European Journal of Medical Genetics 56 (2013) 361–364

Contents lists available at SciVerse ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Short clinical report

A balanced *de novo* inv(7)(p14.3q22.3) disrupting *PDE1C* and *ATXN7L1* in a 14-year old developmentally delayed boy

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ARTICLE INFO

Article history:
Received 27 February 2013
Accepted 22 April 2013
Available online 9 May 2013

Keywords:
Developmental disorder
Inversion (7)(p14.3q22.3)
ATXN7L1
PDE1C
NHEJ
TTTAAA motif

ABSTRACT

We report a 14 year old male patient ascertained for developmental delay, carrying a *de novo* pericentric inversion on chr(7)(p14.3q22.3). Sequencing revealed that the breakpoints overlap a LTR sequence on 7q22.3 and a LINE on 7p14.3. A TTTAAA motif was found in proximity of the breakpoints on both arms. In addition the sequencing detected several small micro-rearrangements, deletion, duplication, insertion, at the breakpoints. No significant sequence identity exists between the 7p14.3 and 7q22.3 breakpoints. These features at the breakpoint junctions suggest that the inversion was triggered by the TTTAAA motif, LTR and LINE and healed by a Non Homologous End Joining (NHEJ) mechanism. The genes *ATXN7L1* and *PDE1C* are disrupted by the inversion. PDE1C is responsible for the hydrolysis of the second messenger molecules cAMP and cGMP and is highly expressed in the human heart and certain brain regions. In mice, *Pde1c* is expressed in migrating neuronal cells within the central nervous system during early embryo development. Although neuronal migration disorder was not seen in our patient, this is the first patient described with haploinsufficiency of *PDE1C* possibly causing developmental delay.

1. Clinical description

We describe a 14 years old male patient, born to a non-consanguineous, Caucasian couple. He was the youngest of three siblings. One more sibling died at 12 days of age due to complications from aortic stenosis and ventricular hypertrophy. The proband's mother also had a miscarriage at 12–13 weeks. The proband's father and eldest brother had dyslexia, but no learning difficulties.

The patient was born at 37 weeks by normal vaginal delivery after a pregnancy complicated by pre-eclampsia. Birth weight, length and occipito frontal circumference (OFC) were recorded as 3.2 kg (75th centile), 49 cm (50–75th centile) and 35.5 cm (97th centile) respectively, and APGAR scores were 8 and 9 after 1 and 5 min respectively. He was born with adducted feet. He started physiotherapy at 8 weeks, sat at 7–8 months, walked at 18 months and spoke a couple of words at 2 years of age.

At 2.5 years he was referred to a neurological examination because of language and motor delay. No focal neurological deficit was found, although developmental delay was evident. Electroencephalography (EEG) recording was normal. At this age he started speech therapy and special educational assistance including practice of sign language.

At the age of 5 years his weight was 18.7 kg (50th centile), his length was 109 cm (25th centile) and his OFC was 51.5 cm (10th centile). He had developed more vocabulary, but was difficult to understand leading the patient to prefer a sign language. Oral cavity malformations were excluded. At a new neurological examination the patient manifested hypotonic face, protruding tongue, drooling, breathing with open mouth, and stiffness in the right side of the body. Reflexes were normal. Shyness and quietness were also noted. A cerebral magnetic resonance imaging (MRI) showed two cystic lesions, one in the left periventricular region and one in the right temporal region in cranial direction from the choroid fissure. Both were interpreted as developmental anomalies without pathological significance. Additionally, he had strabismus and astigmatism.

At 7 years of age he started school. His pronunciation was still unclear and presented enuresis both day and night. Uroflowmetry test was normal. At age of 8 years evaluation with Wechsler Intelligence Scale for Children-III (WISC-III) showed general learning difficulties.

At 11.5 years, he showed abilities at the level of age 9 years (evaluated with WISC-IV). His reading and writing abilities were at a level of 8 years and in mathematics he scored at a level of 7–8 years, indicating mild intellectual disability with severe writing and

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http://dx.doi.org/10.1016/j.ejmg.2013.04.005

reading difficulties. At clinical examination the patient showed thick smooth hair, the hairline on the left side of neck was lower than on the right, well-formed arched eyebrows, freckles, full lips, facial hypotonia and drooling (Fig. 1). At the age of 12 years the patient was medicated with methylphenidate, with positive effect on diurnal enuresis, but no effect on his ability to concentrate.

2. Methods of detection and confirmation

G-Banding at standard resolution performed at the age of 11 years on leucocytes from peripheral blood detected an apparently balanced *de novo* pericentric inversion 46,XY,inv(7)(p13q22) (Fig. 2a). 244K Array comparative genomic hybridization (aCGH) (Agilent Technologies, Santa Clara, CA) was performed on DNA extracted from peripheral blood, according to manufacturer's instructions. No pathological copy number changes were detected. Test for Fragile X syndrome was negative.

The inversion breakpoints were mapped by fluorescent *in situ* hybridization (FISH) using BAC and fosmid clones as probes. The BACs RP11-28D14 (chr7:31,947,708–32,104,560 bp) and RP11-96B13 (chr7:105,168,713–105,320,846 bp) gave splitting signals, therefore identifying the breakpoints, which were further restricted to the fosmids G248P84835D10 (chr7:32,034,016–32,075,468 bp) and G248P800740E10 (chr7:105,252,001–105,288,792 bp) (Fig. 2b) on 7p14.3 and 7q22.3, respectively. All genomic positions refer to the human reference sequence (GRCh37, hg19), produced by the Genome Reference Consortium.

