Sex related expressivity of the phenotype in coronal craniosynostosis caused by the recurrent P250R FGFR3 mutation

Elisabeth Lajeunie, Vincent El Ghouzzi, Martine Le Merrer, Arnold Munnich, Jacky Bonaventure, Dominique Renier

Abstract
A recurrent point mutation in the fibroblast growth factor receptor 3 (FGFR3) gene that converts proline 250 into arginine is commonly associated with coronal craniosynostosis and has allowed definition of a new syndrome on a molecular basis. Sixty-two patients with sporadic or familial forms of coronal craniosynostosis were investigated for the P250R FGFR3 mutation. It was identified in 20 probands originating from 27 unrelated families (74%), while only 6/35 sporadic cases (17%) harboured the mutation. In both familial and sporadic cases, females were significantly more severely affected than males. Hence, while 68% of females carrying the P250R mutation showed brachycephaly, only 35% of males had the same phenotype. In the most severe forms of the disease, the association of bicoronal craniosynostosis with hypertelorism and marked bulging of the temporal fossae were common hallmarks that might be helpful for clinical diagnosis.

Taken together, these results indicate that the P250R FGFR3 mutation is mostly familial and is associated with a more severe phenotype in females than in males. The sex related severity of the condition points to the possible implication of modifier genes in this syndrome.

Keywords: coronal craniosynostosis; P250R FGFR3 mutation; sex related expressivity

The coronal synostoses may exist as isolated defects or in association with extracranial features, commonly including facial or limb anomalies, that define genetically determined syndromes. The frequencies of bilateral and unilateral coronal synostoses are known to differ. Unicoronal craniosynostosis is frequently sporadic and commonly associated with non-syndromic conditions. By contrast, bicoronal synostosis is more frequently familial and is usually a main feature of craniofacial syndromes. In the last four years, the molecular bases of most of the syndromic craniosynostoses have been elucidated. Phenotypically distinct entities including Crouzon, Apert, Pfeiffer, and Jackson-Weiss syndromes have been ascribed to mutations in three members of the fibroblast growth factor receptors (FGFRs) gene family, while mutations in the TWIST gene specifically account for the Saethre-Chotzen syndrome.

Although non-syndromic coronal synostoses are mostly sporadic, genetic factors may be involved in their aetiology as 14% of cases are familial. Recently, a single recurrent P250R FGFR3 mutation has been reported to cause familial and sporadic forms of unicoronal (plagiocephaly) and bicoronal (brachycephaly) craniosynostosis and to be associated with a wide clinical spectrum including pancraniosynostosis, syndromic craniosynostosis originally diagnosed as Crouzon, Pfeiffer, and Saethre-Chotzen syndromes, as well as Adelaide type craniosynostosis. The recent findings that familial deafness with or without craniosynostosis could be the result of the P250R FGFR3 mutation has further illustrated the intrafamilial variability of the phenotype. Indeed, in some families, subjects carrying the mutation presented variable clinical features extending from extremely mild conditions (minor bone anomalies of the extremities with no sign of craniosynostosis) to more severe phenotypes (brachydactyly associated with plagiocephaly or brachycephaly).

To determine the prevalence of the P250R FGFR3 mutation in familial and sporadic forms of coronal craniosynostosis, we studied a series of patients comprising 35 sporadic cases and 27 familial forms. The recurrent P250R mutation was found in 74% of familial cases but only in 17% of sporadic cases. The presence of an unexpected number of affected females in our series prompted us to examine closely sex related phenotypes in an attempt to delineate a spectrum of features that would better define patients carrying the mutation. Our study suggests that severity of the phenotype caused by the mutation is higher in females than in males. The relevance of previously reported cases to this study is discussed.
Figure 1 Pedigrees of 20 families with coronal synostosis showing variable expressivity of skull anomalies. Phenotypes include brachycephaly, plagiocephaly, or no sign of craniosynostosis. Presence or absence of the P250R mutation is denoted by + or −, respectively. In the last two families (F19, F20), only the proband was available for DNA analysis.

Legend:

- Brachycephaly
- Plagiocephaly
- No sign of craniosynostosis
Material and methods

A total of 35 sporadic and 27 familial cases (62 probands) were examined at the Craniofacial Surgery Department of the Necker Enfants-Malades Hospital in Paris. They were referred as apparently non-syndromic coronal synostosis. Patients with maxillary hypoplasia, ocular proptosis, or anomalies of the hands that fit any recognizable syndrome were excluded from the study. Skull abnormalities were confirmed during surgical repair in the probands and by clinical and radiographic examination in their relatives. No mutations were identified in the TWIST, FGFR1, or FGFR2 genes.

