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

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Craniosynostoses are caused by premature fusion of one or more sutures of the infant's skull with an incidence between 1:1000 and 1:10 000.¹ Isolated and syndromic forms can be differentiated and are involved in over 150 genetic disorders.² 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.³ 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.*⁴ 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.^{3–7}

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 syndrome⁸) and *FGFR2* (P253R, Apert syndrome⁹). Moloney *et al.*¹⁰ 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.¹¹

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.¹² 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.*¹² 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 by 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.*⁸ and Paznekas *et al.*⁷ PCR primers for *FGFR3* were designed by us using the Vector NTI Suite 7.0¹³ and the sequence data given at GeneBank (Accession No Y08089–Y08094) except exon 7.⁴ 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.*⁴ The programme consisted of an

Table 1 Genetic involvement in craniosynostoses

Gene	Chromosomal assignment	Disorder	OMIM	Ref
<i>FGFR1</i>	8p11	Pfeiffer syndrome type 1	101600	8
<i>FGFR2</i>	10q26	Crouzon syndrome	123500	15
		Apert syndrome	101200	9
		Jackson-Weiss syndrome	123510	16
		Pfeiffer syndrome type 2 and 3	101600	17, 18
		Saethre-Chotzen syndrome	101400	7
		Beare-Stevenson syndrome with cutis gyrata	123790	19
<i>FGFR3</i>	4p16	Crouzon syndrome with acanthosis nigricans	100600	20
		Muenke syndrome	602849	4, 3

Figure 1 Direct sequencing of the sense strand of the exon 7 amplicon of the *FGFR3* gene in (A) the patient and (B) her mother.

initial denaturing step (94°C for five minutes) followed by 35 PCR cycles (58°C, one minute; 72°C, one minute; 94°C, one minute) and a terminal cycle (58°C, one minute; 72°C, 10 minutes).

Single strand conformation polymorphism analysis was carried out on PAGE gels containing 8% polyacrylamide as reported previously.¹⁴ PCR products showing aberrant banding patterns were subjected to direct sequencing. The mutation was confirmed by reverse strand sequencing in an independent PCR product.

RESULTS

We could identify a novel mutation in *FGFR3* (P250L, 749C→T) (fig 1A) in a patient clinically diagnosed as having isolated craniosynostosis (plagiocephaly). The mutation could also be shown in the maternal DNA (fig 1B), but was excluded in 30 chromosomes from patients with isolated or syndromic craniosynostoses and their relatives.

The patient was first examined clinically at the age of 7 months. A frontal bossing of the left side and flattened forehead on the right side was diagnosed. At 18 months she was referred to the University of Regensburg because of exotropia associated with a vertical deviation. On neuro-ophthalmological examination, a V pattern and overactivity of the right inferior oblique eye muscle was diagnosed together with a minor blunt optic disc. Neuroimaging showed a unilateral stenosis of the right coronal suture underlying the right plagiocephaly (fig 2A). A fronto-orbital advancement was performed at the age of 3 years. At this age the patient's head circumference was 52 cm (slightly above the 97th centile). Asymmetry of the skull was obvious with diagonals measuring 15.2 cm from right to left and 17.5 cm from left to right.

Radiographs of the hands and feet were performed at the age of 4 because of the molecular genetic diagnosis but did not show any abnormalities. Ophthalmoscopy at the same age disclosed bilateral sectorial hypopigmentation of the upper

Figure 2 Facial appearance of (A) the patient at 17 months and (B) her mother at the age of 33 years.

and lower retina starting from the optic disc. At the same time, the mother was also examined clinically. She was reported to have been suspected of having hydrocephalus in infancy because of her increased head circumference but no increased intracranial pressure had been found. On examination, she showed a prominent and large forehead, a slightly smaller right side of the face, and a slight facial scoliosis to the right (fig 2B). 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,³ 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.

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