3. Sequencing and analysis of the inversion breakpoints

Primers were designed inside the splitting fosmid sequences using Primer 3 software[1], in order to PCR amplify the breakpoint junctions. PCR fragments were obtained by long range PCR using LA TAKARA Taq polymerase kit (TAKARA BIO INC., Japan) according to manufacturer's instructions. A PCR fragment of 3.5–4 kb, bridging the breakpoint on 7p, was obtained with primers, P7_primer 1 (5′-GCTGATGCTTTATTTCCCTGTC-3′ chr7: 32,055,024–32,055,045(+)) and P7 primer 2 (5′-CGACGATGACTTCCTAACATCC-3′ chr7: 105,270,805–105,270,826 (+)). Similarly, a ~8 kb fragment bridging the breakpoint on 7q was obtained with the primers Q7_primer1 (5′-AGATGGCAGACCAGAGGATG-3′ chr7: 32,065,932–32,065,951 (−)) and Q7_primer2 (5′-ACTTCTCCAGCCACATCCTG-3′ chr7: 105,271,677–105,271,696 (−)). PCR products were sequenced on the ABI 3730xl DNA Analyzer (Life Technologies Corporation, Carlsbad, CA). Sequences were aligned using BLAT (genome.ucsc.edu) and MEGA4 software[2]. The breakpoint sequence on 7p14.3 revealed a fusion between chr7:32,058,441 bp and chr7:105,270,938 bp, and at 7q22.3 the sequences fused were chr7:105,270,936 bp and chr7:32,058,444 bp (Fig. 3 and Supplementary file 1). The breakpoint at the 7p14.3 is located within a Long Interspersed Nuclear Elements (LINE) sequence, belonging to the L1 family, and the 7q22.3 breakpoint is located within a Long Terminal Repeat (LTR) sequence in the ERVL-MaLR family. The motif "TTTAAA" was detected less than 30bp from the 7p14.3 breakpoint (chr7:32,058,408–32,058,413 bp and chr7:32,058,408–32,058,413 bp) and also at the breakpoint on 7q22.3 (chr7: 105,270,905–105,270,910 bp and chr7: 105,270,956–105,270,961 bp) (Fig. 3 and Supplementary file 1). At the inv7p14.3 breakpoint junction, a 3 bp duplication (chr7:105,270,936–105,270,938 bp) was detected. At the inv7q22.3 breakpoint junction, a few micro-rearrangements were detected: a 2 bp deletion (chr7:32,058,442–32,058,443 bp), an 11 bp insertion (of unknown origin) (Fig. 3 and Supplementary file 1), and an additional 4 bp deletion was found at less than 300 bp from the 7q22.3 breakpoint (chr7:105,271,211–105,271,216 bp) (not shown). After accurate mapping of the breakpoint the karyotype could be revised to 46,XY,inv(7)(p14.3q22.3). No significant sequence identity can be found between the two repetitive elements at the breakpoints (Blast2 at ncbi.nlm.nih.gov).

4. Discussion

The patient carried a *de novo* chromosome 7 inversion likely causing his developmental delay. The breakpoints were located within a LINE and a LTR and lacked sequence identity. In addition, TTTAAA motifs and several micro rearrangements were found in proximity of the breakpoints. All these features are indicative of a NonHomologous End Joining (NHEJ) mechanism as the origin of the chromosome aberration. As pointed out by Nobile et al. [3] and Toffolati et al. [4], LINE, LTR, *Alu*, MIR and MER2 DNA elements are detected in a large fraction of breakpoints in non-recurrent rearrangements that were healed by NHEJ. The authors also point at the presence of TTTAAA motifs in vicinity of the breakpoint [3, 4]. TTTAAA is one of the DNA motifs known to induce double strand breaks or curving of DNA, which would predispose to recombination and other nuclear processes [4–6]. Thus the TTTAAA sequence present close to the inversion breakpoints in our patient may have

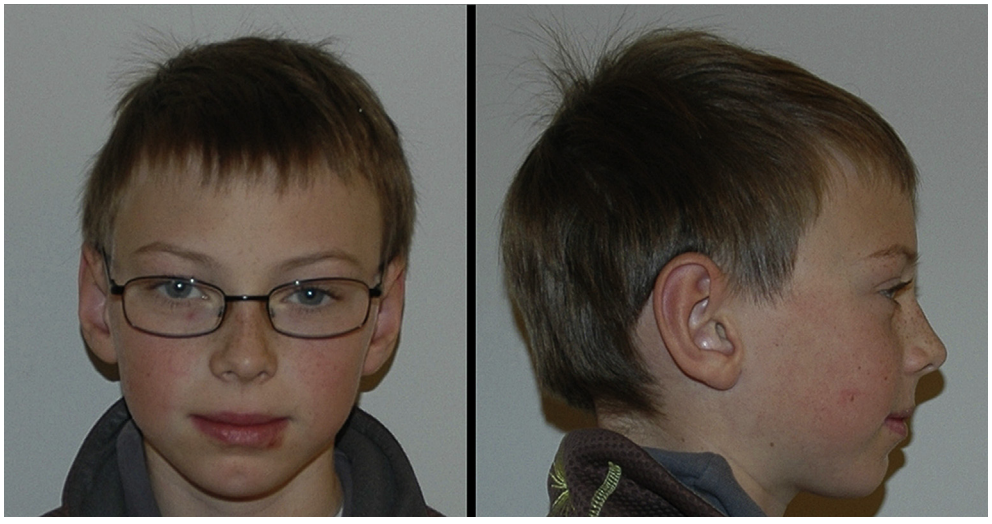

Fig. 1. Picture of the propositus at the age of 12.