Craniosynostosis Associated With Ocular and Distal Limb Defects Is Very Likely Caused by Mutations in a Gene Different From FGFR, TWIST, and MSX2


1Centro de Estudo do Genoma Humano, Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil
2Disciplina de Genética, Escola Paulista de Medicina, São Paulo, Brazil
3Hospital do Servidor Público Estadual de São Paulo, São Paulo, Brazil
4Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Craniosynostosis caused by genetic factors includes a heterogeneous group of over 100 syndromes, most with autosomal dominant inheritance. Mutations in five genes (FGFR1, -2, -3, TWIST, and MSX2) causing craniosynostosis as the main clinical feature were described. In most of these conditions, there are also limb malformations. We report a two-generation kindred segregating microcornea, optic nerve alterations and cataract since childhood, craniosynostosis, and distal limb alterations, with a great clinical intrafamilial variability. The ophthalmological problems here described seem to be unique to this genealogy while similar feet alterations were apparently only described in two other affected siblings with acro-cranial-facial dysostosis syndrome (ADS). However, ADS has an autosomal recessive inheritance instead of the dominant pattern of the present genealogy. The candidate exons of the five genes previously mentioned were tested through sequencing analysis presenting normal results in all cases. Therefore, clinical and laboratory analyses in our patients suggest that their phenotype represents a new syndrome very likely caused by mutation in a gene different from those studied.

KEY WORDS: craniosynostosis; autosomal dominant inheritance; FGFR; TWIST; MSX2; ophthalmological problems; distal limb malformations

INTRODUCTION

Craniosynostosis represents a heterogeneous group of disorders, which by definition involve premature fusion of the cranial sutures. Many of these conditions are genetically determined and family studies have been an important element in the emergence of clinically distinct craniosynostosis syndromes [Winter and Baraitser, 1996; Cohen and Maclean, 2000]. Among the clinically best-characterized autosomal dominant syndromic craniosynostoses are Crouzon, Jackson-Weiss, Pfeiffer, Apert, and Saethre-Chotzen. Except for Crouzon syndrome, all the others have also limb alterations besides craniofacial abnormalities, suggesting that aspects of craniofacial and limb development utilize common molecular pathways [Wilkie, 1997]. Recently, the molecular basis for these syndromes has been partly elucidated. Dominant gain of function mutations in exons related to extracellular domain of the fibroblast growth factor receptor genes 1, 2, and 3 (FGFR1, -2, and -3) can be associated with most of these syndromes [Webster and Donoghue, 1997; Wilkie, 1997; Burke et al., 1998; Paznekas et al., 1998; Passos-Bueno et al., 1999; Kan
et al., 2002]. In addition, Saethre-Chotzen phenotype can also be caused mainly by null mutations or deletions in the TWIST gene [El Ghouzzi et al., 1997; Howard et al., 1997; Paznekas et al., 1998]. A fifth gene, the homebox MSX2, has been associated with one particular subtype of craniosynostosis, the Boston type [Jabs et al., 1993]; interestingly, null or deletion mutations in this gene lead to parietal foramina [Wilkie et al., 2000].

A common clinical feature associated with all these craniosynostotic syndromes is the involvement of coronal suture. In the present report, we describe the clinical and molecular findings of three individuals from a two-suture. In the present report, we describe the clinical and molecular findings of three individuals from a two-generation kindred segregating an apparently novel type of syndrome with ocular and limb defects associated with craniosynostosis. Analysis of the candidate exons for the FGFR1–3, TWIST, and MSX2 genes, which are primarily associated with craniosynostosis, showed no alteration. These results further suggest that the phenotype in this family is very likely caused by mutations in another gene.

**CLINICAL REPORT**

**Patient 1**

This boy was born at term after an eventful pregnancy. He is the second child of unrelated Brazilian Caucasian parents. Birth weight was 3,200 g (below the 50th centile), length 52 cm (at 50th centile), head circumference 35 cm (at 50th centile). Craniofacial and lower limbs abnormalities were noted at birth. Development was delayed; he walked at 2 and had his first words at 4 years of age.

On referral for diagnostic evaluation at age 4 years, this boy was 102 cm tall, weighed 18 kg, and had a head circumference of 53 cm. He had macrocephaly, dolichocephaly and prominent forehead, sparse and slow-growing hair, short palpebral fissures, long eyelashes, shallow orbits, blue sclera and ptosis of the eyelids (particularly at the right side), beaked nose, and pectus carinatum (Fig. 1A–C). Feet X-ray showed metatarsus adductus, severe hypoplasia of first metatarsals and of the distal phalanges, and fingerlike halluces (Fig. 1D,E). Hands were normal (not shown).

Ophthalmological examination showed amblyopia (OD 20/200; OS finger count, best corrected), congenital cataract, bilateral pseudophacica (surgery performed at 11 years old), microcornea (.9.0 mm horizontal and vertical AO), optic disk C/D 7/10 OD and 4/10 OS, median intraocular pressure 16 mm Hg, convergent strabismus; axial length and interpupillary distance were not obtained.

