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Increased *FGF3* and *FGF4* gene dosage is a risk factor for craniosynostosis

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ABSTRACT

Interstitial duplications involving chromosome 11q have rarely been reported in the literature and mainly represent large, cytogenetically detectable rearrangements associated with a wide and variable spectrum of neurodevelopmental disorders. We report on a patient affected by intellectual disability, craniosynostosis, and microcephaly. Array-CGH analysis identified a de novo 290 kb interstitial duplication of chromosome 11q13.3 including the *FGF3* and *FGF4* genes. Clinical comparison of our patient with those previously reported with overlapping 11q duplications allows us to define the minimal duplicated region associated with craniosynostosis and strongly supports the hypothesis that the constitutional increased dosage of the *FGF3* and *FGF4* genes is a risk factor for craniosynostosis in humans.

1. Introduction

Interstitial duplications involving chromosome 11q have rarely been reported in the literature and mainly represented large, cytogenetically detectable rearrangements associated with a wide and variable spectrum of neurodevelopmental disorders (Zarate et al., 2007).

Jehee et al. (2007) described the first case of a mosaic 46,XY, dup(11)(q11–q13.3)(29)/46,XY(6) duplication in a patient with multiple craniosynostoses, congenital heart defect and developmental delay. In the same study, they also refined by FISH analysis the extension of the rearrangement in a previously reported patient carrying a 11q13.5–q21 duplication, and identified an overlapping region of about 1.2 Mb including the *Fibroblast Growth Factor 3* (*FGF3*) and *Fibroblast Growth Factor 4* (*FGF4*) genes that they suggested as candidates for craniosynostosis.

More recently, DNA microarray techniques have permitted the identification of shorter rearrangements associated with specific clinical features, allowing a better genotype–phenotype correlation.

We report on a patient affected by intellectual disability (ID), craniosynostosis and microcephaly harboring a de novo 290 kb interstitial duplication of chromosome 11q13.3 including the *FGF3* and *FGF4* genes. Clinical comparison with previously reported patients carrying overlapping duplications allows us to define the minimal duplicated region associated with craniosynostosis. Gain of function mutations in the FGF receptor coding genes, such as the *Fibroblast Growth Factor Receptor 1* (*FGFR1*), *Fibroblast Growth Factor Receptor 2* (*FGFR2*) and *Fibroblast Growth Factor Receptor 3* (*FGFR3*) genes, have already been associated with craniosynostosis. In the light of these considerations, we strongly support the hypothesis that the duplication of the *FGF3* and *FGF4* genes is a risk factor for craniosynostosis.

2. Clinical report

The patient is an 8-year-old Italian male, born to non-consanguineous parents. Family history is negative for ID. He was born by spontaneous premature delivery after 27 weeks and 4 days of pregnancy characterized by threatened abortion. Apgar scores were 4 and 7, at 1 and 5 min, respectively. At birth, he showed a depressed cardiorespiratory activity, requiring immediate resuscitation by Intermittent Positive Pressure Ventilation and orotracheal intubation. He was then admitted to the local neonatal intensive care unit (NICU). The clinical evaluation showed normal values of birth weight, length and occipito-frontal circumference (OFC) for gestational age (1070 g, 35 and 24 cm, respectively), generalized cyanosis, overlapping cranial sutures, and an anterior fontanelle of normal tension, spanning 0.5 cm both in width and length. On admittance in the NICU, he was mechanically ventilated according to the

Abbreviations: CT, cranial tomography; MRI, magnetic resonance imaging; g, grams; cm, centimeters; kg, kilograms; <, below; 3D, three dimensional; EEG, electroencephalogram; ECG, electrocardiogram; EMG, electromyography; CGH, comparative genomic hybridization; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; BAC, bacterial artificial chromosome; kb, kilobases; bp, base pairs; dup, duplication; q, long arm of a chromosome; OMIM, Online Mendelian Inheritance in Man.

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High Frequency Oscillatory Ventilation procedure for 36 h. Subsequently, he was shifted toward the Synchronized Intermittent Mechanical Ventilation procedure up to the 16th day of postnatal life, and eventually to Continuous Positive Airways Pressure for the subsequent six days. The umbilical catheter, placed in the delivery room, was replaced a week afterwards by a Central Venous Catheter, allowing total parenteral feeding. A blood product transfusion was administered for septic shock and disseminated intravascular coagulation during the first week. Three brain ultrasounds were performed at 16, 70, and 85 days of postnatal life, respectively, without any clear demonstration of brain damage. No brain CT scan or MRI was performed at that time. On discharge from the hospital, at the age of five months and 14 days, weight was 2760 g, length 48.5 cm, and OFC 34.5 cm, all below the 3rd centile; anterior fontanelle was 2 × 2 cm in size. Developmental milestones were delayed: first steps at 20 months and first words at 21 months. He has been clinically evaluated by us for the first time at age 25 months. Psychomotor evaluation, with Griffiths Mental Development Scales and Vineland Adaptive Behavior Scale, revealed Not Otherwise Specified ID. His weight was 12.3 kg (10th–25th centile), his height 83 cm, and he was microcephalic (45.1 cm, <2nd centile). Clinical phenotype showed generalized hypotonia, plagiocephaly, prominent metopic suture, coarse face, thick lips, anteverted nostrils, long philtrum, four fingers crease, and low-set ears. Subsequent check-ups at age $3^{7/12}$ and $8^{5/12}$ years, confirmed the phenotype, showing at last observation weight on 90th centile (31 kg), height on 75th centile (132 cm), and microcephaly (OFC = 49 cm, <2nd centile). Last psychometric evaluation with Wechsler Infantile Scale for Children-III, Leiter International Performance Scale-Revised, Autism Diagnostic Interview-Revised, and Autism Diagnostic Observation Schedule, gave the diagnosis of mild ID and Not Otherwise Specified Pervasive Developmental Disorder. Brain MRI, performed at age $3^{7/12}$ years, showed hypoplasia of the corpus callosum and cerebellar vermis, without any sign of perinatal brain damage. Skull 3D CT scan, performed at age $8^{5/12}$ years, showed closure of all cranial sutures (Fig. 1). Last odontoiatric evaluation showed a mixed dentition, a narrow palate, and nothing relevant on dental occlusion or teeth. Eye examination showed myopic astigmatism. Routine blood tests, EEG, hearing evaluation, ECG, and EMG were normal. Clinical and molecular data of this patient have been added to the Decipher database (patient code: 274615).

