

Peter Heutink<sup>a</sup>  
Christl Vermeij-Keers<sup>b</sup>  
Ben A. Oostra<sup>a</sup>

<sup>a</sup> Department of Clinical Genetics,  
and

<sup>b</sup> Department of Plastic and  
Reconstructive Surgery and  
Department of Anatomy,  
Erasmus University Rotterdam,  
The Netherlands

## The Genetic Background of Craniosynostosis Syndromes

.....  
**Key Words**

Craniosynostosis syndromes  
Positional cloning  
Homeobox  
Zin finger  
Fibroblast growth factor  
receptors

.....  
**Abstract**

Craniosynostosis syndromes are developmental disorders that cause an abnormal shape of the skull due to the premature fusion of cranial sutures. Enormous progress has been made recently in understanding the genetic background of these disorders and a classification of syndromes on a genetic basis is beginning to emerge. Members of at least three gene families that play an important role in vertebrate development are associated with different craniosynostosis syndromes. Here we review the genetic aspects of this fast-moving field.  
.....

### Introduction

Craniosynostosis, the premature (pre- and postnatal) fusion of one or more cranial sutures, is a relatively common developmental anomaly that causes an abnormal shape of the skull. Normal development of the human skull requires differentiation and coordination of all the outgrowing bones, particularly at the site of the sutures. Sutural growth is necessary to accommodate the enlarging brain. During normal embryonic and fetal development, some bone centers can fuse directly and others form sutures [1]. Therefore, the pathogenesis of craniosynostosis may involve the direct fusion of bone centers with-

out suture formation at that site, the premature fusion of sutures, accelerated bone maturation preventing the formation of sutural ligaments, or secondary changes of the sutures due to forces mediated by the abnormal development of the base of the skull [2, 3].

Apart from skull deformations such as brachycephaly, turriccephaly, plagiocephaly and cloverleaf configuration, other craniofacial features are present, e.g., maxillary hypoplasia, shallow orbits, hyper- or hypotelorism, midface problems and mental retardation. Other findings can be (poly-)syndactyly of hands and feet, fusion of cervical vertebrae, and cardiac, vascular and intestinal malformations. Craniofacial surgery is indicated to

**Table 1.** Human craniosynostosis syndromes that have been localized or cloned

Disorder	Gene/location	References
Greig cephalopolysyndactyly	GLI3	Vortkamp et al. [16]
Boston type	MSX2	Jabs et al. [26]
Crouzon syndrome	FGFR2	Reardon et al. [30] Jabs et al. [46] Oldridge et al. [47]
Jackson-Weiss syndrome	FGFR2	Jabs et al. [46]
Pfeiffer syndrome	FDFR1/FGFR2	Muenke et al. [54] Lajeune et al. [55] Rutland et al. [56] Schell et al. [57]
Apert syndrome	FGFR2	Wilkie et al. [60]
Saethre-Chotzen syndrome	7p	Brueton et al. [63] Herwerden et al. [66]
Adelaide type	4p16	Hollway et al. [67]

correct intracranial pressure, exorbitism, malocclusion, obstructive apnea and overall craniofacial appearance. Craniosynostosis can occur as an isolated anomaly, or as part of a syndrome and is found in all ethnic and racial groups. The estimated incidence is as high as 1 in 3,000 infants [4]. More than 100 different forms of isolated craniosynostosis and craniosynostosis syndromes are known, showing etiologic and pathogenetic heterogeneity [5]. In about half of the syndromes, a genetic cause has been established or suggested. Most syndromes with a genetic background are inherited as monogenic autosomal dominant traits [2, 6]. The familial cases of craniosynostosis syndromes are quite rare but provide a way of mapping genes that are involved in the pathogenesis of craniosynostosis and in the development of the skull. A large overlap in phenotypes between the different syndromes and the intrafamilial variability in the expression of symptoms have led to uncertainty in diagnosis. Recent studies on families with

craniosynostosis using positional cloning strategies and cytogenetic analysis have demonstrated their power to link these disorders with candidate genes (table 1). This can lead to a better classification and understanding of the etiology of these disorders and eventually provide better patient management and counselling.

### Greig Syndrome

The finding of chromosomal abnormalities in patients often provides a first indication for the localization of a gene involved in the pathogenesis of a disorder. A number of cytogenetic abnormalities have been reported in association with craniosynostosis involving multiple chromosomes [7, 8]. Cytogenetic abnormalities of chromosome 7p13 and 7p21 are fairly frequently associated with craniosynostosis suggesting the presence of at least two important genes in this chromosomal re-

gion. Three balanced translocations involving chromosome 7p13 were associated with Greig cephalopolysyndactyly syndrome (GCPS) in different families [9–11]. Two additional sporadic cases showed deletions in 7p13 [12].

