Fibroblast growth factor receptor 3 mutation in nonsyndromic coronal synostosis: clinical spectrum, prevalence, and surgical outcome

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Object. A recurrent point mutation in the fibroblast growth factor receptor 3 gene that converts proline 250 into arginine has been reported recently in cases of apparently nonsyndromic coronal craniosynostosis. The goal of the present study was to examine the phenotype of patients in whom this mutation was present, to determine the prevalence of the condition, and to assess the functional and the morphological outcome of the surgically treated patients.

Methods. A DNA analysis was performed in 103 children suffering from apparently isolated coronal synostosis, 41 of whom had bilateral and 62 of whom had unilateral disease. There were 31 boys and 72 girls in the study group. Sixty cases were sporadic and 43 were familial; the 43 familial cases arose in 33 unrelated families. The mutation was found in seven (12%) of 60 sporadic cases and in 24 (73%) of the 33 families. The functional and morphological results were assessed in all surgically treated patients who had at least 1 year of follow up and who were at least 3 years of age at the time of assessment. A comparison was made between patients with the mutation and those without.

Conclusions. The most typical presentation was seen in girls and consisted of a bicoronal synostosis resulting in a severe brachycephaly associated with mild hypertelorism and marked bulging of the temporal fossae, which resulted in a huge enlargement of the upper part of the face. The most frequently associated extracranial anomaly was brachydactyly, identified either clinically or radiologically. Based on the proportion of bilateral and unilateral coronal synostoses, the present data indicate that the mutation is associated with more severe cases and that girls with the mutation are more severely affected than boys. The functional and morphological results were worse in patients in whom the mutation was present as compared with those in whom it was not.

KEY WORDS • craniosynostosis • brachycephaly • plagiocephaly • fibroblast growth factor receptor 3 mutation • children

Mutations in the FGFR gene family are involved in syndromic craniosynostoses: Apert’s, Crouzon’s, Pfeiffer’s, and Saethre–Chotzen syndromes. The coronal sutures are the ones most frequently involved in these syndromes. In apparently nonsyndromic coronal craniosynostoses, a genetic origin is suspected because 14% of cases are familial. Recently, a single mutation, C749G, in the FGFR3 gene that converts proline 250 into arginine, P250R, was found in nonsyndromic coronal craniosynostosis, of either sporadic or familial origin. However, the clinical presentation of the mutation appeared to be extremely variable: plagiocephaly, brachycephaly, and even pancraniosynostosis were reported. Hypertelorism, brachyactyly, and radiological anomalies of the hands and feet were also described. In familial cases, some carriers of the mutation presented with anomalies of the hands and feet but not with craniosynostosis.

The aim of this study is to determine the prevalence and to attempt to delineate a clinical spectrum of the P250R FGFR3 mutation in familial and sporadic nonsyndromic coronal synostosis.

Clinical Material and Methods

Patient Population

Of 2094 patients with craniosynostoses referred to the craniofacial team of the Department of Pediatric Neurosurgery at the Necker–Enfants Malades Hospital in Paris between 1976 and 1999, there were 352 cases classified as apparently nonsyndromic coronal synostosis. Patients with facial anomalies such as ocular proptosis, bifid nose, maxillary retrusion, or anomalies of the extremities that fit any recognizable syndrome were excluded. Two hundred forty-six cases were classified as plagiocephalies and 106 as brachycephalies. Suture fusion was confirmed intraoperatively in the patients who underwent surgery, as well as by clinical and radiological studies conducted in their rel-
atives. We were able to perform a DNA analysis in 103 of the children with apparently isolated coronal synostosis and in relatives for familial cases. No mutation was identified in the \textit{TWIST}, \textit{FGFR1}, and \textit{FGFR2} genes.

Sixty cases were sporadic and 43 were familial, from 33 families. Among sporadic cases, 14 of 60 children (two boys and 12 girls) had bilateral and the other 46 (12 boys and 34 girls) had unilateral coronal synostosis. Among familial cases, 27 of 43 children (nine boys and 18 girls) had bilateral and the other 16 (eight boys and eight girls) had unilateral coronal synostosis. In 15 of 33 families, all affected individuals had bicoronal synostosis; in five of 33, all affected patients had unilateral coronal synostosis, and in the remaining 13 there was an intrafamilial variability, with a mixture of unilateral and bilateral coronal synostosis.