3. Materials and methods

3.1. Patient's sample

Informed consents were obtained from the parents of the propositus. Genomic DNA from the propositus, his parents and controls was isolated from peripheral blood according to standard procedures.

3.2. Genome wide aCGH analyses

Array-CGH analysis was performed using the Human Genome CGH Microarray 400K (Agilent Technologies, Palo Alto, CA, USA) which consists of 400,000 60-mer oligonucleotide probes that span both coding and non-coding sequences with an overall median probe spatial resolution of 1 kb. Normal Human male DNA (Agilent Technologies) was used as the reference. The procedures were performed according to the manufacturer's instructions.

3.3. Multiple-ligation-probe amplification (MLPA)

A region specific multiplex ligation-dependent amplification assay was designed and performed in the propositus, his parents and in a group of ten normal individuals (reagents and enzyme were from MRC-Holland, Amsterdam, Netherlands). Amplification products were identified and quantified by capillary electrophoresis on an ABI 3130 Genetic Analyser (Applied Biosystems, Foster City, CA) and the accompanying software. The tracing data were then normalized by dividing each probe's peak area by the average area of all peaks of the sample and then dividing this value by the average normalized peak's area of the corresponding locus of all the samples.

3.4. Paternity testing

Segregation pattern was performed in the proband and his parents using the AmpFlSTR Profiler Plus Kit Reagents (Applied Biosystems). The PCR products were electrophoresed on ABI 310 Genetic Analyser (Applied Biosystems) and analyzed by Genescan software.

3.5. Fluorescent in situ hybridization (FISH)

Fluorescent in situ hybridization (FISH) experiment was performed on metaphase chromosome by standard techniques using BAC insert clone RP11-266K14 mapping inside the duplicated region and RP13-317D12 as control clone for the 11p chromosome (BlueGnome, Technogenetics).

3.6. Web resources

The URLs for data present herein are as follows:

Online Mendelian Inheritance in Man (OMIM):http://www.ncbi.nlm.nih.gov/Omim
UCSC Genome Browser: http://genome.ucsc.edu/cgi-bin/hgGateway
Database of Genome Variants: http://projects.tcag.ca/variation
DECIPHER: http://decipher.sanger.ac.uk
e-Array: http://earray.chem.agilent.com/earray/
MLPA: http://www.mrc-holland.com/WebForms/.

4. Results

According to human reference sequence (NCBI Build GRCh37) array-CGH analysis revealed a duplication of about 290 kb in chromosome 11q13.3 (centromeric breakpoint between 69384046 and 69395184 bp, telomeric breakpoint between 69685539 and 69824968 bp) (Fig. 2). Further MLPA analysis confirmed the duplication in our proband but not in his parents, indicating a de novo event while paternity testing confirmed that father and mother were the biological parents.

The FISH result was compatible with a tandem duplication since the RP11-266K14 BAC probe hybridized to 11q chromosomes only, although no intensity difference was observed between FISH signals.

5. Discussion

We report on a patient with mild ID, postnatal microcephaly, plagiocephaly due to craniosynostosis, muscular hypotonia, myopic astigmatism, corpus callosum and cerebellar vermis hypoplasia.

Array-CGH analysis in our patient revealed a de novo 290 kb interstitial duplication of 11q13.3 encompassing the *CCND1*, *ORAOV1*, *FGF19*, *FGF3* and *FGF4* genes.

The previously reported 11q duplicated patients carried a larger rearrangement: Legius et al. (1996) described a de novo dup11q13.3–14.2 and Zhao et al. (2003) described a de novo dup11q13–q25; Jehee et al. (2007) reported the first case of a mosaic duplication 46,XY, dup(11)(q11–q13.3)(29)/46,XY (6) and refined by FISH analysis the extension of the dup11q13.5–q21 described by Yelavarthi & Zunich (2004).

Recently Ziebart et al. (2013) have described a familial 11q13.3 microduplication including the *FGF3* and *FGF4* genes.

As shown in Table 1, abnormal facial features and head shapes are frequently present in patients carrying 11q overlapping duplications.