GCPS is an autosomal dominant disorder affecting limb and craniofacial development in humans [13, 14]. GCPS-affected individuals are characterized by pre- and postaxial polysyndactyly of the hands, preaxial polysyndactyly of the feet, macrocephaly, a broad base to the nose with mild hypertelorism and a prominent forehead. Craniosynostosis is present in only 5% of patients with GCPS. Additional evidence for a locus on chromosome 7p13 came from Brueton et al. [15] who showed linkage of GCPS to the epidermal growth factor receptor on chromosome 7p12-p13. Vortkamp et al. [16] looked at the chromosomal abnormalities in more detail and found that the three translocation breakpoints on 7p13 fall within a 630-kb *NotI* restriction fragment. Two of these translocations interrupt the *GLI3* gene, a zinc finger gene of the *GLI-Krüppel* family that had already been localized to 7p13 [17, 18]. The breakpoints of the translocations are within the first third of the coding sequence of *GLI3*. In the third translocation, chromosome 7 is broken about 10 kb downstream from the 3' end of *GLI3*. It is not clear how this last translocation can cause GCPS, but there are possibly cis-acting sequences downstream of the gene that influence expression. Further evidence that the *GLI3* gene is responsible for GCPS was obtained by studies on a naturally occurring mouse mutant [19]. The spontaneous semi-dominant mutation *extra toes* (*Xt*), which affects limb development, has almost complete penetrance in heterozygotes but variable expressivity. The mutation has been mapped to mouse chromosome 13 in a region homologous to human 7p13 [20, 21]. *Xt* heterozygotes show the same unusual combination as

GCPS patients of predominantly preaxial polydactyly of the hind limbs and postaxial nubbins on the fore limbs. Heterozygous *Xt* mice also often show an enlarged interfrontal bone, analogous to the broad forehead and broad nose seen in GCPS patients, and some have hydrocephaly. The mouse mutant brachyphalangy (*Xt<sup>bph</sup>*), also referred to as *Xt<sup>3H</sup>*, is a radiation-induced allele that has similar but distinguishable effects to *Xt* in both heterozygotes and homozygotes [21, 22]. In addition, *Xt<sup>bph</sup>* heterozygotes usually have an abnormal sternum and occasionally show syndactyly. All *Xt* alleles are homozygous lethal with more extreme limb and craniofacial defects plus other abnormalities like exencephaly and edema that are not observed in heterozygotes. *Xt* mutants contain an intragenic deletion of *Gli3* and in normal mice, all tissues affected in *Xt* mutants express *Gli3* during embryogenesis [23]. *Xtj* homozygous mutants do not express *Gli3* during embryogenesis. Normal *Gli3* expression is most prominent in developing limb buds and facial primordia which are the most affected structures in *Xt* heterozygotes. The expression of *Gli3* in the precursors of craniofacial mesenchyme and interdigital mesenchyme, cells that are programmed for cell death, suggests a role in apoptosis.

### **Craniosynostosis: Boston Type**

Linkage studies are becoming increasingly important in mapping single-gene disorders. Evidence for a craniosynostosis locus on chromosome 5qter came from study of a large three-generation family with Boston-type craniosynostosis [24]. A spectrum of expression of synostosis was noted in the cranial morphology, ranging from simple brachycephaly with forehead recession through turribrachycephaly and frontal bossing to klee-

blattschädel deformity [24, 25]. None of the individuals had stigmata of the well-known craniosynostosis syndromes, i.e. midfacial retrusion, dental malocclusion, exorbitism, hypertelorism, blepharoptosis, brachsyndactyly or angulated/broad thumbs or toes. A first indication for the localization of the gene came from a linkage report of Müller et al. [25] localizing the gene close to D5S211. Using a candidate gene approach, Jabs et al. [26] identified a mutation in the muscle segment homeobox gene 2 (MSX2) in all affected individuals in an independent large three-generation family. A single base pair change (C to A) in patients results in the substitution of a histidine for a proline at amino acid position 7 of the homeodomain. This amino acid is invariant in Msx homeobox domains from other species. The region of the homeodomain affected by the mutation is believed to be involved in protein-protein interaction and protein-DNA interaction. Jabs et al. [26] suggest that the geometry of the N-terminal portion of the homeobox domain is changed by the mutation in the region that is involved in protein-protein interaction but not protein-DNA interaction.

In avian and mammalian embryos, Msx2 is expressed in osteogenic tissue of the face. In neonatal mice it is expressed in the facial region. Expression of Msx2 was detected by *in situ* hybridization on day-15.5 embryonic mouse heads in the membranous bone of the calvaria and in adjacent mesenchymal cells. In neonatal mouse heads, Msx2 is expressed along a line parallel to the edge of the calvarial bones in the region of the sutures. The spatial pattern of Msx2 expression strongly suggests that Msx2 plays a role in the development or maintenance of the sutures.

