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Paracentric Inversion of Chromosome 2 Associated With Cryptic Duplication of 2q14 and Deletion of 2q37 in a Patient With Autism

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Abstract

We describe a patient with autism and a paracentric inversion of chromosome 2q14.2q37.3, with a concurrent duplication of the proximal breakpoint at 2q14.1q14.2 and a deletion of the distal breakpoint at 2q37.3. The abnormality was derived from his mother with a balanced paracentric inversion. The inversion in the child appeared to be cytogenetically balanced but subtelomere FISH revealed a cryptic deletion at the 2q37.3 breakpoint. High-resolution single nucleotide polymorphism array confirmed the presence of a 3.5 Mb deletion that extended to the telomere, and showed a 4.2 Mb duplication at 2q14.1q14.2. FISH studies using a 2q14.2 probe showed that the duplicated segment was located at the telomeric end of chromosome 2q. This recombinant probably resulted from breakage of a dicentric chromosome. The child had autism, mental retardation, speech and language delay, hyperactivity, growth retardation with growth hormone deficiency, insulin-dependent diabetes, and mild facial dysmorphism. Most of these features have been previously described in individuals with simple terminal deletion of 2q37. Pure duplications of the proximal chromosome 2q are rare and no specific syndrome has been defined yet, so the contribution of the 2q14.1q14.2 duplication to the phenotype of the patient is unknown. These findings underscore the need to explore apparently balanced chromosomal rearrangements inherited from a phenotypically normal parent in subjects with autism and/or developmental delay. In addition, they provide further evidence indicating that chromosome 2q terminal deletions are among the most frequently reported cytogenetic abnormalities in individuals with autism.

Key words: paracentric inversion; 2q37 deletion syndrome; duplication; chromosome 2; autism; mental retardation; DNA microarray

INTRODUCTION

Autism is a neurobehavioral syndrome characterized by impairment in social interaction and communication and by restricted and repetitive patterns of interests and activities [American Psychiatric Association, 1994]. The prevalence of autism spectrum disorders (ASDs) is 0.6% and is four times higher in males than in females [Chakrabarti and Fombonne, 2005]. Twin studies, sibling recurrence rates, and the association with genetic disorders and chromosomal abnormalities indicate that ASDs have a strong genetic component with complex inheritance [Abrahams and Geschwind, 2008]. Linkage and association studies have failed to identify any definite susceptibility genes for idiopathic autism. Chromosomal abnormalities are detected by routine karyotyping in about 4%-5.8% of individuals with idiopathic autism [Veenstra-Vanderweele et al., 2004; Marshall et al., 2008]. Recent studies using higher resolution approaches such as comparative genomic hybridization or single nucleotide polymorphism (SNP) arrays show that approximately 10% of patients with sporadic ASD have *de novo* copy number variations (CNVs) [Autism Genome Project Consortium, 2007; Sebat et al., 2007; Marshall et al., 2008]. A great variety of structural chromosomal abnormalities have been reported for all chromosomes [Castermans et al., 2004; Veenstra-Vanderweele et al., 2004; Vorstman et al., 2006]. The occurrence of autism or autistic features in children with deletion of the terminal band of chromosome 2q has been reported in a growing number of cases [Conrad et al., 1995; Ghaziuddin and Burmeister, 1999; Smith et al., 2001; Wolff et al., 2002; Lukusa et al., 2005; Reddy, 2005; Wassink et al., 2005; Sebat et al., 2007; Galasso et al., 2008]. In a review of 66 individuals with 2q37 terminal deletion, autism or autistic behavior were reported in 24% of patients [Casas et al., 2004]. However, few of the reported 2q37 deletions have been delineated with precision, precluding the identification of a minimal deleted region that might contain one or more genes involved in the autistic phenotype.

Here we report on a paracentric inversion of chromosome 2q in a boy with autism. The inversion was inherited from the healthy mother and appeared to be cytogenetically balanced but fluorescent in situ hybridization (FISH) as well as high-resolution SNP microarray revealed a 2q37.3 deletion at the distal breakpoint and a 2q14.1q14.2 duplication of the proximal breakpoint of the inversion, with the duplicated material located at the distal end of chromosome 2q. We present the phenotypic, cytogenetic, and molecular genetic findings in this patient.

MATERIALS AND METHODS

Subject

The patient was ascertained as part of a study of genetic and perinatal risk factors in autism, performed in an epidemiological cohort of children born between 1985 and 1998 and living in three neighboring French counties (Isère, Savoie, and Haute-Savoie). Children were evaluated by a child neurologist and a laboratory workup was performed to identify the underlying etiology, including

screening for chromosome anomalies, fragile X syndrome and inborn errors of metabolism. The study was approved by the local research ethics board. Written informed consent was obtained from all families.

Cytogenetic and Molecular Analyses

Cytogenetic analysis. Conventional cytogenetic investigations were performed according to standard methods on lymphocytes from phytohemaglutinin-stimulated peripheral blood cultures. Chromosome spreads were processed for RHG and GTG banding.

FISH. We performed multi-subtelomeric FISH using a multiprobe system (Cytocell, Oxfordshire, UK). Chromosome denaturation, hybridization and signal detection were done according to the manufacturer's instructions. Slides were analyzed on a Zeiss epifluorescent microscope equipped with appropriate filters and a Metasystems image analysis system was used to analyze the subtelomeric region of every chromosome for deletions and balanced translocation events. The p-arm probes were labeled with digoxigenin and detected using a fluorescein isothiocyanate (FITC)-conjugated amplification system. The q-arm probes were labeled with biotin and detected with Cy3 conjugated antibodies. Cells were counterstained with 4,6-diamino-2-phenylindole (DAPI).