**Patient 2**

Patient 2, the oldest brother of the propositus, was born at term after an uneventful pregnancy. Birth weight was 3,070 g (between 25th and 50th centiles), birth weight was 3,070 g (between 25th and 50th centiles), length 53 cm (at 50th centile), head circumference 35 cm (at 50th centile). Craniofacial and lower limbs abnormalities were noted at birth. Development was delayed; he walked at 2 and had his first words at 4 years of age.

On referral for diagnostic evaluation at age 4 years, this boy was 102 cm tall, weighed 18 kg, and had a head circumference of 53 cm. He had macrocephaly, dolichocephaly and prominent forehead, sparse and slow-growing hair, short palpebral fissures, long eyelashes, shallow orbits, blue sclera and ptosis of the eyelids (particularly at the right side), beaked nose, and pectus carinatum (Fig. 1A–C). Feet X-ray showed metatarsus adductus, severe hypoplasia of first metatarsals and of the distal phalanges, and fingerlike halluces (Fig. 1D,E). Hands were normal (not shown).

Ophthalmological examination showed amblyopia (OD 20/200; OS finger count, best corrected), congenital cataract, bilateral pseudophacica (surgery performed at 18 years old), microcornea (.9.0 mm horizontal and vertical AO), asymmetric axial myopia (OD 23.50 mm, OS 27.00 mm), asymmetric axial myopia (OD 23.50 mm, OS 27.00 mm), optic disk C/D 9/10 OD and 10/10 OS, median intraocular pressure above 24 mm Hg, interpupillary distance 56.5 mm (below the 50th centile), convergent strabismus, and horizontal pendular nystagmus.

**DNA Extraction**

Blood samples were drawn from the mother and all three patients following informed consent and DNA was extracted according to standard methods [Miller et al., 1988].

**PCR**

PCR primer sequences (available under request) were designed to amplify exons 5, 6, 7, 8, and 9 of FGFR1. A typical 25 µl PCR reaction consisted of 100 ng genomic DNA, 20 mM tris-HCL (pH 8.4), 50 mM KCL, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1µM of each primer, and 1U Taq DNA polymerase (Life Technologies). The cycling conditions were denaturation at 94°C for 40 sec, annealing temperature, suited for each primer pair, for 40 sec, and extension at 72°C for 30 sec for 35 cycles.

The primers and conditions used for FGFR2, FGFR3, TWIST, and MSX2 amplifications were the same as previously described [Jabs et al., 1993; Rutland et al., 1995; Wilkie et al., 1995; Bellus et al., 1996; Howard et al., 1997].

Craniosynostosis 201

54 cm. He showed dolichocephalic head shape, low-set ears, ptosis of the eyelids, shallow orbits, beaked nose, and pectus carinatum (Fig. 2A–C). Both feet showed oligodactylly, agenesia of both halluces, and large second toes (Fig. 2D,E). Hands were normal (not shown). A G-banded karyotype (500–800 bands) was normal, 46,XY.
Fig. 1. Patient 1. Face (A,B) and skull X-ray (C) showing dolichocephalic head shape, prominent forehead, sparse hair, small palpebral fissures, shallow orbits, blue sclera, ptosis of the eyelids, and beaked nose. Feet (D) and foot X-ray (E) showing metatarsus adductus, hypoplasia of first metatarsals and digital phalanges, and fingerlike halluces.
Fig. 2  Patient 2. Face (A, B) and skull X-ray (C) showing dolichocephalic head shape, prominent forehead, strabismus, ptosis of the eyelids, beaked nose, and narrow face. Feet (D) and feet X-ray (E) showing bilateral agenesis of halluces and large second toes.
Fig. 3. Patient 3. Face (A, B) and skull X-ray (C) showing brachycephalic cranium, shallow orbits, strabismus, ptosis of the eyelids, and narrow face. Feet (D) and feet X-ray (E) showing metatarsus adductus, hypoplasia of first metatarsals and digital phalanges, and fingerlike halluces.
SSCP Analysis

SSCP was done with 3 μl of the PCR-amplified product and 7 μl of SSCP loading buffer. Samples were denatured at 95°C for 5 min prior to loading. Electrophoresis was done in MDE TM (2.5% glycerol; Bio Whittaker Molecular Applications, Rockland, ME) gel at 8 W for 10–14 hr at room temperature. Products were visualized by silver staining [Bassam et al., 1991].

DNA Sequencing

PCR products were purified with shrimp alkaline phosphatase (SAP) and exonuclease I (Amersham Pharmacia Biotech), then sequenced in an ABI Prism Model 377 (version 3.0) in both directions using the BigDye Terminator Cycle sequencing kit (PE Biosystems, Foster City, CA).