Among our 27 families, 59 subjects had coronal craniosynostosis and 60 were apparently unaffected. In 15/27 families, all affected subjects had bicornal synostosis while in 3/27 families, affected members had unicoronal synostosis. In 9/27 families there was intrafamilial variability with either unilateral or bilateral synostosis in affected subjects. Among sporadic cases, 8/35 children (two males and six females) had bilateral and 27/35 (nine males and 18 females) had unilateral coronal synostosis. Macrocephaly was not observed in our series.

The chi-square test was used for statistical analysis.

DNA Analysis

Genomic DNA was extracted from blood samples and used for PCR amplification of a 351 bp FGFR3 product. Primers and PCR conditions were the same as described by Moloney et al. Restriction digestion with NciI and analysis on 4% metaphor gels (FMC Bioproducts, Rockland, ME, USA) allowed identification of the C749G mutation that converts proline 250 of the receptor into arginine. Relatives of patients found to have the mutation were also tested (when available) regardless of their apparent phenotype.

Results

Twenty-six of the 62 probands (42%) were found to carry the C749G point mutation in the FGFR3 gene. Twenty cases out of 27 (74%) were familial and 6/35 (17%) were sporadic (table 1).

In 16 out of the 20 families in which the FGFR3 mutation was detected, complete clinical data and DNA samples from all the subjects were available (families F1 to F16, fig 1). In those families completely ascertained, the mutation was identified in 50 subjects (22 males and 28 females). Coronal synostosis was documented in only 40 carriers of the mutation; among them craniosynostosis was bilateral in 28 (70%) and unilateral in 12 (30%). Brachycephaly was observed in 20 out of the 28 female carriers while the other eight had either plagiocephaly (5/28) or no craniosynostosis (3/28). Eight out of the 22 males had brachycephaly and the other 14 had plagiocephaly (7/22) or no sign of craniosynostosis (7/22). In summary, three female carriers and seven males from seven unrelated families had no evidence of craniosynostosis, either clinically or radiologically (families F1 to F7, fig 1). These data suggested that females positive for the P250R mutation might be more severely affected than male carriers. In order to assess the validity of this hypothesis, the offspring of each mutation carrier were divided into two groups based on the severity of the phenotype: group 1 with brachycephaly and group 2 with plagiocephaly or no sign of craniosynostosis (table 2). Thirteen out of 19 carrier females (68%) had brachycephaly while it was present in only 6/17 of the males (35%). Statistical analysis of the
two groups according to the sex of the carriers showed a significant difference ($\chi^2=4.25, p<0.05$).

Only 6/35 of sporadic cases were positive for the mutation: 3/10 males (one bicoronal and two unicoronal synostoses) and 3/25 females (all bicoronal synostoses). Interestingly, the mutation was found to be preferentially associated with brachycephaly (4/8 cases, 50%) and was scarcely found in sporadic cases of plagiocephaly (2/27 cases, 7%) (table 1). Fathers of children carrying the P250R mutation had a mean age of 39.7 years at the time of birth. Four fathers out of six were older than 40. The increased age of the fathers is consistent with a possible paternal origin of the mutation.

Clinical re-examination of 32 children including the six sporadic cases and 26 children of the 20 familial cases positive for the mutation confirmed that coronal synostosis was unilateral in 12/32 cases and bilateral in 20/32 cases. In addition, the bicoronal synostosis was usually associated with specific features including severe forehead retrusion together with hypertelorism and severe bulging of the temporal fossae, resulting in a marked enlargement of the face (13/20 patients). This peculiar morphology was observed in four sporadic cases and nine familial forms (representative photographs are shown in fig 2). In the familial cases, mother-child pairs had similar phenotypes. In unicoronal synostosis, hypertelorism was restricted to 4/12 cases. Other facial findings included downward slanting palpebral fissures (6/32), ptosis (4/32), and low set frontal hairline (6/32). The only relevant extracranial manifestation consisted of minor abnormalities of the hands, irrespective of the clinical and radiological presence or absence of craniofacial defects within the kindreds. Clinical brachydactyly was observed in 12/32 cases but was never detected during infancy.