Jabs et al. [26] showed that the acrocephalosyndactyly syndromes (ACSs) are not allelic to this locus by recombination analysis with an intragenic marker for MSX2. During the

last year it has become clear that this group of craniosynostosis syndromes is caused by mutations in a distinct class of genes.

### ACSs

The ACSs comprise a clinically similar group among the autosomal dominant craniosynostosis syndromes, characterized by coronal or multiple synostosis in association with distal fore and hind limb anomalies, particular syndactyly. Blank [27] proposed a classification into a typical and an atypical form. The typical form is Apert syndrome, characterized by syndactyly of hands and feet of a special type (complete distal fusion with a tendency for fusion also of the bony structures). The second group, atypical non-Apert (ACS), was thought to comprise a heterogeneous collection of disorders and includes Crouzon, Jackson-Weiss, Pfeiffer and Saethre-Chotzen syndromes. ACS syndromes through their overlap in phenotype cause large diagnostic dilemmas and there has been extensive discussion about which syndromes should be regarded as separate entities. Now that the genetic background of ACS syndromes has become clearer a new classification based on genetic evidence is starting to evolve.

From the atypical ACS syndromes, Crouzon syndrome was the first for which the genetic defect was found. Crouzon syndrome is characterized by craniosynostosis, hypertelorism, exophthalmos and external strabismus, with a beaked nose, short upper lip, hypoplastic maxilla and a relative mandibular prognathism [28]. The main distinction from Apert, Pfeiffer, Saethre-Chotzen and Jackson-Weiss syndromes is that patients usually have no hand and/or feet malformations.

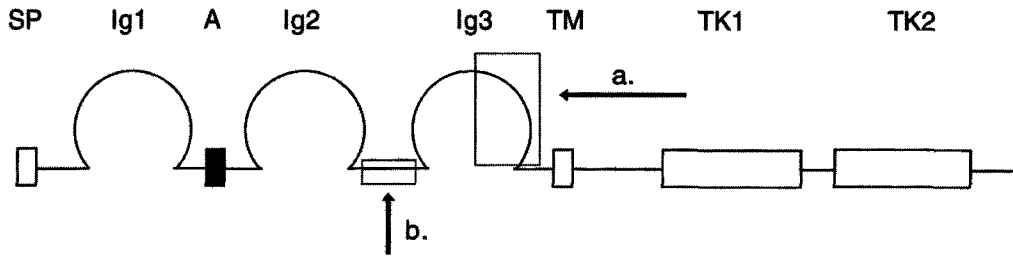
Crouzon syndrome, like many other craniosynostosis syndromes shows a variable phenotype expression and is inherited as an

**Table 2.** Mutations found in fibroblast growth factor receptors

Disorder	Amino acid substitution or base change		
	FGFR1	FGFR2	
	exon 5	exon 7	exon 9
Crouzon		Ser267Pro Cys278Phe Gln289Pro Trp290Arg Del 287-289	Tyr328Cys Tyr340His <i>Cys342Tyr</i> <i>Cys342Arg</i> Cys342Ser Ser347Cys Ser354Cys splice mutations
Jackson-Weiss			Ala344Gly
Pfeiffer	Pro252Arg		Asp321Ala Thr341Pro <i>Cys342Tyr</i> <i>Cys342Arg</i> intronic 952A → G splice mutations
Apert		Ser252Trp Pro253Arg	

autosomal dominant disorder. The Crouzon locus was mapped to chromosome 10q using a linkage approach [29]. Tight linkage was shown to markers on chromosome 10q25-q26. This prompted Reardon et al. [30] to use a candidate gene approach to identify the responsible gene. A fibroblast growth factor (FGF) receptor (FGFR) had been previously mapped to this chromosomal region [31, 32]. These FGFR are part of an intercellular signalling pathway that regulates cell proliferation, differentiation, migration and survival in embryonic development, malignancy and angiogenesis (see Appendix). Therefore, FGFR2 was considered a candidate gene for Crouzon syndrome. In the study performed by Reardon et al. [30], twenty Crouzon cases and 89 unaffected individuals were tested for mutations in the FGFR2 gene. In nine patients single-stranded conformational poly-

morphism (SSCP) changes were found in exon 9, the exon that is present in the splice variant of FGFR2 that is involved in osteogenesis. In three families, these SSCP changes cosegregated with the disease. Direct sequencing of exon 9 revealed amino acid changes in the second half of the third immunoglobulin (Ig) loop of the FGFR2 gene (table 2, fig. 1). In the same Ig loop, a G-to-A transition at nucleotide 1044 was detected, creating a new donor splice site [45]. In separate studies by Jabs et al. [46] and Oldridge et al. [47], three additional mutations in patients with Crouzon syndrome were identified (table 2). Two mutations introduce an additional cysteine residue in the third Ig domain; the third substitutes the cysteine by a phenylalanine. The Cys342 is highly conserved in FGFRs, presumably because the structural integrity of Ig domains is maintained by Cys-Cys bonds.