In addition, FISH investigations on chromosome 2 were carried out using a whole chromosome painting probe (WCP 2 Oncor, Gaithersburg, MD), a subtelomeric Tel 2q probe (TelVysion probe 2q; Vysis, Downers Grove, IL), 10 BAC clones located at 2q37.3 (Table I) and one clone at 2q14.2 (RP11-77A13).

Whole-genome SNP array. The patient and both parents were analyzed with the HumanCNV370-Duo DNA Analysis BeadChip (Illumina, San Diego, CA) containing over 370,000 markers. Approximately 750 ng of genomic DNA were used to genotype each sample. Samples were processed according to the Infinium II assay manual. Briefly, each sample was whole-genome amplified, fragmented, precipitated, and re-suspended in an appropriate hybridization buffer. Denatured samples were hybridized on the HumanCNV370-Duo BeadChip for a minimum of 16 h at 48°C. After completion of the assay, the BeadChips were scanned with a two-color confocal BeadArray reader. Image intensities were extracted and analyzed using Illumina's BeadStudio 3.0 software.

Real-time quantitative PCR. Quantitative PCR (qPCR) was used to confirm the deletion and duplication identified at the inversion breakpoints. We used the Universal Probe Library (UPL) system (Roche, Indianapolis, IN), which consists of a library of 90 fluorescence-labeled probes covering over 98% of the genome when paired with region-specific primers. Probes were chosen according to the gene sequences specified using ProbeFinder v2.04 software (Roche, http://www.universalprobelibrary.com). Primers to be used with the UPL probes were also designed with the ProbeFinder application. Ten µl reactions were assembled with 25 ng DNA, 400 nM of each primer, 100 nM UPL probe (Roche), and 1X

Platinum Quantitative PCR SuperMix-Uracil-D-Glycosylase (UDG), with Rox (Invitrogen, Carlsbad, CA). All reactions were performed in triplicate. In addition to the DNAs and the genes to be assayed for copy number, each 384-well plate included three control samples and three reference genes, as well as a no-template control for each gene. PCR conditions were as follows: 2 min UDG activation at 50°C, 2 min denaturation at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The plate was analyzed with an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Raw data were obtained with the SDS v2.3 software (Applied Biosystems) and exported for analysis with qBase [Hellemans et al., 2007].

RESULTS

Clinical Report

The patient is a Caucasian 14-year-old boy, the second child of non-consanguineous healthy parents. The family history was unremarkable. He was born at 39 weeks gestation by vaginal delivery. Birth weight was 3350 g (25th-50th centile), birth length 49 cm (50th centile) and head circumference 34 cm (10th-50th centile). Apgar scores were 10 and 10 at 1 and 5 min. The mother was 26 years old and the father 31 years old at the time of birth. During the first 6 months he was noted to be somewhat hypoactive, but his motor milestones were normal; he sat at 9 months and walked at 14 months. The parents first realized something was wrong when he was 18 months old: he became hyperkinetic, often banging his head against the wall, did not answer to his name and avoided eye contact. He was fascinated with electrical sockets, the first thing he would explore when he arrived in a new place. Speech and language development were delayed; he started to use meaningful words at 30 months and phrases at 60 months. His non-verbal communication was also delayed: he did not point and his range of facial expressions was limited. He started nursery school at the age of 3 years and had difficulty integrating. He was evaluated at that time by a psychiatrist who prescribed a neuroleptic as a sedative. By the age of 5 he would not show or share things he liked, was aloof, seldom interacting with peers, and had no imaginative play. He had overall speech delay with occasional pronoun reversal and particularities, including excessive questioning and preoccupation with particular topics. He was constantly active and sometimes agitated for no reason. He received speech and occupational therapy and when he was 9 he started to attend a specialized medical institution for children with autism.

At the time of evaluation at the age of 12 years he had made progress in the domains of social interaction and language. Despite some attempts to communicate with others, he was often rejected by other children. He still lacked social reciprocity and was excessively interested in all things electronic. He had been less active since he had started taking Ritalin 2 years before, but its effect tended to decrease and he became more anxious. During the examination he was anxious; he showed no social withdrawal but exhibited poor eye contact. His voice was loud and monotonous and he had a poor range of facial

expressions. His activity level was generally low with occasional outbursts of increased activity and his gestures were clumsy.

Based on his medical history and the above examination, he meets DSM-IV criteria for autistic disorder [American Psychiatric Association, 1994], because of the combination of deficits in reciprocal social interactions and language, and ritualistic interests. On the Childhood Autism Rating Scale (CARS) his score was 30.5, indicating mild-moderate autism. On the Autism Diagnostic Interview-Revised (ADI-R) he met all the cut-offs for autistic disorder [Lord et al., 1994]. The global IQ, evaluated with the Wechsler Intelligence Scale for Children–III, was 46. Verbal IQ and performance IQ were 46 and 50, respectively.

The patient has a personal history of asthma, insulin-dependent diabetes, and growth retardation with growth hormone deficiency. He is treated with an association of corticoids, beta2 agonist, insulin and growth hormone. There was no history of seizures. An electroencephalogram and brain MRI performed at the age of 3 found no abnormalities. On physical examination at 12 years, he had short stature (142 cm, 14th centile), with normal weight (40 kg) and head circumference (54 cm). No pubertal development was observed. He had mild facial dysmorphism with frontal bossing, flattened nasal bridge, deep set eyes, down-slanting palpebral fissures, and thin upper lip (Fig. 1).

