

SHORT REPORT

Haploinsufficiency of *CELF4* at 18q12.2 is associated with developmental and behavioral disorders, seizures, eye manifestations, and obesity

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Only 20 patients with deletions of 18q12.2 have been reported in the literature and the associated phenotype includes borderline intellectual disability, behavioral problems, seizures, obesity, and eye manifestations. Here, we report a male patient with a *de novo* translocation involving chromosomes 12 and 18, with borderline IQ, developmental and behavioral disorders, myopia, obesity, and febrile seizures in childhood. We characterized the rearrangement with Affymetrix SNP 6.0 Array analysis and next-generation mate pair sequencing and found truncation of *CELF4* at 18q12.2. This second report of a patient with a neurodevelopmental phenotype and a translocation involving *CELF4* supports that *CELF4* is responsible for the phenotype associated with deletion of 18q12.2. Our study illustrates the utility of high-resolution genome-wide techniques in identifying neurodevelopmental and neurobehavioral genes, and it adds to the growing evidence, including a transgenic mouse model, that *CELF4* is important for human brain development.

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INTRODUCTION

Only 20 patients with deletions of 18q12.2 have been reported in the literature.^{1–13} In 15 of these patients, the deletions were examined with chromosome analysis, and in the remaining 5 patients molecular investigations identified deletions ranging from 3.2 to 24 Mb in size.^{9,10,12,13} Common clinical findings included intellectual disability, behavioral disorders, seizures, eye manifestations, obesity, and mild or absent dysmorphic facial features.

In recent years, the use of high-resolution cytogenetic techniques has led to the identification of numerous microdeletion syndromes, where patients with overlapping deletions share clinical features. However, the shared deletions often exceed hundreds of kb or even several Mb, encompassing many genes that hamper the identification of the exact gene(s) underlying the associated phenotype.¹⁴ In contrast, truncation of single genes – for example, by small intragenic deletions or by chromosomal translocation or inversion breakpoints – can directly identify a disease-causing gene.¹⁵

Here we describe a patient with a prenatally detected *de novo* translocation involving chromosomes 12 and 18, with febrile seizures in childhood, borderline intelligence, developmental and behavioral disorders, myopia, and obesity. We characterized the rearrangement with SNP array and next-generation sequencing (NGS) and found truncation of *CELF4*, which has previously been suggested as a neurodevelopmental candidate gene.¹³ This report illustrates the utility of high-resolution genome-wide techniques in identifying neurodevelopmental genes.

PATIENTS AND METHODS

The patient was identified through a nation-wide study of prenatally detected *de novo* balanced structural rearrangements. The study was approved by the Danish Scientific Ethics Committee and written informed consent was obtained.

Clinical report

The patient is a 27-year-old male. He is the second child to healthy unrelated parents. Amniocentesis was performed due to advanced maternal age, and a *de novo* reciprocal translocation involving chromosomes 12 and 18 was diagnosed. The child was born by uncomplicated vaginal delivery at 40 weeks of gestation with a birth weight of 4000 g and birth length 55 cm. The neonatal period was unremarkable. According to the mother, he walked independently at 12 months and there was no delay in language development. At 23 months of age, he was hospitalized due to a series of three febrile seizures. He did not experience recurrent seizures and an electroencephalography was not performed. Owing to minor neurological deficits and difficulties with complex motor tasks, he was diagnosed with minimal brain dysfunction at age 4. At the age of 6, he was diagnosed with hyperkinetic disorder and treated with methylphenidate. He was described as aggressive, with immature and oppositional behavior, having problems with impulsivity and attention shifting, and with a lack of emotional reciprocity. At the age of 13, he was tested with Wechsler Intelligence Scale for Children-Revised and found to have an IQ of 71, and he was diagnosed with behavioral disorder, oppositional defiant disorder, and mixed specific developmental disorder (all according to ICD-10).

A physical examination at the age of 25 years revealed no dysmorphic features. His height was 180 cm, weight 110 kg, and head circumference 61 cm. He wore glasses for myopia (estimated –3 diopters). He was obese with a

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body mass index of 34; according to the mother, obesity has been present since the age of 6–7 years as a result of uninhibited eating behavior. At the age of 27 years, he was screened for major psychiatric disease using the Mini International Diagnostic Interview version 5.0.0 that refers to the DSM-IV. No single diagnosis could be pinpointed. Signs of low IQ and autistic behavior with indications of concrete thinking, impairment of social interaction skills, vagueness in description of others, lack of concentration over time, hyperactivity, and impulsive behavior were seen during the interview. There was significant impairment in occupational and social areas, and a general incapacity for labor market affiliation was manifest. In his youth, he was intensely preoccupied with trains and unable to develop peer relationships. He lives in a group home and his social interactions are limited.