Radiographs of the hands and feet were available in 22/32 children. Hand examination showed brachydactyly commonly affecting the middle phalanges (19/22), coned epiphyses (5/22), and carpal fusion (3/22). Radiographs of the feet disclosed broad big toes (8/22), tarsal fusion (2/22), and calcaneo-cuboidal fusion (1/22). In one family, both parents showed a normal skull on clinical and radiological examination, but the proband and her father, who both carried the mutation (family F3, fig 1), had the same radiological anomalies of the extremities, namely tarsal, carpal, and calcaneo-cuboidal fusion (fig 3).

The IQ score was available in 29 children and the mean was 97. Twenty-six patients had an IQ>80. One patient with a poor familial environment had an IQ of 66 and another with associated hydrocephalus had an IQ of 63.

![Image](https://i.imgur.com/3w.png)

Table 3  Comparison of the present study with previously published results showing severity of the phenotype in familial (A) and sporadic (B) cases according to the sex of the carrier of the P250R FGFR3 mutation

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Total offspring</th>
<th>Group 2 P250R carriers</th>
<th>Group 2 No craniosynostosis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
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<tr>
<td>A Familial forms</td>
<td></td>
<td></td>
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<tr>
<td>This study</td>
<td>19</td>
<td>27</td>
<td>17</td>
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<tr>
<td>Previous studies</td>
<td>21</td>
<td>21</td>
<td>13</td>
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<tr>
<td>Total</td>
<td>40</td>
<td>48</td>
<td>30</td>
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<tr>
<td>B Sporadic forms</td>
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<tr>
<td>This study</td>
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<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Previous studies</td>
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<td>6</td>
<td>2</td>
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<tr>
<td>Total</td>
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<td>9</td>
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Data from the present study and previous studies were combined, taking into account the fact that one familial form and six sporadic cases from ref 7 were included in ref 6. M = males, F = females.
Discussion
In this study, 62 familial and sporadic forms of coronal craniosynostosis were first tested for the presence of the P250R FGFR3 mutation which accounted for 42% of our cases, but striking differences were noted between familial and sporadic cases.

Seventeen percent (6/35) of sporadic cases and 74% (20/27) of familial forms were heterozygous for the mutation. Surprisingly, in a previous series reported by Reardon et al, only 8.8% (4/45) of familial cases proved positive for the mutation. However, many of their patients had clinical features consistent with Saethre-Chotzen, Crouzon, and Pfeiffer syndromes. Patients with syndromic craniosynostosis were not included in our study. Also excluded were patients with evidence of proptosis, maxillary hypoplasia, bifid nose, broad thumb, or 2/3 cutaneous syndactyly. The selectivity of our clinical criteria is likely to explain the higher number of mutation carriers in our series.

Based on a large series of non-syndromic coronal craniosynostosis, plagiocephaly has been reported to occur in 70% and brachycephaly in 30% of affected subjects. Surprisingly, our study showed the reverse distribution (30% plagiocephaly and 70% brachycephaly) among carriers of the P250R FGFR3 mutation, suggesting that it was associated with the most severe form of coronal synostoses. Moreover, statistical analysis of our relevant families showed that the rate of severely affected carriers was significantly higher in females than in males ($\chi^2 = 4.25$, p<0.05). In addition, when both our families and those previously published were combined for statistical analysis (table 3A), the significance of the male:female difference was enhanced ($\chi^2 = 6.49$, p<0.05), thus confirming the sex related severity of the disease. Furthermore, the combined analysis of sporadic carriers presented in this study (six cases), the study of Moloney et al (six cases), and that of Muenke et al (two cases) showed that most of them were females (9/14, 64%) and were severely affected (8/9, 89% with brachycephaly, table 3B). This high rate of sporadic female carriers is likely to be the result of poor recruitment of males (5/14, 36%) owing to their milder phenotype.

The incomplete penetrance of the disease and the sex related expression of skull abnormalities are not easily explicable. Although it is tempting to speculate that one or more modifier genes might modulate the effect of the mutant receptor in a sex dependent manner, the possible involvement of unspecified environmental factors should not be ruled out.

Subtle anomalies of the extremities including brachydactyly, carpal and tarsal fusion, and coned epiphyses required thorough radiographic examination to be detected, especially in young children. The relevance of these signs to the clinical diagnosis is thus debatable. In contrast, hypertelorism with marked bulging of the temporal fossae, which occurred in 13/20 patients with bicoronal synostosis, seemed more predictive of the P250R mutation. However, owing to the variable clinical expression of coronal craniosynostosis caused by the P250R mutation within and between families, molecular identification of the C749G transversion in the FGFR3 gene appears to be the most reliable method for clinical diagnosis and genetic counselling.

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