Except for the karyotype (see below), other laboratory investigations performed at the time of evaluation were normal, including FISH for 15q11-q13, 22q11.2 and 22q13, fragile X molecular testing and metabolic screening for inborn errors of metabolism. A new brain MRI was within normal limits.

Cytogenetic and Molecular Analyses

RHG-banding and GTG-banding analysis in the patient showed a large paracentric inversion on the long arm of chromosome 2, inv(2)(q14.2q37.3) (Fig. 2A). This chromosomal abnormality appeared to be balanced and was inherited from the phenotypically normal mother. However, FISH studies with subtelomeric probes in the patient showed a deletion of the Cytocell 2q probe DJ1011O17 (Fig. 2B), which includes markers 2QTEL86 (D2S2987), 2QTEL37 (D2S2985), 2QTEL47 (D2S2986) and 2QTEL44 (D2S2585). Because polymorphisms resulting from the variable length of the target sequence have been reported with clone D2S2986 [Knight and Flint, 2000], we used two other probes, 172113 (D2S447; Cytocell) and the TelVysion 2q probe VIJyRM2112 (D2S447; Vysis), to analyze the subtelomeric 2q region. These non-polymorphic probes were also deleted (Fig. 2C), indicating that the patient has a submicroscopic deletion of the 2q37.3 region. No 2q terminal deletion was observed in the mother.

To further delineate the distal breakpoint in the patient, 10 BACs of the 2q37 region were used. The results are shown in Table I. The BAC clone immediately proximal to the deletion was RP11-320G1, while no hybridization was seen for clones RP11-162P12 and RP11-546M8. According to these results, the distal breakpoint is on 2q37.3.

High-resolution SNP microarray in the patient confirmed the presence of a 3.5 Mb deletion at the distal breakpoint of the inversion on the maternally derived chromosome, extending to the telomere (chr2:239,400,015–tel, hg18) (Fig. 3). The microarray also revealed a 4.2 Mb duplication at the proximal breakpoint, at 2q14.1q14.2 (116,789,226–121,021,018) (Fig. 3). Both parents had normal profiles. The technique did not find any other potentially significant CNVs. The results of the SNP array were confirmed by real-time qPCR. Figure 4 shows duplication of genes *EN1* and *MARCO* located at 2q14.2 as well as deletion of *OTOS*, *C2orf54*, and *HDLBP* located at 2q37.3, with normal dosage of the genes flanking the rearranged regions.

FISH with BAC clone RP11-77A13 (2q14.2) confirmed the duplication in the patient, and showed that the duplicated material was telomeric, distal to the inverted region (Fig. 5A). In the mother, clone RP11-77A13 hybridized to the expected region on both chromosomes 2 (Fig. 5B and C), indicating that the proximal breakpoint of the inversion was located distally. RP11-475A20 (2q37.3) was inverted and hybridized very closely but distal to RP11-77A13 (2q14.2) (Fig. 5B), whereas RP11-546M8 (2q37.3), previously shown to be deleted in the patient, was not deleted in the mother and hybridized to the telomeric region (Fig. 5C). This locates the proximal breakpoint of the paracentric inversion in 2q14.2, just distal to BAC RP11-77A13. From these analyses, the abnormal karyotype in the patient is: 46,XY,rec(2)inv(2)(q14.2q37.3)mat.ish rec(2)del(2)(q37.3qter)(wcp2+,RP11-162P12–,qter–)dup(2)(q14.2)(RP11-77A13++)*dn*.

DISCUSSION

Paracentric inversions are intrachromosomal rearrangements that result from a two break event occurring on the same chromosome arm, followed by the insertion of the intercalary segment after a 180° rotation. Because they do not alter chromosome arm ratios, paracentric inversions can remain undetected. In addition, they are usually not associated with an abnormal phenotype. For these reasons, the incidence of paracentric inversions has not been clearly established; estimations range from 0.09/1000 to 0.49/1000 [Pettenati et al., 1995]. Paracentric inversions of nearly all chromosomes have been reported [Madan, 1995], with most being inherited [Fryns et al., 1986; Madan, 1995; Pettenati et al., 1995]. A high incidence of mental retardation (26%) and congenital malformations has been reported in the inversion carrier offspring of phenotypically normal parents with apparently identical chromosomal rearrangements [Fryns et al., 1986]. These findings could be explained by the non detection of a small chromosomal imbalance [Fryns et al., 1986]. In a review of 446 paracentric inversions, there were 15 cases of recombinant chromosomes with duplication and/or deletion [Pettenati et al., 1995]. With the higher resolution techniques available today, this figure would likely be much higher. According to classic genetic mechanisms, a crossover in a paracentric inversion loop produces dicentric chromosomes and acentric fragments with deletions and duplications. The instability of these chromosome structures reduces the frequency of viable recombinants. Alternative mechanisms have been proposed to explain

unabalanced products in liveborn children, including breakage of a dicentric bridge, unequal crossover, or U-type exchange within the inversion loop [reviewed in Madan and Nieuwint, 2002]. Others have suggested that the paracentric inversions may be in fact insertions, which carry a high risk of recombination products [Madan and Nieuwint, 2002].