**Fig. 1.** Structure and organization of the human FGFR genes. SP = Signal peptide; Ig1, Ig2 and Ig3 = immunoglobulin-like domains 1, 2 and 3; A = acidic region; TM = transmembrane domain; TK1 and TK2 = tyrosine kinase domains. The arrows indicate mutation hot spots for craniosynostosis syndromes. [Adapted from ref. 43.]

The Cys342 is the only cysteine encoded by exon 9 and therefore the mutations in or close to this codon can be expected to disrupt the extracellular component of FGFR2. The Ser354Cys mutation introduces a second cysteine in the third Ig loop and therefore can also be expected to disrupt the structural integrity of the Ig domain. More recently, Oldridge et al. [47] reported a number of new mutations (table 2) in the first part of the Ig3 domain. Five of them involve the replacement of a cysteine residue.

The finding that an FGFR was involved in craniofacial development prompted investigators to investigate whether other craniosynostosis syndromes could be correlated to one of the known FGFR genes. Indeed soon after the identification of the Crouzon gene, two new reports on FGFRs appeared. One of them described mutations in the FGFR2 gene in patients with Jackson-Weiss syndrome [46]. The Jackson-Weiss syndrome is a craniosynostosis syndrome characterized by foot abnormalities in addition to skull abnormalities [48]. The syndrome had been mapped to chromosome 10q25-q26 [49]. Mutation analysis on FGFR2 revealed a mutation in the same conserved third Ig domain as was found

in Crouzon syndrome [46]. An alanine to glycine transition in codon 344 was found in all affected family members. Again the mutation is close to the cysteine involved in the disulphide bond of this Ig domain.

There has been extensive discussion as to whether or not Jackson-Weiss and Crouzon should be regarded as separate entities. Now that it has become clear that Crouzon syndrome and Jackson-Weiss syndrome are allelic, the question arises why limb abnormalities are only found in Jackson-Weiss syndrome. The mutations that have been found are not identical but are localized close to each other in the same functional Ig domain. With the current knowledge, a similar disruption of gene function by the mutations in both syndromes would be expected and it is unclear why two different phenotypes arise. This situation gets even more confusing now that mutations have been found for Pfeiffer and Apert syndromes. Pfeiffer syndrome is an autosomal dominant disorder with characteristic anomalies of the hands and feet [50, 51]. Apart from the premature fusion of several sutures of the skull, resulting in a short tower-shaped head or cloverleaf skull, hyper- or hypotelorism, a beaked nose and a hypoplas-

tic midface, patients show broad thumbs, syndactyly, shortness of fingers, and broad great toes that are medially deviated. Several families with Pfeiffer syndrome were previously linked to chromosome 8 by Robin et al. [52]. This chromosomal region contains the FGFR1 gene [53]. Using SSCP analysis and direct sequencing, Muenke et al. [54] found a mutation in exon 5 of the FGFR1 gene. A Pro252Arg substitution in the region between the second and third Ig domains was found in all chromosome 8 families. This region of the FGFR1 gene is strongly conserved between species and among all known FGFRs. This 'linker' sequence between two Ig domains is thought to be involved in the orientation of the Ig domains towards each other and can affect binding to FGF or dimerization with FGFRs.

For Pfeiffer syndrome, locus heterogeneity has been reported [52] and mutation analysis on patients with Pfeiffer confirm this. Three reports describe Pfeiffer patients who did not have the reported mutation in FGFR1 but instead had mutations in the FGFR2 gene [55–57]. These mutations are in the second part of the third Ig loop (table 2) and like the mutations found for Crouzon and Jackson-Weiss replace the cysteine residue or are close to it. Other mutations change a neutral residue into an acidic one or are on the intron-exon 9 border disrupting the consensus sequence required for the normal processing of the RNA transcript.

The typical form of ACS syndromes according to Blank [27] is Apert syndrome, an autosomal dominant disorder with a birth prevalence of 1 in 65,000 [58], characterized by coronal suture synostosis, hypertelorism, maxillary hypoplasia, supraorbital retrusion in combination with severe syndactyly of the hands and feet. The upper lip has a trapezoidal configuration; a cleft soft palate can be seen together with other abnormalities of the

skin and internal organs [59]. Apert patients rarely have children due to reduced fitness. The disorder usually arises by a new mutation. A classical linkage approach is therefore difficult, but the finding of the responsible genes for other craniosynostosis syndromes made a candidate gene approach feasible. Wilkie et al. [60] studied a total of 40 patients and found a mutation in FGFR2 in all cases. The mutation was either a Ser252Trp or a Pro253Arg substitution. Both mutations are localized in the linker region between the second and third Ig domains close to the 5' end of exon 7.