Chromosome and copy number variation analyses

Standard G-banding chromosome analysis was performed on cultured peripheral lymphocytes. Genomic DNA was extracted by conventional methods from peripheral blood, and whole-genome copy number variation (CNV) analysis was performed with the Affymetrix Genome-Wide Human

SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA). Raw intensity data files were analyzed with the Genotyping Console software (Affymetrix) according to the manufacturer's recommendations. CNVs larger than 1 kb and encompassing more than eight markers were evaluated.

Next-generation paired-end sequencing

Mate pair libraries were prepared using the Mate Pair Library v2 kit (Illumina, San Diego, CA, USA). Briefly, 10 μ g DNA was sheared using a nebulizer. Two- to three-kilobase pair fragments were isolated, end-repaired using a mix of natural and biotinylated dNTPs, blunt-end ligated using circularization ligase, and fragmented to 200–400 bp. Biotinylated fragments were isolated and end-repaired and A-overhangs were added to the 3'-ends. Paired-end adapters were ligated to the fragments and the library was amplified by 18 cycles of PCR. Mate pair libraries were subjected to 2×36 bases paired-end sequencing on a Genome Analyzer Iix (Illumina), following the manufacturer's protocol. Reads were aligned to a reference genome using Bowtie¹⁶ allowing up to two mismatches in the seed region. Reads not aligning uniquely were discarded from further analysis. Paired reads aligning to different chromosomes or with