In our patient, the paracentric inversion of 2q14.2q37.3 was inherited from the mother. At a standard cytogenetic level, the inversion was identical in the patient and his mother. However, FISH and SNP array analysis revealed the occurrence of a 3.5 Mb deletion at the distal breakpoint (2q37.3) extending to the telomere and a 4.2 Mb duplication at the proximal breakpoint (2q14.1q14.2) only in the patient (Fig. 6). FISH studies in the mother confirmed the presence of a paracentric inversion, and excluded the possibility of an inverted intrachromosomal insertion. The imbalances in the child are most likely the result of crossover in the inversion loop of chromosome 2 during maternal meiosis. We hypothesize that during the first meiotic division, a crossover occurred in the inverted chromosome near the proximal breakpoint (between 2q14.2 and 2q37.3), leading to the formation of a dicentric chromosome (Fig. 7). During anaphase, the dicentric chromosome formed an anaphase bridge that broke when the centromeres joined the opposite pole. The break occurred between the centromeric region and 2q14.2. Thus, the result is the inverted chromosome with a terminal deletion and a duplicated region distal to the inversion. A similar mechanism was suggested for a duplication-deficiency monocentric chromosome 18 resulting from a maternal paracentric inversion [Courtens et al., 1998]. Based on these observations, we suggest that genetic counseling and prenatal diagnosis should be offered to couples where one of the members is a carrier of a paracentric inversion. In the case of inversion carrier offspring, molecular cytogenetic analysis should complete the standard karyotype.

To date, about 100 patients with terminal deletions with breakpoints at 2q37 have been reported [Falk and Casas, 2007]. The most commonly reported features include developmental delay/mental retardation, abnormal behavior including autism or autistic features, hypotonia, mild facial dysmorphism (frontal bossing, round face, depressed nasal bridge, abnormal or prominent ears, deep-set eyes, anteverted nares and thin upper lip), short stature, and short hands or feet [Conrad et al., 1995; Ghaziuddin and Burmeister, 1999; Aldred et al., 2004; Casas et al., 2004]. Albright hereditary osteodystrophy-like brachymetaphalangia has been reported in approximately 50% of patients [Aldred et al., 2004; Casas et al., 2004]. Major malformations are observed in a third of patients with 2q37 monosomy, and include cardiac, gastrointestinal, renal, genitourinary and central nervous system malformations. A phenotype-breakpoint correlation based on 66 individuals with chromosome 2q terminal deletion showed that malformed kidneys and structural brain anomalies were limited to patients with the largest deletions, with breakpoints at or proximal to 2q37.1 [Casas et al., 2004]. However, attempts to define the minimal deleted regions for the major features of the syndrome have been rendered difficult by the considerable clinical variability apparent even among patients with similar breakpoints [Aldred et al., 2004]. Furthermore, most of the reported cases with 2q terminal deletions have not been characterized

molecularly with FISH and high-resolution microarray analysis, so the size of the deletion, the precise breakpoints and the possible presence of other cryptic genomic rearrangements has not been determined. Such studies are essential to establish genotype-phenotype correlations and to provide prognostic information and genetic counseling to parents.

The phenotype of our patient is similar to that described in other individuals with terminal 2q deletions, including mental retardation, autism, and characteristic facial appearance. He did not have brachymetaphalangia or major malformations, although the former maybe difficult to recognize in some preadolescent subjects. His case was complicated by insulin-dependent diabetes mellitus and hyposecretion of growth hormone. Although growth hormone deficiency has been described in association with 2q37 deletions [Kitsiou-Tzeli et al., 2007], diabetes has not been associated with the 2q37 deletion syndrome, so it might be either a rare manifestation or a coincidental finding. Alternatively, the concurrent duplication of 2q14.1q14.2 could play a role in these endocrinopathies.

Autism or autistic features have been repeatedly described in subjects with 2q37 deletions [Conrad et al., 1995; Ghaziuddin and Burmeister, 1999; Smith et al., 2001; Wolff et al., 2002; Lukusa et al., 2005; Reddy, 2005; Wassink et al., 2005; Sebat et al., 2007; Galasso et al., 2008]. Among 66 subjects with 2qter deletion, autistic behavior was observed in 24% [Casas et al., 2004]. However, the prevalence of ASDs in terminal 2q deletions is unknown, because most patients have not been assessed formally. The Autism Chromosome Rearrangement Database (http://projects.tcag.ca/autism/) lists 35 patients with chromosome 2q terminal deletions. Furthermore, in a study of 165 unrelated subjects with autism, Sebat et al. [2007] found two with *de novo* 2q37 deletions. Thus, such deletions are among the most frequent cytogenetic abnormalities reported in patients with autism. By comparing the deletion breakpoints of three patients with autism carrying terminal 2q37 deletions to those of two siblings without autism, Lukusa et al. [2005] suggested that the critical region for autistic disorder appeared to be 1.2 Mb and lied between clones RP11-680O16 (236.2 Mb) and RP11-346I14 (237.6 Mb). The deletion breakpoint of our patient with autism lies distal to the candidate region, between RP11-320G1 (239.5 Mb) and RP11-162P12 (239.8 Mb), and therefore does not support the critical region for autism proposed by Lukusa et al.

Telomeric rearrangements play an important role in the etiology of mental retardation, accounting for ~2.5% of cases [Ravnan et al., 2006]. In a pilot study of 10 patients with autism, one patient with a telomeric 2q37.3 deletion was found [Wolff et al., 2002]. However, subsequent studies have shown that subtelomeric deletions are rare in patients with non-syndromic ASD [Battaglia and Bonaglia, 2006; Di Bella et al., 2006; Wassink et al., 2007].

