



## Short Communication

# A 1.5 Mb terminal deletion of 12p associated with autism spectrum disorder



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## ABSTRACT

We report a patient with a terminal 12p deletion associated with autism spectrum disorder (ASD). This 12p13.33 deletion is 1.5 Mb in size and encompasses 13 genes (*B4GALNT3*, *CCDC77*, *ERC1*, *FBXL14*, *IQSEC3*, *KDM5A*, *LINC00942*, *LOC574538*, *NINJ2*, *RAD52*, *SLC6A12*, *SLC6A13* and *WNK1*). All previous cases reported with partial monosomy of 12p13.33 are associated with neurodevelopmental delay, and we suggest that *ERC1*, which encodes a regulator of neurotransmitter release, is the best gene candidate contributing to this phenotype as well as to the ASD of our patient.

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## 1. Introduction

Autism spectrum disorders (ASDs) describe a range of complex neurodevelopmental disorders, characterized by delayed and/or unusual language, problems with social interactions, repetitive and stereotyped patterns of behavior and restricted interests and activities (Anon, 2000). Specific diagnoses that are types of ASDs include Asperger syndrome, autism and pervasive developmental disorder not otherwise

specified (Veenstra-VanderWeele & Cook, 2004). ASD is one of the most common neurodevelopmental disabilities, with an average estimated global prevalence of 62 cases per 10,000 children (Elsabbagh et al., 2012) and an approximate 4:1 male to female ratio. The first signs of ASD usually appear by the age of 1–2 years, and it can be clearly detected by the age of 2–4 years (Courchesne et al., 2007).

The causes of ASD have not all been clearly defined. However, at least in some cases, there is a genetic basis, demonstrated by the high concordance between monozygotic twins, which can be as high as 90% (Rosenberg et al., 2009). Recently, advances in genomic analysis technologies have found that chromosomal copy number variations (CNVs) significantly contribute to the development of ASD (Shen et al., 2010). Thus, further studies of CNVs in patients with autism can contribute to the identification of new candidate genes and increase the understanding of ASD etiology.

We report an 8-year-old boy with a 1.5-Mb terminal deletion within 12p13.33 associated with ASD. This deletion, detected by microarray-based comparative genomic hybridization, encompasses at least 13 genes, including *ERC1*, which is deleted in all previous reports of partial monosomy 12p13.33.

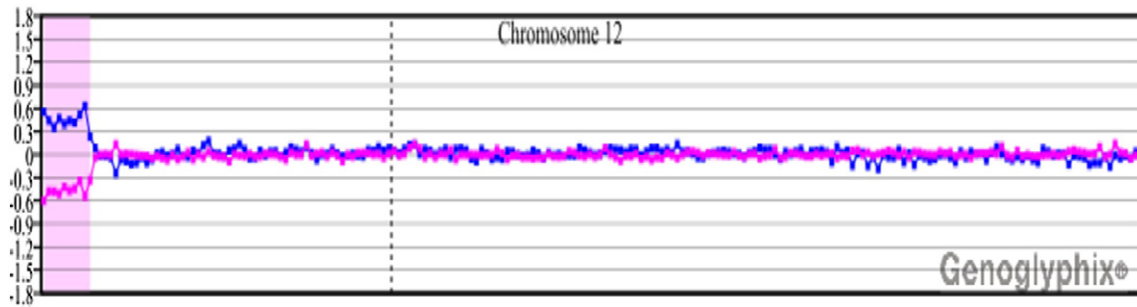
## 2. Clinical report

The proband, an 8-year-old boy of European origin, presented for evaluation of neurodevelopmental delay. He is the first of three children of non-consanguineous healthy parents who, at the time of birth, were