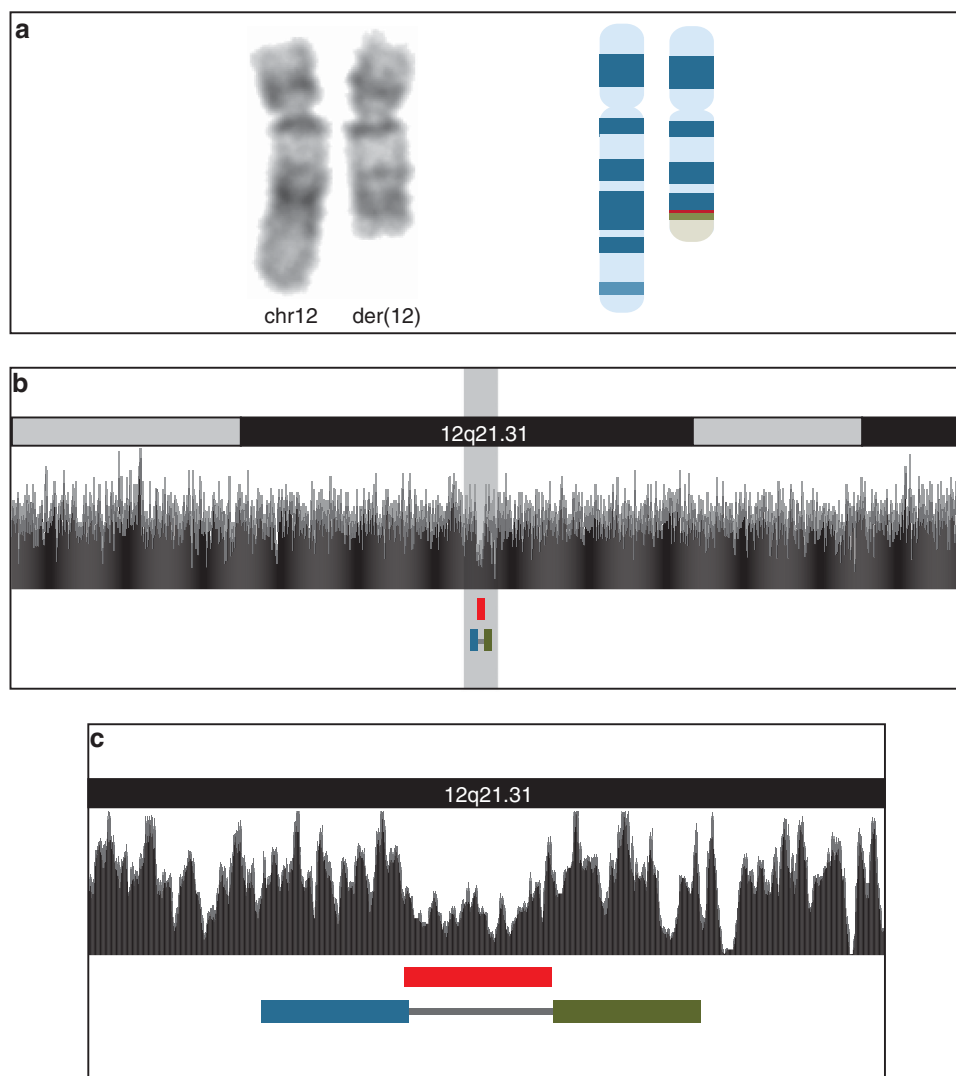


Figure 1 Partial karyotype and molecular findings on the derivative chromosome 12. (a) A partial karyotype showing the normal and the derivative chromosome 12, and a schematic representation of the two chromosomes and the identified rearrangement; the red line represents the deletion associated with the translocation at 12q21.31. (b) The breakpoint region at 12q21.31. The mate pair coverage is shown in black. A deletion detected by SNP 6.0 array is represented by a red bar; note the corresponding low coverage of the mate pair reads. The translocation breakpoint, represented by blue reads mapping to 12q21.31 and green reads mapping to 18q12.2, flanks the deletion. (c) Zoom in on the translocation region (gray shaded area in b). No RefSeq genes were located at the breakpoint.

unexpected strand orientation were extracted to identify potential translocation and inversion breakpoints, respectively. Breakpoints were only considered as candidates if they were confirmed by at least six independent paired reads with end-reads mapping within a 6-kb region. Predicted breakpoints were filtered against known in-house variants based on data from 30 individuals with known breakpoints. The translocation breakpoint was confirmed by PCR amplification and Sanger sequencing of the breakpoint-spanning fragments.

RESULTS

A total of 28 986 673 paired reads passed the chastity filter; 13 689 000 paired reads were aligned uniquely and 204 319 were chimeric pairs (end-reads mapping to different chromosomes). We removed non-clustering chimeric pairs leaving a total of 49 chimeric clusters genome-wide that were visually filtered against known variants.

The translocation breakpoints were identified at 12q21.31 and 18q12.2, respectively. The breakpoint at chromosome 12 affected no annotated genes whereas the breakpoint at chromosome 18 truncated *CELF4*. The translocation breakpoint at chromosome 18 was confirmed by PCR amplification and subsequent Sanger sequencing of the breakpoint-spanning fragments that revealed a small sequence of micro homology (AGGA), likely to facilitate the translocation (Supplementary Figure 1). Additionally, NGS revealed an associated inversion at chromosome 18 with breakpoints at 18q12.2 and 18q22.1 that truncated *CELF4* and *LOC643542*. Re-evaluation of the karyotype confirmed the cryptic complex rearrangement, and CNV analysis revealed small *de novo* deletions (<150 kb in size) corresponding to all four mapped breakpoints (Supplementary Table S1). The revised karyotype was t(12;18)(q21.31;q12.2),inv18(q12.2q22.1).arr12q21.31(83,761,458–83,857,594)x1,18q12.2(34,810,178–34,867,844)x1,18q12.2

