Heterozygous P250L mutation of fibroblast growth factor receptor 3 in a case of isolated craniosynostosis

S Schindler, M Friedrich, H Wagener, B Lorenz, M N Preising

Craniosynostoses are caused by premature fusion of one or more sutures of the infant’s skull with an incidence between 1:1000 and 1:10 000. Isolated and syndromic forms can be differentiated and are involved in over 150 genetic disorders. Syndromic forms tend to be inherited and include variable other malformations of the extremities, the backbone, and the face. Isolated forms of craniosynostoses are often non-hereditary with closure of a single cranial suture. In most cases of syndromic craniosynostoses, multiple sutures are closed and often hydrocephalus and intracranial hypertension occurs. Mutations in the genes for fibroblast growth factor receptors (FGFR) 1, 2, and 3 have been associated with syndromic craniosynostoses in a variety of clinical phenotypes (table 1).

Muenke syndrome has been reported as syndromic craniosynostosis with unilateral or bilateral coronal synostosis, and minimal non-facial features. Muenke syndrome has been diagnosed in patients ascribed to different clinical entities, such as Saethre-Chotzen, Pfeiffer, Crouzon, and Jackson-Weiss syndromes. In these cases, a specific single mutation P250R (749C→G) was found in the FGFR3 gene, first described by Bellus et al. Patients show variable abnormalities on radiographs of the hands and feet, including thimble-like middle phalanges, coned epiphyses, and carpal and tarsal fusions, and in some cases brachydactyly. Sensorineural hearing loss has been described in approximately 30% of cases and developmental delay has rarely been found.

The P250R mutation is located in the linker region between the second and third extracellular Ig-like domains of the FGFR3 protein. The corresponding residues are mutated in two other FGFRs involved in craniosynostoses syndromes, FGFR1 (P252R, Pfeiffer syndrome) and FGFR2 (P253R, Apert syndrome). Moloney et al. pointed out that nucleotide position 749C has one of the highest mutation rates described in the human genome. In the most severely affected cases, bicoronal craniosynostosis was associated with hypertelorism and marked bulging of the temporal fossae has been found. P250R mutation is often familial and associated with a more severe phenotype in females than in males.

A general conclusion given by many of the authors who screened craniosynostosis patients for mutations in FGFR3 was that isolated craniosynostosis patients should be tested for the P250R mutation, since variable clinical expression and minimal clinical signs are common features of this mutation.

**MATERIALS AND METHODS**

We tested 11 isolated cases of isolated craniosynostosis for mutations in FGFR1, FGFR2, and FGFR3. Mutation screening focused on exon 5 of FGFR1, exon 7 and exon 9 of FGFR2, and exons 7 and 9-11 of FGFR3, regions known to be involved in craniosynostoses.

Genomic DNA was extracted from peripheral venous blood using standard procedures. Since premature closure of sutures of the skull tends to occur in single sutures only, DNA was extracted from bone samples obtained at suture surgery (fronto-orbital advancement) to avoid false negative results owing to somatic mosaics. Bone samples were stored at −80°C. For DNA preparation, the bone samples were deep frozen in liquid nitrogen and crushed in a mortar. The powder was digested with PronaseE (Merck Eurolab, Darmstadt) at a concentration of 500 µg/2.675 ml reaction buffer according to Miller et al. The digest was carried out at 37°C overnight in a water bath. Subsequently protein was precipitated with 700 µl of saturated NaCl solution (6 mol/l) and centrifuged at 2300 g in a swing out rotor. The supernatant was recovered and DNA was precipitated with adding 2.5 volumes of iced absolute ethanol. The DNA was centrifuged, dried, and resolved in TE buffer.

The DNA was amplified by PCR. PCR primers were synthesised to amplify FGFR1 and FGFR2 from genomic DNA according to Muenke et al. and Paznekas et al. PCR primers for FGFR3 were designed by us using the Vector NTI Suite 7.0 and the sequence data given at GeneBank (Accession No Y08089-Y08094) except exon 7. PCR was carried out in a 25 µl volume containing 100 ng of template DNA. The forward and reverse primers used to amplify exon 7 of FGFR3 (411 bp) were first described by Bellus et al. The programme consisted of an

**Table 1** Genetic involvement in craniosynostoses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal assignment</th>
<th>Disorder</th>
<th>OMIM</th>
<th>Ref</th>
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<tr>
<td>FGFR1</td>
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<td>Crouzon syndrome</td>
<td>123500</td>
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<td></td>
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<td>101200</td>
<td>9</td>
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<td>Jackson-Weiss syndrome</td>
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<tr>
<td></td>
<td></td>
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<td>100600</td>
<td>17, 18</td>
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<td></td>
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<td>101400</td>
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<tr>
<td>FGFR3</td>
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<td>Crouzon syndrome with acanthosis nigricans</td>
<td>100600</td>
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<td></td>
<td></td>
<td>Muenke syndrome</td>
<td>602849</td>
<td>4, 3</td>
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</table>
Bilateral sectorial hypopigmentation of the upper lids was not evident. Ophthalmoscopy at the same age showed a minor blunt optic disc. Neuroimaging showed a unilaterial stenosis of the right coronal suture underlying the right inferior oblique eye muscle was diagnosed together with a mild esotropia associated with a vertical deviation. On neuro-ophtalmological examination, a V pattern and overactivity of the right side of the face, and a slight facial scoliosis to the right were noted. The ophthalmological examination showed an exophoria at near distances (−12.5). Radiographs of the hands and feet did not show any abnormalities.

**DISCUSSION**

The phenotype in the patient and her mother resembles the milder forms of Muenke syndrome. The mutation (P250L) affects a known mutation hot spot (749C) in FGFR3 and reinforces the conclusion of Moloney et al.10 that 749C is a frequently mutated nucleotide of FGFR3 in craniosynostoses. The novel finding in this regard is that in addition to the well known P250R mutation, which underlies a separate entity of craniosynostoses called Muenke syndrome,1 the substitution of proline 250 by leucine causes a similar phenotype. Therefore, we agree that patients carrying mutations at P250 in FGFR3 should be considered as affected by Muenke syndrome. Expressivity may be mild (as in the index patient) or even minimal (as in the mother). Patients with isolated craniosynostosis should therefore be screened for mutations in the FGFR3 gene.

**REFERENCES**

fibroblast growth factor receptor 3 gene (FGFR3) defines a new craniosynostosis syndrome. Am J Hum Genet 1997; 60:555-64.