**Abbreviations:** aCGH, array CGH; ADD, Attention Deficit Disorder; ADHD, Attention Deficit Hyperactivity Disorder; ASD, Autism Spectrum Disorder; *B4GALNT3*, beta-1,4-N-acetyl-galactosaminyl transferase 3; BAC, bacterial artificial chromosome; CARS, Childhood Autism Rating Scale; *CCDC77*, coiled-coil domain containing 77; CGH, comparative genomic hybridization; CNV, chromosomal copy number variation; DGV, Database of Genomic Variants; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; *ERC1*, ELKS/RAB6-interacting/CAST family member 1; *FBXL14*, F-box and leucine-rich repeat protein 14; FISH, fluorescence in situ hybridization; hg19, Genome Reference Consortium Human Build 37; *IQSEC3*, IQ motif and Sec7 domain 3; *KDM5A*, lysine (K)-specific demethylase 5A; *LINC00942*, long intergenic non-protein coding RNA 942; *LOC574538*, uncharacterized LOC574538; NIMA, Group for Advanced Molecular Investigation; *NINJ2*, ninjurin 2; PUCPR, Pontifícia Universidade Católica do Paraná; *RAD52*, *RAD52* homolog (*S. cerevisiae*); *SLC6A1*, solute carrier family 6 (neurotransmitter transporter, GABA), member 1; *SLC6A12*, solute carrier family 6 (neurotransmitter transporter, GABA), member 12; *SLC6A13*, solute carrier family 6 (neurotransmitter transporter, GABA), member 13; *WNK1*, WNK lysine deficient protein kinase 1.

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**Fig. 1.** Characterization of a 12p13.33 deletion by microarray analysis. Microarray plot showing a single-copy loss of 10 BAC clones from the terminal short arm of chromosome 12 at 12p13.33 (chr12:204 084-1 685 631, hg19 assembly), approximately 1.5 Mb in size. Probes are ordered on the x axis according to physical mapping positions, with the most distal p-arm probes to the left and the most distal q-arm probes to the right. The blue line is a plot of the aCGH data from the first microarray slide (reference Cy5/patient Cy3). The pink line is a plot of the aCGH data from the second microarray slide in which the dyes have been reversed (patient Cy5/reference Cy3).

30 years old. The 39-week pregnancy was uneventful, without any exposures to known teratogens, and he was born by normal spontaneous delivery. The patient's birth weight was 3.156 kg (10th–25th percentiles), length 51 cm (50th–75th percentiles), head circumference 34 cm (10th–25th percentiles), and Apgar scores 9 and 10 at 1 and 5 min, respectively. He was born with spina bifida occulta. Early developmental concerns were raised due to lack of eye contact until 1 year, and language development was delayed. He was able to sit up around 5 months, to crawl around 11 months, to walk by the age of 1 year and 3 months, and his first words were at 3 years. Neurological evaluation at 2.5 years showed that he met the DSM-IV criteria for the diagnosis of ASD, and his symptoms were considered mild-moderate according to the Childhood Autism Rating Scale (CARS) (Schopler et al., 1988). He is currently attending a regular school and, despite good academic performance and good humor, has a tendency for isolation, few friends,

stereotypies, anxiety, hyperactivity, moderate difficulty in changing routines and excessive focus on specific objects and games. He presents an especially good memory, flair for music and high sensitivity to noises. His two younger brothers are developing normally.

On physical examination at 8 years of age, weight was 21 kg (3rd–10th percentiles) height was 1.30 m (50th–75th percentiles) and head circumference was 53 cm (50th–90th percentiles). Electroencephalogram, magnetic resonance imaging of his brain, Fragile X DNA testing, karyotype and plasma organic acids showed normal results.

### 3. Materials and methods

G-banded chromosome analysis was performed on peripheral blood lymphocytes according to standard techniques. Array CGH was performed on DNA extracted from the peripheral blood of the proband