In an attempt to correlate the phenotype of Apert patients with their genotype, the patients were divided into three groups based on their phenotype. The first group comprised patients in whom the thumb and part of the little finger are separate from the syndactylous mass; the second group comprised patients in whom the little finger is not separate, and a third most severely affected group comprised patients in whom all the fingers are fused including the thumb. The more severe syndactyly patients tend to have the mutation at codon 253 but there is no absolute correlation. There is a clear overlap between the groups and, therefore, other factors, either genetic or nongenetic play a role in the variable expression of the phenotype.

Saethre-Chotzen syndrome is relatively common among craniosynostosis syndromes. It shares features with other ACS syndromes such as coronal suture synostosis but in addition there is a facial asymmetry, a high incidence of ptosis, deviated nasal septum, low frontal hairline, brachydactyly, partial cutaneous syndactyly and small ears with prominent crura [61, 62]. Saethre-Chotzen segregates as an autosomal dominant trait and shows variable phenotype expression. The responsible gene has not been identified but chromosomal breaks on chromosome 7p21-

p22 and subsequent family studies have pinpointed it to a region of 8 cM between D7S664 and D7S507 [63–65]. In a patient with a t(2;7)(p23;p22) translocation, in situ hybridization with YAC clones containing D7S664 and D7S507 showed that the gene is distal to D7S664 and that D7S507 is deleted. There is however conflicting evidence for the Saethre-Chotzen locus. van Herwerden et al. [66] mapped the Saethre-Chotzen locus more proximal on the short arm of chromosome 7, between markers D7S493 and D7S516, suggesting that there are two separate loci in the region that can cause Saethre-Chotzen syndrome. Lewanda et al. [65] suggested that clinical heterogeneity is a more likely explanation.

Based upon the similarities with other ACS syndromes, one might expect the responsible gene to be related to a FGFR gene or at least involved in a FGF signalling pathway. However, at this point no obvious candidate gene has been identified.

Adelaide-type craniosynostosis is an autosomal dominant disorder that shares many features with Jackson-Weiss syndrome. There are clinical and radiological differences; coned epiphyses, distal and middle phalangeal hypoplasia and carpal bone malsegmentation on the hands and coned epiphyses, hallux valgus, phalangeal, tarsonavicular and calcaneonavicular fusions and uniform absence of metatarsal fusions on the feet. Using a large family, Hollway et al. [67] mapped the locus to the tip of the short arm of chromosome 4, telomeric to D4S394. Two candidate genes are localized in this region: the MSX1 homeobox gene and the FGFR3 gene. Msx1-deficient mice show abnormalities in craniofacial and tooth development in addition to cleft palate [68]. The FGFR3 gene is known to be involved in achondroplasia [69, 70].

The discussion about the classification of ACS syndromes will enter a new phase now

that the genetic background of these disorders is being unraveled. At least four forms of ACS are due to mutations in FGFRs. Crouzon, Jackson-Weiss, Pfeiffer and Apert appear to be allelic but the classification on a morphological basis that was proposed by Blank [27] into two groups – Apert and non-Apert forms – is still valid. Although Apert syndrome can be caused by mutations in FGFR2, the mutations for Crouzon, Jackson-Weiss and Pfeiffer are generally localized in the third Ig domain that is involved in ligand binding, while the mutations for Apert syndrome are localized in exon 7, the linker region between the second and third Ig domains, a functionally different domain of the FGFR2 gene. It is striking, however, that the FGFR1 Pro252Arg mutation in Pfeiffer syndrome exactly corresponds to the position of the FGFR2 Pro253Arg mutation in Apert syndrome. The craniosynostosis syndromes for which the genes have so far been identified are dominant disorders. The majority of mutations in the FGFR genes cause different ligand-binding properties, which can result in a change in affinity for FGFs. These ‘gain-of-function’ mutations might change the function of the FGFR1 and FGFR2 genes in a very similar way, indicating that the genes show an overlap in ligand-binding properties. The FGFR and FGF gene families encode a network of genes and gene interactions that can explain at least part of the clinical variations in craniosynostosis syndromes. But the distinction between the non-Apert forms on a genetic basis is difficult to explain in this way. In Crouzon syndrome, limb abnormalities are not usually observed, while in Jackson-Weiss this is an important clinical feature. However, on closer inspection, many limb defects and radiographic abnormalities in the hand are found in Crouzon patients. The mutations for both disorders are found in the same functional domain and are expected to disrupt the structure of the disul-



phide bond that stabilizes the third Ig loop. The difference in phenotype is therefore puzzling. Further mutation analysis on patients will determine whether these two disorders should be regarded as separate entities. Alternatively, the differences in phenotype could be the result of differences in genetic background or environmental factors.