The distal 2q37 deletion in our patient removes 42 genes (Fig. 6). Several genes have been proposed as candidates for various aspects of the phenotype, including glypican 1 (*GPC1)*, G-protein-coupled receptor 35 (*GPR35*), serine/threonine protein kinase 25 (*STK25*), and programmed cell death 1 precursor (*PDCD1*). In the context of autism, *KIF1A*, a member of the kinesin family involved in axonal

transport of synaptic vesicles, and *FARP2*, a Rho GTPase involved in neurite growth and axonal guidance, appear as good candidate genes. *FARP2*, together with *HDLBP* and *PASK*, were recently shown to be down-regulated in a patient with autism and 2q37.3 deletion syndrome [Felder et al., 2009]. It is not known at present whether the 2q37 deletion phenotype represents a contiguous gene deletion syndrome or if certain key clinical features result from happloinsufficiency of a single gene.

The microduplication at 2q14.1q14.2 encompasses 19 genes (Fig. 6). Pure duplications of the proximal long arm of chromosome 2 are rare, with only 11 patients reported thus far [Ounap et al., 2005]. The size of the duplications and the clinical signs are variable and no specific 2q duplication syndrome has emerged. No other duplications limited to the 2q14.1q14.2 segment have been reported, so the contribution of this genomic imbalance to the patient's phenotype is not known. However, a recent analysis of CNVs in the general population reported two apparently normal individuals with duplications in 2q14.2 (one encompassing *EN1*, *MARCO*, *C1QL2*, and *STEAP3*, chr2:119,295,788-119,743,129, and the other encompassing *MARCO*, *C1QL2*, *STEAP3*, *C2orf76*, *DB1*, *TMEM37*, and *SCTR*, chr2:119,426,630-119,988,013) [Itsara et al., 2009]. The duplication found in both normal individuals is encompassed within the duplicated interval in our patient, suggesting that these genes probably do not contribute to his phenotype.

In conclusion, our data provide further evidence indicating that chromosome 2q terminal deletions are among the most frequently reported chromosomal aberrations in patients with autism and suggest that haploinsufficiency of one or more genes deleted within a 3.5 Mb segment of the 2q37.3 region may predispose to autism. Furthermore, these findings suggest that the molecular study of apparently “balanced” chromosomal rearrangements inherited from a phenotypically normal parent is warranted in patients with autism and/or developmental delay.

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Table I. Clones spanning the 2q37.3 region used in FISH experiments in the patient

Clone	Location (Mb)	FISH signal
RP11-488K22	238.19	inverted
RP11-526L8	238.66	inverted
RP11-40B20	238.95	inverted
RP11-225M4	239.07	inverted
RP11-475A20	239.27	inverted
RP11-55A23	239.31	inverted
RP11-136O17	239.34	inverted
RP11-320G1	239.52	inverted
RP11-162P12	239.76	deleted
RP11-546M8	239.98	deleted
DJ1011O17	242.50	deleted
VIJyRM2112 (D2S447)	242.53	deleted
NP 172113 (D2S447)	242.53	deleted

Fig. 1. Photo of the patient. Note mild dysmorphic features, with frontal bossing, flattened nasal bridge, deep set eyes, down-slanting palpebral fissures, and thin upper lip.

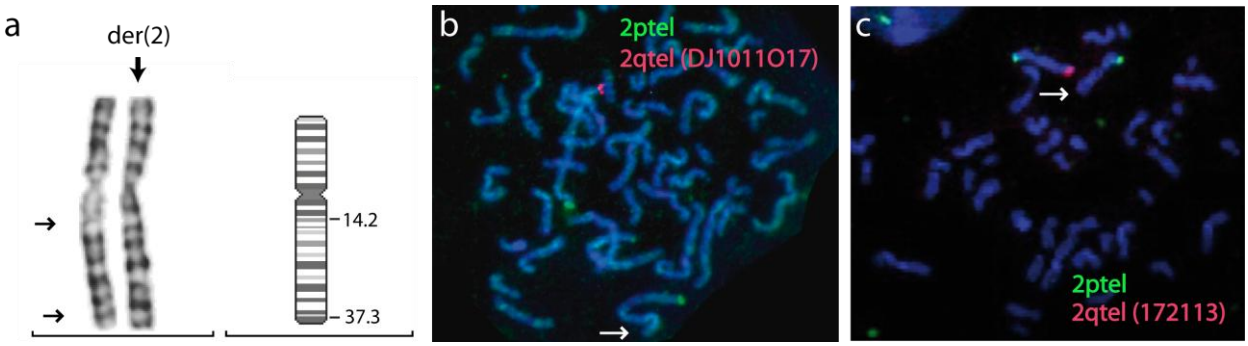

Fig. 2. a. Partial G-banding karyotype of chromosome 2 in the patient showing the paracentric inversion (q14.2-q37.3). The inverted segment is indicated by arrows. **b,c**. FISH analysis with chromosome 2 subtelomeric probes (Chromoprobe Multiprobe System, Cytocell). On the normal chromosome 2, the p and q subtelomeres are labeled green and red, respectively. Both probe DJ1011O17 (**b**) and the non-polymorphic probe 172113 (D2S447) (**c**) show a telomeric deletion of the long arm, indicated by arrows.