**Table 1**  
Summary of the clinical features and deletion size of all patients with 12p13.33 microdeletion.

	Age	Sex	Deletion size	Abnormal features	Behaviors
Baker et al. (2002) – Child	15 years	Male	1.65-Mb	Deep-set eyes; prominent ears; short neck; mild kyphoscoliosis; some primary dentition; heart murmur; a small ventricular septal defect; a squint; asthma	ADD; violent episodes
Baker et al. (2002) – Mother	Not available	Female	1.65-Mb	None	None
Rooryck et al. (2009)	3 years and 8 months	Female	2.3-Mb	Mild hypertelorism; preauricular tag and pit; wide left corner of the mouth; left microtia; and left hemifacial microsomia; axial hypotonia and peripheral hypertonia; lumbar kyphosis; Achilles tendon retraction; patent foramen ovale and moderately shortened QT interval	Not available
Abdelmoity et al. (2011) – Proband	8 years	Female	1.39-Mb	Slight hypertelorism; bulbous nose; mild kinetic tremors and staring episodes	ADD
Abdelmoity et al. (2011) – Brother	13 years	Male	1.39-Mb	Staring episodes	ADD
Abdelmoity et al. (2011) – Father	47 years	Male	1.39-Mb	Staring episodes	Difficulty holding a job
MacDonald et al. (2010)	6 years	Male	2.95-Mb	Microcephaly; short nose; long face and prominent ears	Difficulties interacting with other children
Thevenon et al. (2012) – Patient 1	3 years	Male	3.2-Mb	Square coarse face; mild frontal bossing; enophthalmia; low-set ears; with anteverted and thick ear lobes; a marked philtrum; large nares; thin upper lip and narrowly spaced teeth	Solitariness; low interactions; and communication mostly by shouting
Thevenon et al. (2012) – Patient 2	35 years	Female	3.2-Mb	None	None
Thevenon et al. (2012) – Patient 3	5 years	Male	1.3-Mb	None	ASD; ADHD; solitariness; low interactions and stereotypies
Thevenon et al. (2012) – Patient 4	37 years	Male	1.3-Mb	None	ADHD
Thevenon et al. (2012) – Patient 5	67 years	Male	1.3-Mb	None	ADHD
Thevenon et al. (2012) – Patient 6	3 years	Male	3.1-Mb	Myopathic facies; tented upper lip; highly arched palate; hypotonia and prominent ear lobes	ASD; ADHD; poor communication skills and low interaction
Thevenon et al. (2012) – Patient 7	5 years	Male	2.76-Mb	Long face; large ears; prominent lobes, epicanthus and large incisors with dental malocclusion	Anxiety and ADHD
Thevenon et al. (2012) – Patient 8	10 years	Male	2.5-Mb	Micrognathia, prominent ears and hypothyroidism	Anxiety and ADHD
Thevenon et al. (2012) – Patient 9	16 years	Male	4.79-Mb	Hypotelorism; microcephaly with a prominent metopic suture; moderate joint laxity and brittle first toenails	Abnormal

Abbreviations: ADHD – Attention Deficit Hyperactivity Disorder; ASD – Autism Spectrum Disorder; ADD – Attention Deficit Disorder.

using a whole-genome, bacterial artificial chromosome-based microarray (SignatureChip Whole Genome; Signature Genomic Laboratories, Spokane, WA, USA) (Ballif et al., 2008). The mother was tested using an oligonucleotide-based, 135 K-feature microarray (SignatureChipOligo Solution; custom-designed by Signature Genomics, manufactured by Roche NimbleGen, Madison, WI, USA) (Duker et al., 2010). To visualize the abnormalities identified by aCGH, fluorescence in situ hybridization (FISH) was performed on the patient's metaphase and interphase cells using BAC clone RP11-350L7 from 12p13.33 and RP11-597C23 from Xp22.31 (Traylor et al., 2009).

**4. Results**

aCGH identified a 1.5 Mb terminal deletion at 12p13.33, which encompasses 13 genes (*B4GALNT3*, *CCDC77*, *ERC1*, *FBXL14*, *IQSEC3*, *KDM5A*, *LINC00942*, *LOC574538*, *NINJ2*, *RAD52*, *SLC6A12*, *SLC6A13* and *WNK1*; Fig. 1). The centromeric breakpoint is estimated to be between RP11-73H11 (deleted; chr12:1 542 983-1 685 631, hg19 coordinates) and RP11-636B1 (not deleted; chr12: 1 710 249-1 868 642). Furthermore, a likely tandem duplication at Xp22.31, below the resolution of FISH, was also present. Microarray analysis of the mother showed that she carried the ~210 kb Xp22.31 duplication, which contained no known genes. She did not carry the 12p13.33 deletion, though it is unknown if she carries a balanced chromosomal rearrangement involving the region. The patient's father was unavailable for testing.

