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Methods paper

Phenotype and Micro-array characterization of duplication 11q22.1-q25 and review of the literature

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ABSTRACT

Partial duplication of 11q is related to several malformations like growth retardation, intellectual disability, hypoplasia of corpus callosum, short nose, palate defects, cardiac, urinary tract abnormalities and neural tube defects. We have studied the clinical and molecular characteristics of a patient with severe intellectual disabilities, dysmorphic features, congenital inguinal hernia and congenital cerebral malformation which is referred to as cytogenetic exploration. We have used FISH and array CGH analysis for a better understanding of the double chromosomic aberration involving a 7p microdeletion along with a partial duplication of 11q due to adjacent segregation of a paternal reciprocal translocation t(7;11)(p22;q21) revealed after banding analysis. The patient's karyotype formula was: 46,XY,der(7)t(7;11)(p22;q21)pat. FISH study confirmed these rearrangement and array CGH technique showed precisely the loss of at least 140 Kb on chromosome7p22.3pter and 33.4 Mb on chromosome11q22.1q25. Dysmorphic features, severe intellectual disability and brain malformations could result from the 11q22.1q25 trisomy. Our study provides an additional case for better understanding and delineating the partial duplication 11q.

1. Introduction

Partial duplication of the chromosome 11q was recognized as a known clinical entity and referred to as the duplication 11q21q23 syndrome (De Grouchy and Turleau, 1977; Francke et al., 1977). The majority of patients with partial trisomy 11q reported in the literature are due to the result of meiotic mal segregation of a parental translocation involving other chromosomes and are associated with partial monosomy of other chromosomes (Greig et al., 1985; Pihko et al., 1981). The most common translocation is the partial 11q and 22q trisomy syndrome. This is the result of the unbalanced product of translocation between 11q23 and 22q11 and it carriers a 3:1 tertiary trisomic malsegregation (Fraccaro et al., 1980; Iselius et al., 1983). Trisomy 11q has also been reported as an interstitial duplication (Delobel et al., 1998). The presence of clinical practice in the diagnosis of human chromosome abnormalities of patients with intellectual disability and congenital malformations benefited enormously from the advent of array CGH technology. This allows high resolution pan genomic analysis to detect interstitial and subtelomeric submicroscopic imbalances and to characterize their size at the molecular level and to define the breakpoints of translocation (Sanlaville et al., 2005). Here, we report that this technique was effective to refine the molecular cytogenetic characterization of two genomic imbalances in a 3-year-old boy: a 7p22.3 microdeletion and a partial duplication of the long arm of chromosome 11q22.1-q25. Partial duplication 11q is currently known to share several common clinical features, such as, severe intellectual disability, growth retardation, microcephaly, facial dysmorphism, epilepsy, cardiac, renal and cerebral malformations, and congenital inguinal hernia (Burnside et al., 2009; Delobel et al., 1998; Klaassens et al., 2006; Smeets et al., 1997; Zhao et al., 2003).

Congenital inguinal hernia (CIH) is a relatively common birth defect. Although little is known about its etiology, there are interesting

Abbreviations: FISH, fluorescence in situ hybridization array; CGH, array comparative genomic hybridization; Kb, kilo base; Mb, mega base; CIH, congenital inguinal hernia; PHA, phytohemagglutinin; ISCN 2009, International System for Human Cytogenetic Nomenclature (2009); BAC, bacterial artificial chromosome; dUTP, deoxyuridine triphosphate; DNA, deoxyribonucleic acid; OMIM, Online Mendelian Inheritance in Man; FAM20C, family with sequence similarity 20, member C; NCAM1, neural cell adhesion molecule 1; DRD2, dopamine receptor D2; THY-1, Thy-1 cell surface antigen; GRIK4, glutamate receptor, ionotropic, kainate 4; ROBO4, roundabout, axon guidance receptor, homolog 4 (*Drosophila*); CDON, cell adhesion associated, oncogene regulated; TNNT1, troponin T type 1; CASP1, caspase 1, apoptosis-related cysteine peptidase; IL1B, interleukin 1, beta; IL18, interleukin 18; IL33, interleukin 33; GRIA4, glutamate receptor, ionotropic, AMPA 4; GUCY1A2, guanylate cyclase 1, soluble, alpha 2; DRD2, dopamine receptor D2; NCAM1, neural cell adhesion molecule 1.

