Clinical Report

An 11q11–q13.3 Duplication, Including FGF3 and FGF4 Genes, in a Patient With Syndromic Multiple Craniosynostoses

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Interstitial duplications of 11q are very rare and seldom reported. In this paper we describe the first case of a duplication involving bands 11q11 and 11q12. This newly described patient has multiple craniosynostoses, congenital heart defect and developmental delay, and is a carrier of a mosaic duplication: 46,XY,dup(11)(q11→q13.3)(29)/46,XY(6). The breakpoints were further delimited by comparative genomic hybridization microarray. We also performed fluorescent in situ hybridization analysis to determine the extension of the duplication in a patient described earlier with a duplication 11q13.5–q21. An overlapping region of less than 1.2 Mb was identified and included the duplication of genes FGF3 and FGF4 in both individuals. We discuss the possible implications of dosage effects of these genes in the onset of craniosynostosis.

Key words: fibroblast growth factors; dosage effects; cytogenetic analysis; molecular analysis; FISH; array-CGH; duplication 11q; craniosynostosis; chromosomal abnormalities; FGF3; FGF4


INTRODUCTION

Chromosomal duplications of the long arm of chromosome 11 are more commonly involved in reciprocal translocations leading to the partial trisomy of the terminal bands 11q23–q24 [Takano et al., 1993; Smeets et al., 1997; Hou, 2004]. Interstitial duplications of 11q are apparently very rare and have been reported in a few cases [reviewed by Zarate et al., 2007]. None of the patients reported have duplications extending to bands 11q11–q12.

Herein we describe and characterize with molecular techniques a new patient with an interstitial 11q11–q13.3 duplication present in 82% of analyzed cells. The patient's main clinical features, besides developmental delay, are complex craniosynostoses and congenital heart defects. We also compared the clinical phenotype of our patient with the data of the only three previously reported individuals with overlapping segments. To identify potential candidate genes for the phenotype associated with this duplication, we analyzed by molecular cytogenetic techniques the breakpoints of one of the previously reported patients [Yelavarthi and Zunich, 2004].

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CLINICAL REPORT

Patient 1 (Present Case)

This 6-year-old boy (Fig. 1) was the first child of a healthy nonconsanguineous couple. In a second pregnancy, a phenotypically normal female fetus died in utero due to umbilical cord entanglement. The propositus was born after 39 weeks of gestation by Cesarean section because of breech presentation. Pregnancy had been uneventful. Birth weight was 3,580 g and length was 45 cm. Apgar scores were 9 and 8 at 1 and 5 min, respectively. He developed respiratory distress, and an echocardiogram disclosed a patent ductus arteriosus and patent foramen ovale. At age 6 months he had bacterial meningitis and was hospitalized; on this occasion he suffered two seizure episodes. Another nonfebrile seizure occurred at the age of 4 years. He had recurrent pneumonia and middle ear infections, requiring tympanostomy tube placement. Corrective surgery for inguinal and umbilical hernias was performed. A three-dimensional CT scan at 5 years showed multiple craniosynostoses involving the sagittal, metopic, lambdoid, temporal, and squamosal sutures. The coronal suture was patent and no brain anomalies were documented. Physical examination at 5 years, 11 months showed a weight of 21 kg (50th centile), height of 100 cm (<3rd centile), and OFC of 48 cm (<2nd centile). He also had marked trigonocephalic, turricephalic and scaphocephalic-shaped skull. His neck was short. A right preauricular pit was observed. An audiological evaluation performed at 6 years showed normal results. Blue sclerae, nystagmus, and strabismus were noted. He had a short philtrum and a supernumerary maxillary lateral incisor. Mild brachydactyly of the fingers and clinodactyly of the fifth fingers were present. His thumbs were slightly broad, the distal phalanges were shortened, and the nails were hypoplastic. He was mentally retarded, happy, and friendly able to understand commands and speak short sentences.