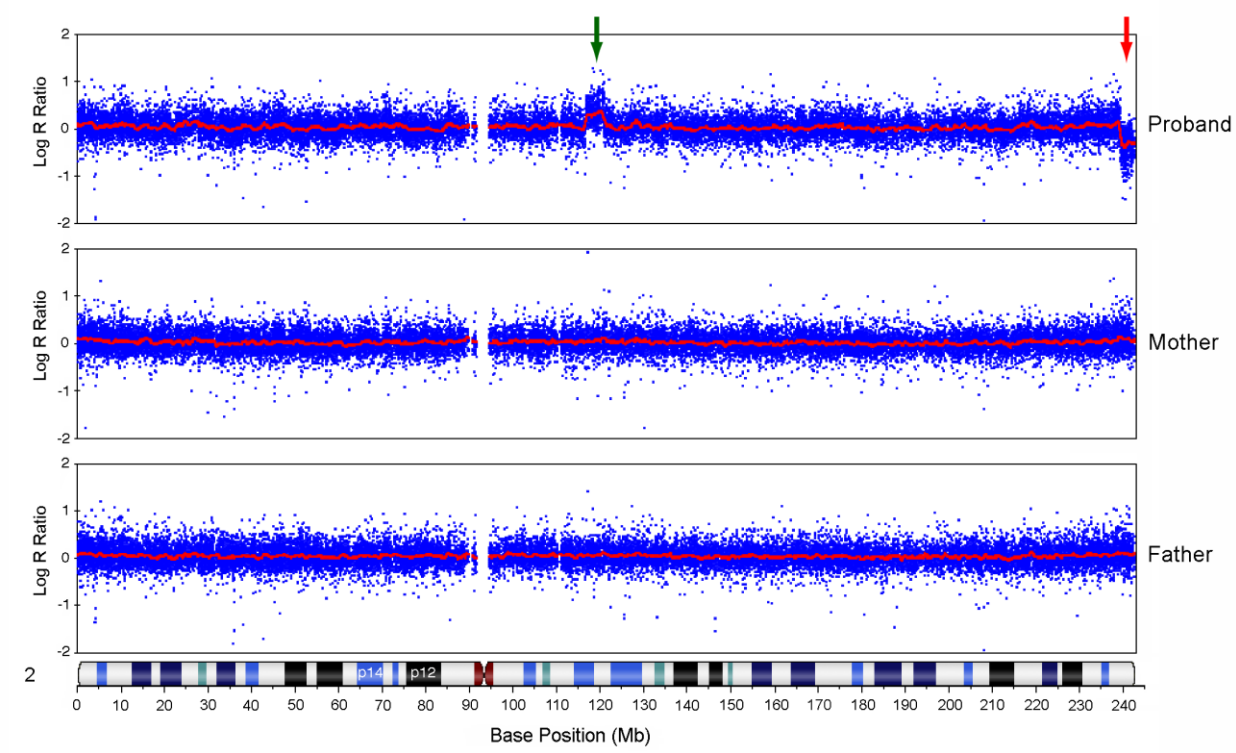

Fig. 3. SNP array results of chromosome 2 in the patient and his parents. SNP array ratio profiles of chromosome 2 showing chromosomal imbalances in the patient and normal profiles in the parents. The duplicated (2q14.1q14.2) and deleted (2q37.3) intervals in the patient are indicated by green and red arrows, respectively. The X axis indicates the chromosomal position (Mb) and the Y axis shows the intensity ratio represented on a log 2 scale. Individual SNPs are represented as blue dots and the average Log ratio is shown as a red line; a ratio of 0 indicates the presence of 2 alleles and is considered normal.

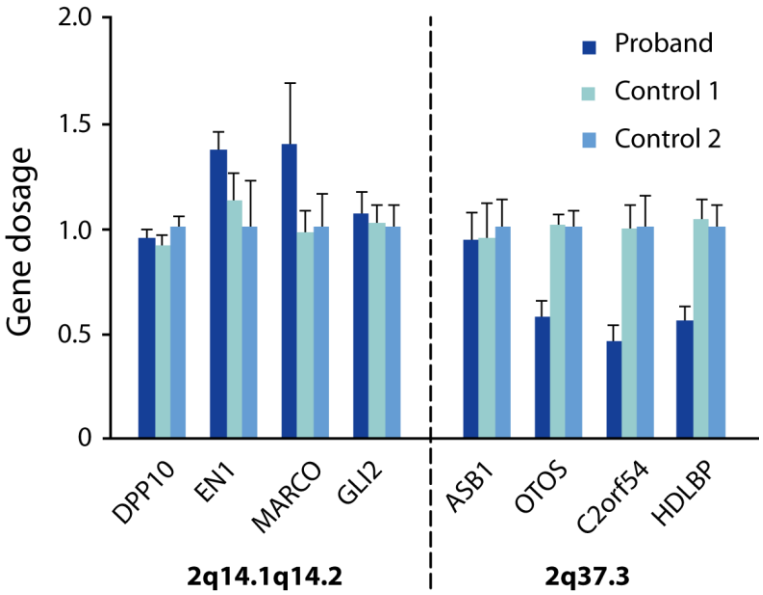

Fig. 4. qPCR gene dosage of duplicated and deleted regions. qPCR was used to confirm the duplication (2q14.1q14.2) and deletion (2q37.3) in the patient. Genes within the deleted or duplicated intervals and the flanking regions were targeted with qPCR probes in the patient and two controls. Data represent mean+SEM. A gene dosage ratio of 1 indicates the presence of 2 alleles and is considered normal; values above correspond to a duplication and values below indicate a deletion.