The situation for Pfeiffer syndrome is even more complicated. It is intriguing that two mutations in FGFR2 have been described (Cys342Tyr, Cys342Arg) that can result in either a Crouzon or a Pfeiffer phenotype and that the Pfeiffer phenotype can be caused by mutations in either the FGFR1 or the FGFR2 gene (table 2). Apart from differences in the genetic background of the patients, it would be interesting to study both the FGFR1 and FGFR2 genes in more detail for additional mutations that could play a role in the pathogenesis of Pfeiffer syndrome. Mutation analysis on craniosynostosis syndromes will be helpful in developing a new classification system on a genetic basis. The understanding of the pathogenesis of craniosynostosis, the variable phenotypes and the effect of each separate mutation will benefit greatly from the development of animal models. At least three classes of genes are now known to be involved in different craniosynostosis syndromes: homeobox genes, genes involved in apoptosis and growth factor receptors. These gene families play a key role in pattern formation, organogenesis and limb development. Understanding the function of the genes involved in craniosynostosis will therefore also lead us to a better understanding of vertebrate development.

### Acknowledgements

We would like to thank Dr. J.M. Vaandrager for critically reading the manuscript, and Prof. Dr. Galjaard for his continuous support.

## Appendix

### *FGFs and Their Receptors*

FGFs constitute a family of related signalling molecules that act to promote the growth and differentiation of cells of different origins. In mammals, nine members of the FGF family have been described so far [33]. FGFs act by binding and activating specific cell surface receptors, which comprise a family of related but individually distinct tyrosine kinase receptors [34–36]. These FGFRs each possess a similar protein structure with three immunoglobulin (Ig)-like domains in the extracellular region, a single membrane-spanning segment and a cytoplasmic tyrosine kinase domain split by a kinase insert (fig. 1). Some cDNA clones have been identified encoding only the second and third Ig domains, which are still functional as a FGFR. Four FGFR genes have been found so far in humans and the diversity of these receptors is further increased by differential RNA splicing [37–41]. Alternative exon usage in the second half of the third Ig loop results in different ligand-binding properties. Ligand-induced receptor activation is mediated by receptor oligomerization via conformation alteration of the extracellular domain. This oligomerization is proposed to stabilize interactions between adjacent cytoplasmic regions and leads to activation of the kinase function [37, 38, 41].

For FGFR1 and FGFR2, alternative splice products have been characterized, and apart from the FGFR form of the genes, a keratinocyte growth factor receptor (KGFR)-like product is found. The two isoforms are identical except for a 49-amino-acid sequence spanning the second half of the third Ig loop in the extracellular region. Control of these alternative splice variants appears to involve trans-acting factors. The variation in the expressed gene product is highly significant since there is a distinction in the ligand-binding characteristics of the two isoforms leading to functional differences.

The expression pattern of the *kgfr* and *fgfr* isoforms of *Fgfr1* and *Fgfr2* has been studied in developing mouse embryos with exon-specific probes [38, 42]. Both forms are expressed as early as gastrulation but show a distinctive expression pattern, with exclusive alternative splicing in different cell types. The *kgfr* isoform plays a role in skin development, the *fgfr* form is preferentially expressed in osteogenesis. This expression pattern is consistent with a role of the *fgfr* isoforms in craniofacial development.