Molecular Results

SSCP and sequencing of the candidate exons at genes FGFR1, FGFR2, and FGFR3 in the family showed no alterations (Table I). Interestingly, the affected siblings share a common haplotype for the 8p11.2-11.1 region containing the FGFR1 gene (data not shown). Therefore, we screened the FGFR1 exons encoding the extracellular domains II and III of this receptor, which are responsible for the protein affinity to the growth factors. No mobility shift in SSCP was identified for the products from these exons. Sequencing was also performed for exon 7/FGFR1, as well as in the TWIST and MSX2 genes. All results were normal (Table I).

DISCUSSION

Here we present a two-generation kindred segregating craniosynostosis associated with ocular and distal limb alterations in apparently autosomal dominant inheritance. Due to the great overlap of the clinical phenotype observed in the FGFR craniosynostotic syndromes, we thought it wise to test this family for the presence of mutation in the candidate exons at the FGFR genes. No sequence alterations were detected in any of the candidate exons, suggesting that mutations in these genes are unlikely to be responsible for the disease in this family.

All the patients here reported have craniosynostosis; however, it varies in presentation, that is, the suture involved in the synostosis of the propositus’s father is the coronal suture, while in his sons is the sagittal one.

The pattern of craniosynostosis most commonly breeds true within families, but exceptions have been reported in the dominant inherited Boston (MSX2) and Muenke (FGFR3) syndromes [Jabs et al., 1993; Muenke et al., 1997]. The lack of mutation in the MSX2 and exon 7/FGFR3 genes in the patients reported here associated with a more complex phenotype supports that this disease represents a distinct disorder.

Craniosynostosis and ptosis of the eyelids are commonly found among patients with Saethre-Chotzen (S-C) syndrome, which has also autosomal dominant inheritance [El Ghouzzi et al., 1997; Howard et al., 1997]. However, the distal limb and eyes abnormalities found in our patients are very peculiar and are not described among patients with the S-C syndrome, even among those with microdeletions enclosing the TWIST gene [Johnson et al., 1998]. These observations, together with the absence of mutation in the TWIST gene in the present family, reinforce that alteration in another locus might be involved with the etiology of the present phenotype.

The Aminopterin-like syndrome sine aminopterin (ASSAS) and the acro-cranial-facial dysostosis syndrome (ADS) deserve special attention since craniosynostosis and limb defects are present in both [Fraser et al., 1987; Kaplan et al., 1988; Verloes et al., 1993]. However, ADS seems to be recessively determined in contrast to dominant inheritance in the patients described here. In addition, our patients do not fulfill the criteria diagnosis for these syndromes and, in contrast, none of the patients with ASSAS and ADS reported so far share the ocular alterations present in the family described here, suggesting that these disorders are not allelic.

Ocular alterations, which include cataracts in early childhood, microcornea, and optic nerve alteration, were observed in all our patients. Association of cataracts in childhood with craniosynostosis seems to be a rare event. Nashimura et al. [1998] reported a family of four affected siblings with apparent autosomal recessive determination with such association; however, the skeletal defects also involved spondyloepiphyseal dysplasia. Martin and Gorski [2001] described a family of three males with apparent autosomal dominant transmission of microphthalmia and related ocular abnormalities, postaxial polydactyly, and interestingly with delayed intramembranous ossification, in opposition to the craniosynostotic phenotype observed in our patients. In this as well as in our family, it seems that the primary gene defect is leading to alterations in structures originated from different germ layers, ectoderm (eye lens) and mesoderm (limbs and skull). Recently, it has been demonstrated that mutations in the same gene can cause craniosynostosis or parietal foramina [Wilkie et al., 2000]; it would be interesting to investigate if the patients of Martin and Gorski [2001] and ours share a common molecular mechanism.

In conclusion, the clinical and laboratory analyzes in the patients here described suggest that their phenotype very likely represents a novel syndrome caused by mutations in a still unknown locus, which might play an important role in craniofacial, limb, and ocular

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Results</th>
<th>Gene</th>
<th>Exon</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>5, 6</td>
<td>Normal</td>
<td>FGFR1</td>
<td>7, 9</td>
<td>Normal</td>
</tr>
<tr>
<td>FGFR2</td>
<td>7, 8</td>
<td>Normal</td>
<td>FGFR3</td>
<td>7</td>
<td>Normal</td>
</tr>
<tr>
<td>TWIST</td>
<td>1</td>
<td>Normal</td>
<td>TWIST</td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>MSX2</td>
<td>1.2</td>
<td>Normal</td>
<td>MSX2</td>
<td>1.2</td>
<td>Normal</td>
</tr>
</tbody>
</table>
development. Identification of genes associated with all these phenotypes will be important to elucidate this hypothesis.

ACKNOWLEDGMENTS

The authors thank the patient family for their collaboration, as well as Dr. Mayana Zatz, Constância Urbani, Elisângela Quedas, Antônia Cerqueira, and Marta Canovas.

REFERENCES