Patient 2

This patient is fully described elsewhere [Yelavarthi and Zunich, 2004]. Clinical features included, among others, sparse hair, trigonocephaly, frontal bossing, prominent occipital area, triangular face with pointed chin, arched eyebrows, broad nasal root, protruding ears, high arched palate, bithoracic narrowing, mid-thoracic spinal curvature, broad thumbs and fingers, and bilateral transverse creases. A MRI disclosed no evidence of craniosynostosis; however, skull radiographs or CT-scans were not available for proper confirmation of suture closure. He had a maternally inherited duplication, defined as 46,XY;dup(11)(q13.5→q21).ish dup(11)(q13.5→q21)(wcp11+mat). His mother was not available for clinical evaluation.

CYTOGENETIC AND MOLECULAR ANALYSIS

Chromosomal analyses were done in peripheral blood lymphocytes. Conventional ~550 G-banded analysis was performed on the patient and his parents. Fluorescent in situ hybridization (FISH) experiments were performed by standard techniques using a library of whole chromosome 11 and large BAC insert clones (RP11-804L21, RP11-30016, and RP11-554A11). Array-CGH (comparative genomic hybridization microarray) was carried out with slides containing 3,527 clones spaced at an average distance 1 Mb over the full genome as described elsewhere [Fiegler et al., 2003; Vermeesch et al., 2005].
RESULTS

Patient 1

Chromosomal analysis showed mosaicism for a partial duplication of the long arm of chromosome 11 initially described as 46,XY,dup(11)(q12→q13)(29)/46,XY(6) (Fig. 2A). Parental chromosomes were normal. FISH studies using a library of whole chromosome 11 uniformly painted the two chromosomes 11 showing that the extra segment was indeed from chromosome 11 (data not shown). Array-CGH analysis showed that this duplication comprised 20 clones spanning at maximum 15 Mb. The proximal breakpoint lays between clones RP11-10E21 (non-duplicated) and RP11-131J4 (duplicated) at 11q11, and the distal breakpoint lays between clones RP11-804L21 (duplicated) and clone RP11-21D20 (non-duplicated) at 11q13-3, as shown in Figure 2B–D. FISH analyses, using the clone RP11-804L21, disclosed the presence of three copies of this sequence in Patient 1's metaphases, two of them located on the duplicated chromosome 11 and one mapped in the normal homolog of the pair 11. Fluorescent signals of apparently equal intensity support the array-CGH data, which indicate that the sequence of this clone is duplicated but not disrupted by the rearrangement. His final karyotype was defined as 46,XY,dup(11)(q11→q13.3)(29)/46,XY(6).

Patient 2

New FISH analysis was carried out for clones RP11-300I6 and RP11-554A11 that map inside the duplicated segment of Patient 1. Duplication of the sequence contained in the clone RP11-300I6 was clearly detected (Fig. 3) but results for the proximal clone RP11-554A11 were nonconclusive. This indicates that Patient 2's duplication extends at least to band 11q13.3, overlapping with Patient 1's duplication over a region of less than 1.2 Mb as shown in Figure 4.

DISCUSSION

We report a de novo mosaic interstitial 11q duplication in a patient with developmental delay and dysmorphic features. This duplication encompasses less than 15 Mb from 11q11 to q13.3 and is present in 82% of the analyzed metaphases. Our patient is the first to be reported with a chromosome 11 duplication involving bands 11q11 and 11q12. It has been speculated that the paucity of reported partial trisomies of these bands is due to lethality [Brewer et al., 1999]. The duplicated segment in our

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Fig. 2. A. Partial karyotype depicting on the left the duplicated chromosome 11 and on the right the normal pair. B. Duplication at 11q11→q13.3 demonstrated by array-CGH. C. Segmental ideogram of chromosome 11 showing the duplicated segment and respective duplicated clones RP11-131J4 and RP11-804L21 flanking the duplication breakpoints. D. Further enlargement of the distal breakpoint showing the exact position of genes *FGF3* and *FGF4*. 

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patient is a gene-rich region in which approximately 520 genes are mapped and the viability of this abnormality could be due to its mosaic constitution.