## References

- 1 Vermeij-Keers C: Craniofacial embryology and morphogenesis: Normal and abnormal; in Stricker M, van der Meulen JC, Raphael B, Mazzola R, Tolhurst DE, Murray JE (eds): Craniofacial Malformations. Edinburgh, Churchill Livingstone, 1990, pp 27–60.
- 2 Cohen MM Jr: Craniosynostosis: Diagnosis, Evaluation and Management. New York, Raven, 1986.
- 3 Cohen MM Jr: Sutural biology and the correlates of craniosynostosis. *Am J Med Genet* 1993;47:581–616.
- 4 Lammer EJ, Cordero JF, Wilson MJ, Oimette D, Ferguson S: Investigation of a suspected increased prevalence of craniosynostosis: Colorado, 1978–1982. *Proc Greenwood Genet Cent* 1987;6:126–127.
- 5 Winter RM, Baraitser M: The London Dysmorphology Database. Oxford, Oxford University Press, 1994.
- 6 On Line Mendelian Inheritance of Man. Baltimore, Johns Hopkins University, 1995.
- 7 Gorlin RJ, Cohen MM, Levin LS: Syndromes of the Head and Neck. Oxford, Oxford University Press, 1990, pp 519–539.
- 8 Frysns JP: Structural abnormalities of the Y chromosome and craniosynostosis. *Clin Genet* 1992;42:100–101.
- 9 Tommerup N, Nielsen F: A familial reciprocal translocation t(3;7)(p21.1;p13) association with the Greig polysyndactyly-craniofacial anomalies syndrome. *Am J Med Genet* 1983;16:313–321.
- 10 Kruger G, Gotz J, Kvist U, Dunker H, Erfurth F, Pelz L, Zech L: Greig syndrome in a large kindred due to reciprocal chromosome translocation t(6;7)(q27;p13). *Am J Med Genet* 1989;32:411–416.
- 11 Vortkamp A, Gessler M, Le Paslier D, Elasarapy R, Smith S, Grzeschik KH: Isolation of a yeast artificial chromosome contig spanning the Greig cephalopolysyndactyly syndrome (GCPS) gene region. *Genomics* 1994;22:563–568.
- 12 Wagner K, Kroisel PM, Rosenkranz W: Molecular and cytogenetic analysis in two patients with microdeletions of 7p and Greig syndrome: Hemizygoty for PGAM2 and TCRG genes. *Genomics* 1990;8:487–491.
- 13 Greig DM: Oxycephaly. *Edinburgh Med J* 1928;33:189–218.
- 14 Gollop TR, Fontes LR: The Greig cephalosyndactyly syndrome: Report of a family and review of the literature. *Am J Med Genet* 1985;22:59–68.
- 15 Brueton L, Huson SM, Winter RB, Williamson R: Chromosomal localisation of a developmental gene in man: Direct DNA analysis demonstrates that Greig cephalopolysyndactyly maps to 7p13. *Am J Med Genet* 1988;31:799–804.
- 16 Vortkamp A, Gessler M, Grzeschik KH: Glij3 zinc-finger gene interrupted by translocations in Greig syndrome families. *Nature* 1991;352:539–540.
- 17 Ruppert JM, Kinzler KW, Wong AJ, Bigner SH, Kao FT, Law ML, Seanez HN, O'Brien SJ, Vogelstein B: The GLI-Kruppel family of human genes. *Mol Cell Biol* 1988;8:3104–3113.
- 18 Ruppert JM, Vogelstein B, Arheden K, Kinzler KW: GLI3 encodes a 190-kilodalton protein with multiple regions of GLI similarity. *Mol Cell Biol* 1990;10:5408–5418.
- 19 Johnson DR: *Extra-toes*: A new mutant gene causing multiple abnormalities in the mouse. *J Embryol Exp Morphol* 1967;3:543–581.
- 20 Lyon MF, Morris T, Searle AG, Butler J: Occurrences and linkage relations of the mutant 'extra-toes' in the mouse. *Genet Res* 1967;9:383–385.
- 21 Lyon MF, Kirby MC: Mouse chromosome atlas. *Mamm Genome* 1992;90:22–43.
- 22 Johnson DR: Brachyphalangy, an allele of extra-toes in the mouse. *Genet Res* 1969;13:275–280.
- 23 Hui C, Joyner AL: A mouse model of Greig cephalopolysyndactyly syndrome: The extra-toes<sup>1</sup> mutation contains an intragenic deletion of the Gli3 gene. *Nat Genet* 1994;3:241–246.
- 24 Warman ML, Mulliken JB, Hayward PG, Muller U: Newly recognized autosomal dominant disorder with craniosynostosis. *Am J Med Genet* 1993;46:444–449.
- 25 Müller U, Warman ML, Mulliken JB, Weber JL: Assignment of a gene locus involved in craniosynostosis to chromosome 5qter. *Hum Mol Genet* 1993;2:119–122.
- 26 Jabs EW, Müller U, Li X, Ma L, Luo W, Hawoth IS, Klisak I, Sparkes R, Warman ML, Mulliken JB, Snead ML, Maxson R: A mutation in the homeodomain of the human MSX2 gene in a family affected with autosomal dominant craniosynostosis. *Cell* 1993;75:443–450.
- 27 Blank CE: Apert's syndrome (a type of acrocephalosyndactyly): Observations on a British series of thirty-nine cases. *Ann Hum Genet* 1960;24:151–164.
- 28 Crouzon O: Dysostose cranio-faciale héréditaire. *Bull Mém Soc Méd Hôp Paris* 1912;33:545–555.
- 29 Preston RA, Post JC, Keats BJB, Aston CE, Ferrell RE, Priest J, Nouri N, Losken HW, Morris CA, Hurtt MR, Mulvihill JJ, Ehrlich GD: A gene for Crouzon craniofacial dystosis maps to the long arm of chromosome 10. *Nat Genet* 1994;7:149–153.
- 30 Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S: Mutations in the fibroblast growth factor receptor 2 cause Crouzon syndrome. *Nat Genet* 1994;8:98–103.
- 31 Mattei MG, Moreau A, Gesnel MC, Houssaint E, Breathnach R: Assignment by in situ hybridisation of a fibroblast growth factor receptor gene to human chromosome band 10q26. *Hum Genet* 1991;87:84–86.
- 32 Dionne CA, Modi WS, Crumley G, O'Brien SJ, Schlessinger J, Jaye M: BEK, a receptor for multiple members of the fibroblast growth factor (FGF) family, maps to human chromosome 10q25.3-q26. *Cytogenet Cell Genet* 1992;60:34–36.
- 33 Basilico C, Moscatelli D: The FGF family of growth factors and oncogenes. *Adv Cancer Res* 1992;59:115–165.