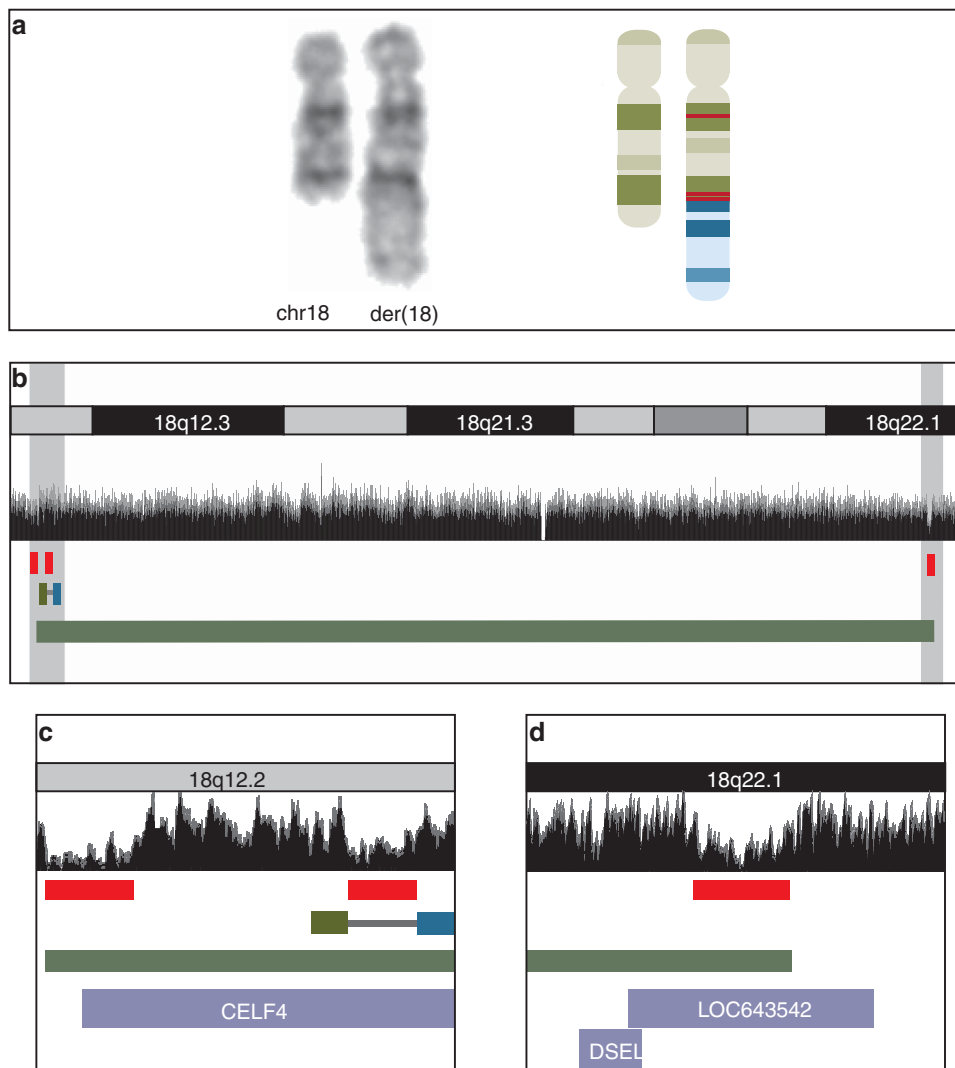


Figure 2 Partial karyotype and molecular findings on the derivative chromosome 18. (a) A partial karyotype showing the normal and the derivative chromosome 18, and a schematic representation of the two chromosomes and the identified rearrangements; the red lines represent deletions associated with the translocation breakpoint at 18q12.2 and the inversion breakpoints at 18q12.2 and 18q22.1. (b) The breakpoint region at 18q. The mate pair coverage is shown in black. Deletions detected by SNP 6.0 array is represented by red bars; note the corresponding low coverage of the mate pair reads. The missing coverage of mate pair reads in 18q21.3 corresponds to a gap in the annotated reference genome (UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly). The translocation breakpoint is represented by green reads mapping to 18q12.2 and blue reads mapping to 12q21.31, and the inversion is represented by a green bar. (c, d) Zoom in on the breakpoint regions (gray shaded areas in b). The protein coding gene *CELF4* is truncated by the translocation and two deletions at 18q12.2 (c), and the non-coding RNA *LOC643542* is truncated by the inversion breakpoint and an associated deletion at 18q22.1 (d).

Table 1 Clinical features in patients with molecularly characterized and overlapping interstitial deletions involving the 18q12.2 region

Reference	Present report	Gilling <i>et al</i> ¹³	McEntagart <i>et al</i> ¹⁰	Published patients ^a	Total
				(not fine mapped and/or not phenotyped in detail)	
Number of patients	1	1	1	18	21
Examination method	SNP array, NGS	aCGH, FISH	aCGH	Karyotype/aCGH/FISH	—
Deletion	18q12q12.2	18q12.1q12.2	18q11.2q12.2	Unbalanced ^b	—
Age (years)	27	38	4	2–67	2–67
Gender	M	F	M	6F, 12M	7F, 14M
ID/DD	(+) ^c	—	+	18/18	20/21
Motor delay	+	+	+	12/12	15/15
Seizures	+	—	+	11/16	13/19
Hypotonia	+	—	NR	8/11	9/13
Behavioral difficulties	+	+	+	11/13 ^d	14/16
ASD/autistic traits	+	+	NR	4/5	6/7
Obesity	+	NR	NR	9/13 ^d	10/14
Strabismus	—	NR	NR	8/11 ^d	8/12
Myopia	+	+	NR	1/4	3/6
Hypermetropia	—	—	NR	2/4	2/6
Dysmorphic features	—	—	—	15/16	15/19

Abbreviations: aCGH, array comparative genome hybridization; ASD, autism spectrum disorder; F, female; FISH, fluorescence *in situ* hybridization; ID/DD, intellectual disability or developmental delay; M, male; NR, not reported; SNP, single-nucleotide polymorphism.