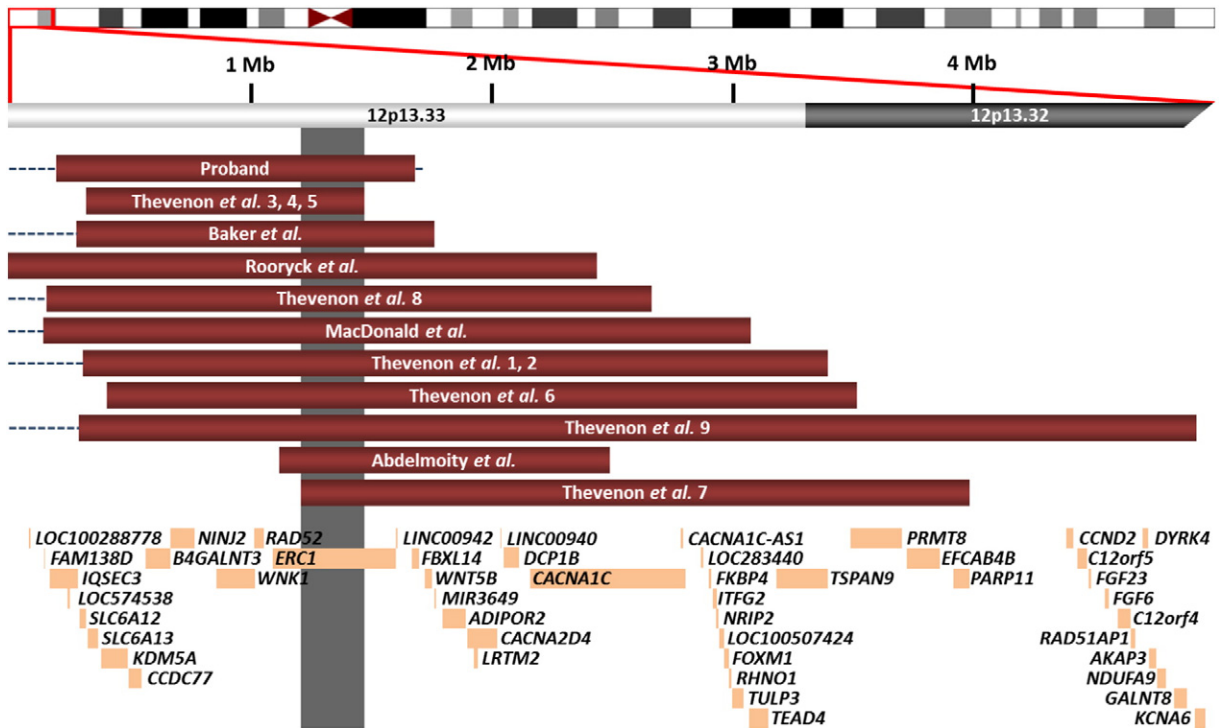
**5. Discussion**

Here we describe a patient who carries a 1.5-Mb terminal deletion at 12p13.33 associated with ASD, a more severe version of the abnormal behaviors previously associated with 12pter deletions. There have been five previous reports about 12p13.33 microdeletions (<5-Mb) (Abdelmoity et al., 2011; Baker et al., 2002; Macdonald et al., 2010; Rooryck et al., 2009; Thevenon et al., 2012), with all cases showing

variable phenotypes possibly due to the different sizes and gene content of the deleted segments. However, there seems to be no relation between the size of the deleted segments and the severity of the reported phenotypes.