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evidences of its genetic cause. Both numerical and structural chromosomal abnormalities have been described in patients with CIH as the duplication 11q21q23 syndrome (Smeets et al., 1997; Yelavarthi and Zunich, 2004). As the phenotype repercussions of both rearrangements seem to be attributed to the duplication 11q, we thus performed a genotype-phenotype correlation analysis to ascertain the contribution of duplication in the clinical features of our case study.

2. Materials and methods

2.1. Patient

The patient is a male child born via a cesarean section after 40 weeks of uncomplicated pregnancy. The boy was the first child of healthy unrelated parents. At the time of diagnosis, the patient's mother had a second pregnancy. The details are as follows: birth weight 2450 g (3rd percentile), length 45 cm (10th percentile), head circumference 34 cm (30th percentile). The neonatal period was complicated by hypotonia, muscular hypertonia with a pyramidal syndrome, frequent respiratory, urinary tract infections and epilepsy (type syncope, with a brief loss of consciousness, controlled by the depakine). The boy show significant retardation in all his developmental features. The face showed dysmorphic features with microcephaly, hypertelorism, structural asymmetry of face, low set ears, beaked nose, up-slanting palpebral fissures, higher arched palate and micrognathia (Fig. 1 Supplementary Data). He had congenital inguinal hernia operated at the age of 11 months, hiatal hernia, congenital dislocation of the hip and a small penis. Magnetic resonance imaging showed agenesis of corpus callosum, atrophic cerebellum and simple cerebral gyration.

2.2. Cytogenetic analysis

Metaphase chromosome preparations were obtained from PHA stimulated lymphocyte culture according to standard procedures. Chromosomal analysis was carried out by applying R-banding at a 500 band level according to ISCN 2009 (Shaffer et al., 2009) in the patient, parents and fetus.

2.3. Fluorescence in situ hybridization

The presence of derivative chromosomes of the translocation (7;11) was assessed using commercial probes (TelVysion 11p/11q, Vysis®) and four specific probes of the short arm of chromosome 7 obtained from BACs: RP11-420P20 (40,248,195-40,427,632), RP11-611P20 (18,088,141-18,132,520), RP11-42B7 (4,126,462-4,281,305) and RP11-713A20 (106,471-298,664) were generously provided by the genomic public bank, Bari (http://www.biologia.uniba.it/rmc/) selected from the location 7p14.1, 7p21.1, 7p22.2 and 7p22.3 regions, respectively. The clone probes were labeled with tetramethylrhodamine-6-dUTP and fluorescein-12-dUTP (Abott-Vysis®, Downers Grove, IL, USA), respectively, using nick translation kit (Abott-Vysis®, Downers Grove, IL, USA). FISH was performed on blood lymphocytes blocked at metaphases according to the standard protocol.

The hybridized chromosomal spreads were analyzed using a fluorescent microscope equipped with appropriate filters and cytovision FISH system image capture software (Zeiss Axioskop 2 plus). Slides were scored by the number of probe signals for each metaphase. For each target area, ten hybridized metaphase were analyzed.