Analysis of DNA

Genomic DNA was extracted from blood samples and used for polymerase chain reaction amplification of a 351-bp \textit{FGFR3} product. Primers and conditions for polymerase chain reaction were the same as those described by Moloney, et al.\textsuperscript{10} Restriction digestion with the NciI enzyme and analysis on 4\% Metaphor gels (FMC Bio-products, Rockland, ME) allowed identification of the C749G mutation that produces P250R. This mutation was located in the linker region between the second and third extracellular immunoglobulin-like domains of the FGFR3 protein (Fig. 1). When available, family members of patients in whom the mutation was found were also tested, regardless of whether they had apparent craniosynostosis.

Functional Outcome and Morphological Results

The functional outcome and morphological results were assessed in all surgically treated children who had completed at least 1 year of follow up and who were at least 3 years of age at the time of assessment. The functional outcome was assessed using IQ scores. The morphological results were assessed using the following codes:\textsuperscript{16} 1, excellent result; 2, incomplete result; 3, poor result necessitating a surgical correction; and 4, failure (reoperation mandatory). For functional and morphological results, comparisons were made between children with and without the mutation.

Statistical Analysis

The statistical software used for this study was SPSS for Windows 8.0 (SPSS, Inc., Chicago, IL). The Pearson chi-square and Fisher’s exact tests were used to compare percentages, and Student’s t-test was used to compare means.

Results

Thirty-seven (36\%) of the 103 children were found to carry the C749G point mutation in the \textit{FGFR3} gene. The mutation was found in 24 (73\%) of 33 families and in seven (12\%) of 60 sporadic cases (Table 1).

The sex ratio in the group of patients with craniosynostosis and with the P250R mutation of the \textit{FGFR3} gene was 0.76 (16 boys and 21 girls). In the group of patients without the mutation, the sex ratio was 0.29 (15 boys and 51 girls).

In the 24 families, the mutation was identified in 72 individuals. In nine of these 72 patients in whom the mutation was found no evidence of craniosynostosis was demonstrated, either clinically or radiologically. Sixty-three presented with a coronal synostosis, which was bilateral in 46 (73\%) and unilateral in 17 (27\%). Among 29 males with the mutation, 13 (45\%) had brachycephaly, nine (31\%) had plagiocephaly, and in seven (24\%) there was no evidence of craniosynostosis. In 43 female patients with the mutation, the distribution was 33 (77\%) brachycephalies, eight (18\%) plagiocephalies, and two (5\%) without craniosynostosis (Table 2).

In sporadic cases, the mutation was found in four (29\%) of 14 brachycephalies and in three (6\%) of 46 plagiocephalies (Table 3). The mean age (± standard deviation) of the fathers was 34 ± 10 years when the child was born. Sixteen fathers (27\%) were older than 40 years, and the P250R mutation was identified in four of their children.

Phenotype of Affected Children With the Mutation

Clinical reexamination of 37 children who underwent operation (seven sporadic cases and 30 children from 24...
families) revealed that coronal synostosis was unilateral in 12 of 37 (plagiocephaly) and bilateral in 25 of 37 cases (brachycephaly). The bicoronal synostoses were usually associated with specific features including severe forehead retrusion associated with moderate hypertelorism and severe bulging of the temporal fossae, resulting in a marked enlargement of the upper part of the face (15 of 25 patients; Fig. 2). This peculiar morphology was observed in four sporadic and 11 familial cases. In familial cases, mother–child pairs shared similar phenotypes (Fig. 3). In unicoronal synostoses, hypertelorism was restricted to four of 12 cases. Other facial findings included downslanting palpebral fissures (eight of 37), ptosis (five of 37), and low-set frontal hairline (seven of 37).

The only relevant extracranial manifestation consisted of minor abnormalities of the hands, regardless of the clinically and radiologically documented presence of craniofacial defects within the kindreds. Clinical brachydactyly was very difficult to detect in infants. In older children, brachydactyly was observed in 17 of 30 cases (Fig. 4). Radiological studies of the hands and feet were available in 27 of 37 children and revealed brachydactyly commonly affecting middle phalanges (24 of 27), coned epiphyses (six of 27), and carpal fusions (four of 27). Radiological studies of the feet demonstrated broad big toes (10 of 27), tarsal fusions (three of 27), and calcaneocuboidal fusion (two of 27). In one family, both parents were shown to have a normal skull on clinical and radiological examination, but the proband and her father presented the same radiological anomalies of the extremities, namely tarsal, carpal, and calcaneocuboidal fusions.