^aChudley *et al*⁷; Engelen *et al*⁹; Feenstra *et al*¹²; Krasikov *et al*⁶; Poissonnier *et al*⁸; Schinzel *et al*⁴; Surh *et al*⁵; Wilson *et al*^{2,3}

^bCytogenetic unbalances (range 18q11–18q21.1), all include 18q12.2.

^cIntelligence quotient = 71.

^dFeenstra *et al*¹² note that common features in the studied group of 29 patients include obesity, strabismus, and behavioral problems. Since it is not specified if the 2 reported patients with deletions overlapping 18q12.2 have these features, they are not counted here.

(35,007,705–35,049,456)x1,18q22.1(65,284,854–65,430,839)x1 dn (hg19). The molecular characterization of the rearrangements is depicted in Figures 1 and 2.

DISCUSSION

Here, we report a male patient with a translocation involving chromosomes 12q and 18q with borderline IQ, developmental and behavioral disorders, myopia, obesity, and a history of febrile seizures in childhood. Using SNP array and NGS, we identified a cryptic complex rearrangement that truncated 2 *RefSeq* genes: the protein coding gene *CELF4* (also known as *BRUNOLA*) at 18q12.2 and the non-coding RNA *LOC643542* at 18q22.1.

Few of the previously reported patients with deletions of 18q12.2 were characterized sufficiently both clinically and molecularly to allow direct phenotype–genotype comparisons. However, as illustrated in Table 1 there is a considerable phenotypic overlap with our patient. Gilling *et al*¹³ reported a patient with childhood autism, severe myopia, and a *de novo* translocation, t(5;18)(q34;q12). The patient had normal intelligence (IQ 88) and no dysmorphic facial features. The breakpoint region contained a 3.2-Mb deletion encompassing *CELF4*. McEntagart *et al*¹⁰ described a patient with a *de novo* del(18)(q11.2q12.2) and a phenotype remarkably like the one reported here consisting of mild intellectual disability (IQ 61), developmental delay, febrile seizures, clumsiness, behavioral difficulties including poor concentration and hyperactivity, distractibility and learning difficulties, and no facial dysmorphism. Tinkle *et al*¹¹ described a 67-year-old female patient with del(18)(q12.2q21.1). This patient was only examined with conventional karyotyping but the reported phenotype included intellectual disability, a history of fine motor problems, and seizures in childhood. Her behavior was described as impulsive, aggressive, defiant, restless, and labile autistic-like. She was obese and had problems with food seeking obsession.

Although *CELF4* was previously found disrupted in patients with overlapping phenotypes,^{10,13} no phenotype has been associated with the non-coding RNA *LOC643542*, thus we find it highly likely that

haploinsufficiency of *CELF4* causes the described phenotype. *CELF4* belongs to a family of evolutionary highly conserved genes.^{17,18} The encoded protein binds to RNA and act as a splicing regulator. *CELF4* is expressed in various tissues with high expression in both fetal and adult human brain.¹⁹ In frog embryos (*Xenopus laevis*), *Celf4* is solely expressed in the nervous system, including the optic vesicles,²⁰ suggesting an important role in both brain and eye development. Furthermore, *Celf4* is highly expressed in mouse brain^{13,19,21} and *Celf4* insufficient transgenic mice develop convulsive seizures, hyperactivity, and late-onset body weight gain.^{21,22} Not only is *CELF4* conserved from *Drosophila* to mouse and human, but the genomic landscape next to *CELF4* is also evolutionary conserved²³ harboring ultra-conserved elements (UCE),²⁴ and it has been suggested that UCES may be directly involved in neurodevelopmental disorders.^{25,26}

We found *CELF4* to be disrupted in a patient with borderline IQ, developmental and behavioral disorders, myopia, obesity, and a history of febrile seizures in childhood. The phenotype resembles that of patients with 18q12.2 deletions illustrating that *CELF4* haploinsufficiency has a key role in explaining their phenotype. This report, and the finding of *Celf4*-deficient mice with overlapping traits, for example, seizures, hyperactivity, and obesity,^{21,22} adds to the growing evidence that *CELF4* is important for the brain development and in the disposition to obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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