2.4. Comparative genomic hybridization

180000 Agilent Technologies® oligonucleotides array were used according to the manufacturer's instructions « Human Genome 180 K OligoMicroarray ». The patient's DNA and the reference DNA were digested with RsaI and AluI. Digested DNA produced were labeled by random priming with Al5-dUTP or Al3-dUTP. After Column-purification, the probes were denaturized and pre-annealed with 50 μg of human Cot-1 DNA (Invitrogen®, Calif., USA). The hybridization was performed at 65 °C for 24 h. After washing, the microarray was then scanned by the Agilent Microarray Scanner. Data analysis was performed by Agilent Feature Extraction® 9.1 software. Interpretations of results were carried out with CGH analytics® 4.5 Software with the following parameters: z-score threshold: 2.0, window: 0.5 Mb. If 3 contiguous oligonucleotides presented an abnormal $\log^2$ ratio ($>+0.5$ or <-0.5) then a copy number variation was determined. The results was compared to the data recorded in the database of genomic variants. Web resources included the Database of Genomic Variants: (http://projects.tcag.ca/variation/), OMIM: (http://www.ncbi.nlm.nih.gov/sites/entrez), DECIPHER$^{v5.0}$ database: (http://decipher.sanger.ac.uk/) and Ensembl genome browser 59 (http://www.ensembl.org/homo_sapiens/location/).

3. Results

Initially conventional cytogenetic analysis showed a patient's karyotype 46,XY,add(7)(?::p22→qter), the cytogenetic analysis of the father revealed a balanced translocation apparently de novo between the short arm of chromosome 7 and the long arm of chromosome 11 (Fig. 1A). The mother showed a normal karyotype. Therefore the karyotype of the boy was 46,XY,der(7)t(7; 11)(p22; q21)pat as a result of an adjacent disjunction of paternal reciprocal translocation 46,XY,t(7; 11)(p22; q21). He was monosomic for the segment 7p22→7pter and trisomic for the segment 11q21→11qter. A rapid prenatal karyotype was performed on the amniotic fluid and this resulted in a balanced translocation in the karyotype of the fetus, probably without phenotypic repercussions after birth.

FISH performed on metaphasic lymphocytes of peripheral venous blood (father's) using subtelomeric probes (Vysis) of chromosome 11q, showed hybridization to the derivative chromosome 7 and to the father's normal chromosome 11 (Fig. 1C1), confirming the reciprocal translocation t(7;11)(p22;q21), Another series of probes mapping on chromosome 7 were used to characterize this rearrangement. The 7p breakpoint was localized in the distal terminal region below the subtelomeric region (Fig. 1C2) because the BAC clones RP11-420P20, RP11-611P20 and RP11-42B7 were not translocated to the derivative chromosome 11(Fig. 1C3, C4).

Array-CGH exploration of the patient DNA revealed a small loss of 140 Kb on the short arm of chromosome 7 and a large duplication of 33.4 Mb on the long arm of chromosome 11. The chromosome breakpoints were precisely localized by array CGH on 11q22.1 [arr 11q22.1q25 (100,505,885-133,951,370)×3 pat] and 7p22.3 [arr 7p22.3 (149,268-289,592)×1 pat] (Fig. 2)

4. Discussion

Partial duplication 11q is well described in the literature which explains the occurrence of intellectual disability, dysmorphism and severe congenital malformations especially in “duplication 11q21-23.3 syndrome” (Burnside et al., 2009; Delobel et al., 1998; Klaassens et al., 2006; Zhao et al., 2003). We describe a proband with an inherited unbalanced translocation of chromosome 11 and 7, although the proband is concordant for most of the features and share a phenotype that is consistent with partial “trisomy 11q syndrome”. In comparison with a series of seven partial duplication 11q patients (Table 1) previously reported (Burnside et al., 2009; Delobel et al., 1998; Forsythe et al., 1988; Klaassens et al., 2006; Pfeiffer and Schutz, 1993; Smeets et al., 1997; Zhao et al., 2003), To our knowledge, our patient had the second largest distal duplication after the duplication published by Zhao et al. (2003) with similar dysmorphic features and the absence of upper airway obstruction associated with the large duplication distal to 11q21 (Fig. 3; R1). Chromosomal rearrangements including the long arm of chromosome 11q can be divided into four groups: (1) partial trisomy 11q and a partial monosomy of another chromosome; (2) double