Patient 1 represents the fourth case of an interstitial duplication involving band 11q13. The affected segments in the other three cases were 11q13.3–q14.2 [Legius et al., 1996], 11q13–q25 [Zhao et al., 2003] and 11q13.5–q21 [Yelavarthi and Zunich, 2004]. Table I reviews the main clinical features of all patients with duplications extending to band 11q13. With the exception of the case reported by Legius et al. [1996], all patients had abnormal facial features and abnormal head shapes.

The breakpoints of the previously reported cases were originally determined by conventional cytogenetics. We had the opportunity to further delimit the extension of the duplication of the patient described by Yelavarthi and Zunich [2004] (Patient 2). FISH analysis showed that he had two copies of clone RP11-300I6 and that his duplication contained band 11q13.3. This enabled us to determine that the overlapping duplicated region between Patients 1 and 2 was less than 1.2 Mb in which ten known genes are mapped (Fig. 4). Candidate genes for cranial suture development include fibroblast growth factors FGF3, FGF4, and FGF19. FGF3 is proximally mapped in the last duplicated clone of Patient 1 (RP11-804L21, at 11q13.3). FISH analyses showed three sets of fluorescent signals with similar intensity, confirming the duplication of the clone RP11-804L21 and indicating that FGF3 is probably fully duplicated in Patient 1 (Fig. 3).

FGF19 encodes a ligand exclusive to FGFR4, whose function is not well known to date. This receptor is only expressed in muscle and was never related to any craniofacial malformation [Xie et al., 1999]. On the other hand, gain-of-function mutations in the fibroblast growth factor receptors 1, 2, and 3 (FGFR1, FGFR2, and FGFR3) induce, among others, constitutive receptor activation (craniosynostosis syndromes) or receptor-ligand enhancement in craniosynostosis syndromes in the linker region between IgII and IgIII (Pfeiffer syndrome on FGFR1, Apert syndrome on FGFR2, and Muenke syndrome on FGFR3) [Neilson and Friesel, 1995; Anderson et al., 1998; Cohen, 2004; Ibrahimi et al., 2004]. The
increased expression of FGFRs ligands, such as FGF2, FGF3, and FGF4, has also been reported in craniosynostotic animal models. Carlton et al. [1998] described the upregulation of Fgf3 and Fgf4 in mice with craniofacial abnormalities similar to some human craniosynostosis syndromes. Craniosynostosis was also induced by the administration of FGF4 near the developing coronal suture in normal mouse embryos [Mathijssen et al., 2000].

The duplication of FGF3 and/or FGF4 in our patients could, theoretically, mimic the gain-of-function mechanism derived by the craniosynostotic mutations in FGFRs and be the cause of the abnormal head shape in these individuals. We could also hypothesize that the increased doses of FGFRs ligands act as a predisposition factor, that in conjunction with the genetic background of each patient, including the other duplicated genes, will determine the presence and severity of the cranial malformation. In the duplicated segment of Patient 1, for instance, two other genes seem to be of relevance to osseous malformations: TCIRG1, mutated in autosomal recessive osteopetrosis and LRP5, related to bone diseases,
such as osteoporosis-pseudoglioma syndrome and autosomal dominant osteosclerosis, recently associated with craniosynostosis [Kwee et al., 2005].

At present, we cannot affirm that the increased doses of FGF3 and FGF4 are responsible for the craniosynostosis in our patient nor can we prove that Patient 2 had trigonocephaly due to craniosynostosis. However, these are the first reports of overdoses of these ligands in humans and it is intriguing that these two patients share an abnormal head shape. It is also of note that the patient described by Zhao et al. [2003] with a duplication of 11q13–q25 also shares a phenotype of abnormal head shape. On the other hand, Legius et al. [1996] suggest that FGF4 is not included in their patient’s duplication due to the lack of craniofacial and hand abnormalities. We hope that reporting other patients with such duplications will help elucidate the effect of the constitutional increased doses of FGFs in humans.

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**REFERENCES**


