preferred to use his right or left hand, or had no lateral preference.

	Proband		Father	Mother
	Time 1	Time 2		
Trails A	98 (45)	106 (66)	105 (63)	101 (53)
Trails B	98 (45)	109 (73)	115 (84)	119 (90)
Grooved Pegboard				
Left Hand	87 (19)	107 (68)	113 (81)	93 (32)
Right Hand	<50 (<.1)	80 (9)	101 (53)	77 (6)
Finger Tapping				
Left Hand	115 (84)	102 (55)	100 (50)	87 (19)
Right Hand	112 (79)	97 (27)	85 (16)	74 (4)
Wechsler Abbreviated Scale of Intelligence				
Full Scale IQ	126 (96)		120 (91)	111 (77)
Verbal IQ	129 (97)		122 (93)	119 (90)
Performance IQ	117 (87)		112 (73)	101 (53)
Edinburgh Handedness				
Left Hand	0			
Right Hand	16			
Ambidexterity	7			