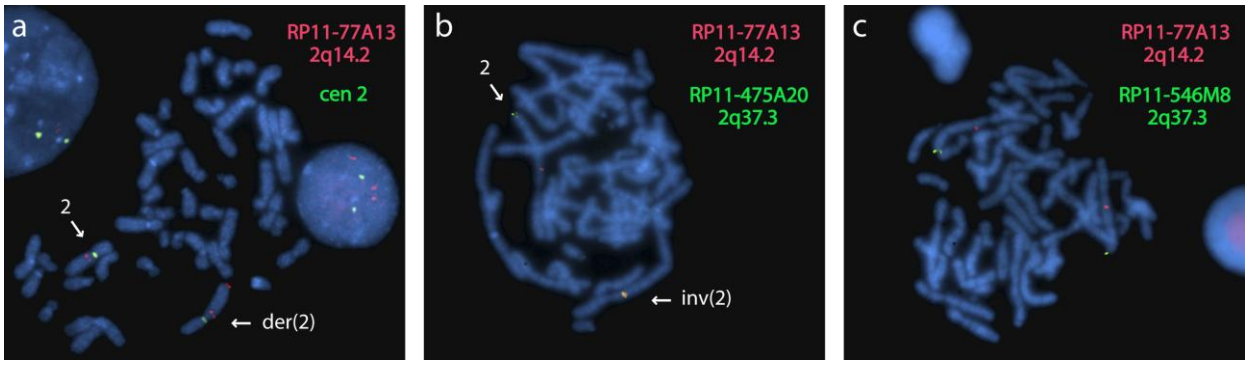

Fig. 5. a. FISH analysis with clone RP11-77A13 (2q14.2, red) and a centromeric probe (green) in the patient showed that the duplicated 2q14.2 material was located at the telomeric end of chromosome 2q. **b,c.** FISH analysis with clone RP11-77A13 (2q14.2, red) in the mother shows normal hybridization to both chromosomes 2, indicating that the proximal breakpoint of the inversion was located distally. Clone RP11-475A20 (2q37.3) was inverted and hybridized very close to RP11-77A13 (2q14.2) on one of the homologues (**b**), whereas clone RP11-546M8, which hybridizes to the 2q telomeric region, showed normal signals (**c**).