Functional Outcome

An IQ assessment was available in 21 children with the mutation and in 29 without the mutation (Table 4). In the group with the mutation, the mean IQ score was 94 (range 63–116), and in 14 patients (66.7%) the IQ score was higher than 90. In the group without the mutation, the mean IQ score was 103 (range 64–135), and in 26 patients (89.7%) the IQ score was higher than 90. These differences were statistically significant: Student’s t-test for comparison of the means yielded a probability value of 0.034; Fisher’s exact test for comparison of the proportions of normal IQ scores yielded a probability value of 0.05.

Morphological Results

In the group of children with the mutation, we were able to assess 33 for morphological results. Twenty were coded as 1, eight were coded as 2, and five were coded as 3 (Fig. 5); the mean score was 1.55. In the group without the mutation, we were able to assess 55: 42 were coded as 1 and 13 were coded as 2; the mean score was 1.24 (Table 5). These differences were statistically significant: Pearson
chi-square for distributions yielded a probability value of 0.011; Student’s t-test for means yielded a probability value of 0.016.

Discussion

One of the questions arising from the discovery of a gene mutation in apparently nonsyndromic coronal synostosis is this: should we reconsider the classification of craniosynostoses? In other words, have we found a new clinical syndrome or should we describe the craniosynostoses on the basis of molecular anomalies? The scope of this investigation probably does not allow a definitive answer to this recurrent problem in genetics, but it brings new information to this difficult discussion. Another aspect of the question could be this: do so-called nonsyndromic coronal synostoses really exist?

The attempt to determine whether coronal craniosynostosis caused by P250R mutation of the FGFR3 gene can be clinically delineated is very important, to allow a clinical diagnosis, to improve genetic counseling, and to study the prognosis of this condition. Unfortunately, the published cases and the present ones show that the clinical presentation of this condition is extremely variable, and no definitive single phenotype can be delineated. In our series, of 37 patients in whom the mutation was found, 17 familial cases and three sporadic ones (54%) had an apparently simple coronal synostosis with no associated clinical anomaly. Moreover, in familial cases, the mutation was found in patients without craniosynostosis, in whom, it is interesting to note, skeletal anomalies of the extremities were demonstrated on x-ray films. Hollway, et al., reported cases of deafness in patients without craniosynostosis, in whom the P250R FGFR3 mutation was demonstrated. However, some nuances in the clinical aspect of the coronal synostosis have been observed in the carriers of the FGFR3 mutation, which could be described as highly suggestive of the mutation. The most typical signs are seen in girls, who present with a bicoronal synostosis resulting in a severe brachycephaly associated with hypertelorism and marked bulging of the temporal fossae, which results in a huge enlargement of the upper part of the face. The most frequently associated extracranial anomaly is a brachydactyly, which can be confirmed either clinically or radiologically, but in most cases these anomalies are subtle and therefore not easily detectable in young children. Radiological examination of the extremities should be performed in all patients with coronal synostosis, and the other members of the family should be

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<th>Mutation</th>
<th>No. of Patients</th>
<th>Mean IQ</th>
<th>&gt;90 (%)</th>
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<tr>
<td>present</td>
<td>21</td>
<td>94</td>
<td>66</td>
</tr>
<tr>
<td>absent</td>
<td>29</td>
<td>103</td>
<td>89.7</td>
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FIG. 4. Photograph showing brachydactyly in a child with craniosynostosis and the P250R FGFR3 mutation.

FIG. 5. Photograph showing morphological results in three children with the P250R FGFR3 mutation who had undergone surgery for brachycephaly. Left: Code 1 (good result). Center: Code 2 (incomplete result): depression of the lateral parts of the forehead. Right: Code 3 (poor result): reoperation recommended because of asymmetry of the arches of the eyebrows and persistent temporal bulging.
The FGFR3 mutation in craniosynostosis

Table 5

<table>
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<tr>
<th>Mutation</th>
<th>No. of Patients</th>
<th>Code*</th>
<th>Mean Score</th>
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<tr>
<td>absent</td>
<td>55</td>
<td>4, 2, 3</td>
<td>1.24</td>
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* See Functional Outcome and Morphological Results for description of codes.

Fig. 6. Drawings showing floating forehead for brachycephaly. Note correction of the temporal bulging (asterisks): the bulging bone is removed, turned around, and replaced without fixation.
synostosis lead to the conclusion that molecular analysis should be performed in all cases of brachycephaly and plagiocephaly, whether of sporadic or familial origin.

References