Thevenon et al. (2012) recently reported nine patients with different sizes of 12p13.33 subtelomeric interstitial and terminal deletions, the majority of them *de novo*. Neurodevelopmental delay was observed in all, intellectual disability in most and autistic features in patients 1, 3 and 6. The first (patient 1), a 3-year-old boy, had neurodevelopmental delay and minimal dysmorphic features (square coarse face, mild frontal bossing, enophthalmia, low-set ears, thin upper lip and irregular and narrowly spaced teeth) and a 3.2-Mb terminal deletion inherited from his mother (patient 2), who had severe speech and learning difficulties in early childhood. The second (patient 3), a 5-year-old boy, shares with his father (patient 4) and paternal grandfather (patient 5) a 1.3-Mb terminal deletion. He displayed neurodevelopmental delay, behavioral abnormalities including anxiety, solitariness, limited social interaction and stereotypies, and the father and grandfather had a similar past history of speech delay and learning difficulties. The third (patient 6), also a 5-year-old boy, carrying a 3.1-Mb deletion, had developmental delay, intellectual disability, mild hypotonia, tented upper lip, myopathic facies, prominent ear lobes and behavioral problems (limited social interaction).

In the four other reports in the literature, the largest deletion (2.95-Mb) was described by Macdonald et al. (2010) in a six-year-old boy with developmental delay, microcephaly, mild dysmorphism (short nose, long face and prominent ears) and problems with social interaction. Abdelmoity et al. (2011) identified the smallest deletion (1.39 Mb) in an eight-year-old girl, her father and brother, who all showed developmental delay and staring episodes. There were no dysmorphic features except for hypertelorism and a bulbous nose in the girl. A summary of the clinical features and deletion size of all patients with 12p13.33 microdeletion are listed in Table 1. Neurodevelopmental delay is the only feature found in all reported individuals.



**Fig. 2.** Schematic showing deletions involving 12p13.33. Full ideogram of chromosome 12 is across the top, with a partial ideogram of chromosome band 12p13.33p13.32 below (hg19). Red bars represent the minimum deletion sizes of the patients in this report and in the literature. When available, horizontal dashed lines extend through gaps in coverage to show maximum deletion sizes. Tan bars represent the genes in the region. The smallest region of overlap of all reported cases is represented by the vertical gray bar.

The deleted region of our patient encompasses 13 genes and is approximately 1.5 Mb in size. Similar deletions have not been reported in healthy controls, either in the Database of Genomic Variants (DGV — <http://dgv.tcag.ca/dgv/app/home>) or a control group of 2026 healthy children (Shaikh et al., 2009). The smallest region of overlap among our patient and the previously reported individuals is within the *ELKS/RAB6-interacting/CAST* family member 1 gene (*ERC1*; Fig. 2).

*ERC1* is more than 500 kb in size (Nakata et al., 2002) and encodes 24 different transcripts, generated through alternative splicing. Many isoforms show tissue-specific expression, including one brain-specific isoform (*ERC1b*) present in the active zone, a presynaptic region where synaptic vesicles dock and neurotransmitter release is regulated (Takao-Rikitsu et al., 2004). *ERC1b* interacts with other active zone-specific proteins to form a large protein complex implicated in the molecular organization of this zone (Nomura et al., 2009).

Based mainly on those reported cases with some autistic features (patients 1, 3 and 6 (Thevenon et al., 2012)) and in the fact that alterations in genes affecting synaptic processes are enriched in ASD (Swanwick et al., 2011), we suggest that *ERC1* could be considered as a new candidate gene contributing to the autism phenotype as well as to the neurodevelopmental delay present in all patients. The wide range of phenotypic severity, from learning difficulties and speech delay in early childhood to autism, could be better explained by variable expression. Other recurrent clinical findings such as low-set ears, prominent nose, dental and digit abnormalities, hypotonia, microcephaly and growth retardation, may be caused by the deletion of surrounding genes.

To our knowledge this is the first time *ERC1* has been associated with autism, and we believe that these data can contribute to the understanding of how alterations in different genes within the same or related pathways can cause ASD. A deeper molecular analysis of the *ERC1* transcripts is required to fully understand its functional role in the neurotransmission process and its etiological association with ASD.

## 6. Conclusions

In conclusion we describe a patient with ASD and a 12p13.33 deletion. While there are no other new reports of partial monosomy of distal 12p13.33 nor additional information about the genes within this region, we suggest that *ERC1* is the best candidate for the neurodevelopmental delay and ASD.

## Conflict of interest

Jill Rosenfeld is an employee of Signature Genomic Laboratories, a subsidiary of PerkinElmer, Inc.

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