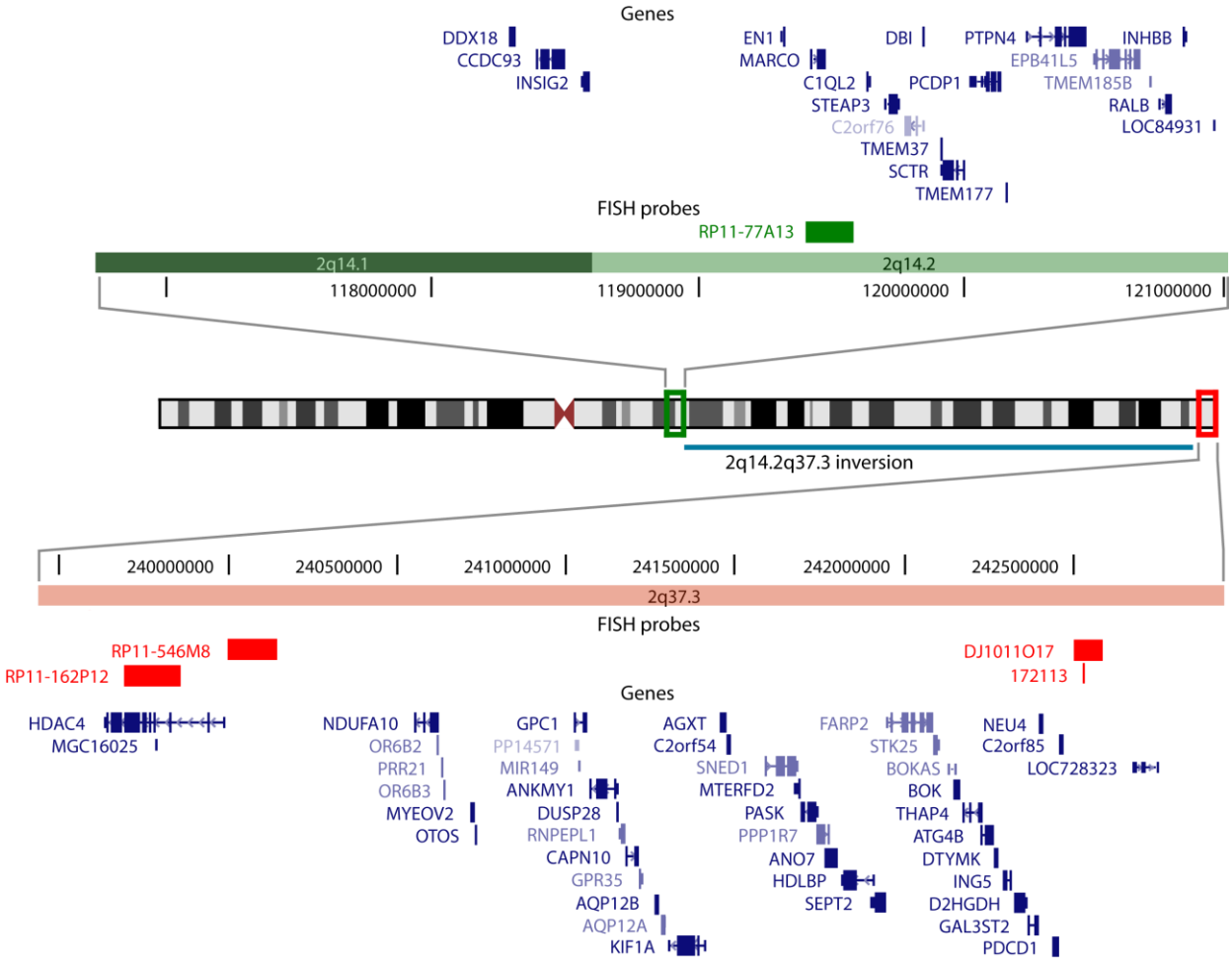

Fig. 6. Schematic representation of chromosome 2 rearrangements in the patient. The duplicated (2q14.1q14.2) and deleted (2q37.3) intervals are surrounded by green and red rectangles, respectively, on the ideogram; close-ups of both regions including the chromosomal position and gene content are depicted above and below, respectively. The inverted region is indicated by a blue bar. Deleted FISH probes are shown in red, duplicated probes in green. The additional copy of the 2q14.2 segment was shown by FISH to be located near the 2q telomere.

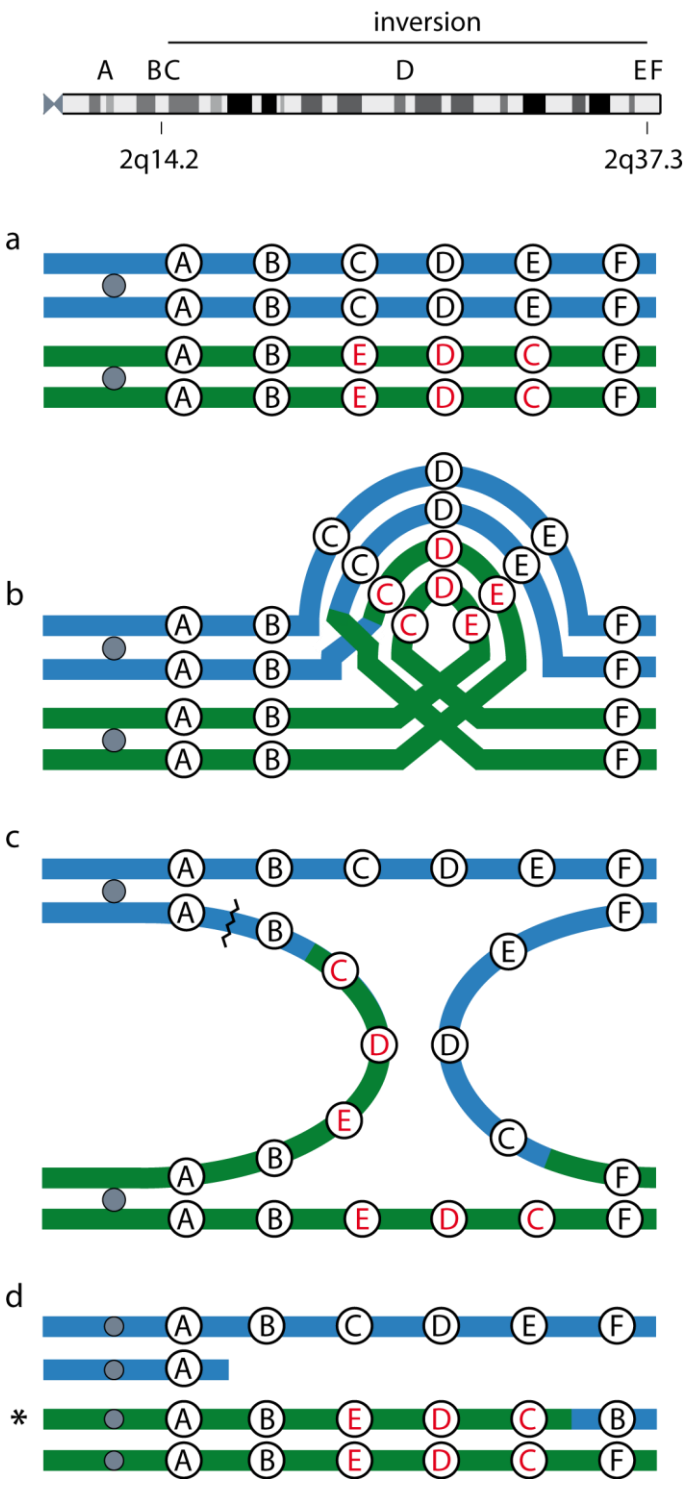

Fig. 7. Breakage of a dicentric chromosome as a mechanism for the formation of the recombinant observed in the patient. Letters A-F correspond to the chromosomal regions indicated at the top: C and E are within the inversion, while B and F flank the inversion. **a.** One of the two maternal chromosomes carries a paracentric inversion of 2q. **b.** During meiosis I, the inverted chromosome forms a loop; crossover between nonsister chromatids within the loop results in abnormal chromosome structures. **c.** As the chromosomes separate to opposite poles, a dicentric chromosome and an acentric fragment are formed. The acentric fragment is lost, while the dicentric bridge is pulled in opposite directions and breaks. **d**. Meiosis II generates a normal chromosome, a deleted chromosome, an inverted chromosome carrying a deletion distal to the inversion and a proximal duplication, with the duplicated segment located at the telomeric end, and an inverted chromosome. The recombinant transmitted to the patient is indicated by an asterisk.