- 34 Johnson DE, Williams LT: Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 1993;60:1-41.
- 35 Ornitz DM, Leder P: Ligand specificity and heparin dependence of fibroblast growth factor receptors 1 and 3. *J Biol Chem* 1992;267:16305-16211.
- 36 Partanen J, Makela TP, Eerola E, Korhonen J, Hirvonen H, Claesson-Welsh L, Alitalo K: FGFR-4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern. *EMBO J* 1991;10:1347-1354.
- 37 Johnson DE, Lee PL, Lu J, Williams T: Diverse forms of a receptor for acidic and basic fibroblast growth factors. *Mol Cell Biol* 1990;10:4728-4736.
- 38 Reid HH, Wilks AF, Bernard O: Two forms of the basic fibroblast growth factor receptor-like mRNA are expressed in the developing mouse brain. *Proc Natl Acad Sci USA* 1990;87:1586-1600.
- 39 Johnson DE, Lu J, Chen H, Werner S, Williams LT: The human fibroblast growth factor receptor genes: A common structural arrangement underlies the mechanisms for generating receptor forms that differ in their third immunoglobulin domain. *Mol Cell Biol* 1991;11:4627-4634.
- 40 Werner S, Duan DS, De Vries C, Peters KG, Johnson DE, Williams LT: Differential splicing in the extracellular region of fibroblast growth factor receptor 1 gene generates receptor variants with different ligand-binding specificities. *Mol Cell Biol* 1992;12:82-88.
- 41 Avivi A, Yayon A, Givol D: A novel form of FGF receptor-3 using an alternative exon in the immunoglobulin domain III. *FEBS Lett* 1993;330:249-252.
- 42 Eisenmann A, Ahn J, Graziana G, Tronick S, Ron D: Alternative splicing generates at least five different isoforms of the human basic-FGF receptor. *Oncogene* 1991;6:1195-1202.
- 43 Miki T, Bottaro D, Fleming P, Smith C, Burgess W, Chan A, Aaronson S: Determination of ligand binding specificity by alternative splicing: Two distinct growth factor receptors encoded by a single gene. *Proc Natl Acad Sci USA* 1992;89:246-250.
- 44 Orr-Urtreger A, Bedford MT, Burakova T, Arman E, Zimmer Y, Yayon A, Givol D, Lonai P: Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2). *Dev Biol* 1993;158:475-486.
- 45 Li X, Park WJ, Pyeritz RE: Effect on splicing of a silent FGFR2 mutation in Crouzon syndrome. *Nat Genet* 1995;9:232-233.
- 46 Jabs EW, Li X, Scott AF, Meyers G, Chen W, Eccles M, Mao J, Charnas LR, Jackson CE, Jaye M: Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. *Nat Genet* 1994;8:275-279.
- 47 Oldridge M, Wilkie AOM, Slaney SF, Poole MD, Pulleyn LJ, Rutland P, Hockley AD, Wake MJC, Goldin JH, Winter RM, Reardon W, Malcolm S: Mutations in the third immunoglobulin domain of the fibroblast growth factor receptor-2 gene in Crouzon syndrome. *Hum Mol Genet* 1995;4:1077-1082.
- 48 Jackson CE, Weiss L, Reynolds WA, Forman TF, Peterson JA: Craniosynostosis midface hypoplasia, and foot abnormalities: An autosomal dominant phenotype in a large Amish kindred. *J Pediatr* 1976;88:963-968.
- 49 Li X, Lewanda AF, Eluma F, Jerald H, Choi H, Alozie I, Proukakis C, Talbot CC Jr, Kolk CV, Bird LM, Jones MC, Cunningham M, Clarren SK, Pyeritz RE, Weissenbach J, Jackson CE, Jabs EW: Two craniosynostosis syndrome loci, Crouzon and Jackson-Weiss, map to chromosome 10q23-q26. *Genomics* 1994;22:418-424.
- 50 Pfeiffer RA: Dominant erbliche Akrocephalosyndactylie. *Z Kinderheilkd* 1959;160:168-171.
- 51 Cohen MM: Pfeiffer syndrome update, clinical subtypes, and guidelines for differential diagnosis. *Am J Med Genet* 1993;45:300-307.
- 52 Robin NH, Feldman GJ, Mitchell HF, Lorenz P, Wilroy RS, Zackai EH, Allanson JE, Reich EW, Pfeiffer RA, Clarke LA, Warman ML, Mulliken JB, Brueton LA, Winter RM, Price RA, Gasser DL, Muenke M: Linkage of Pfeiffer syndrome to chromosome 8 centromere and evidence for genetic heterogeneity. *Hum Mol Genet* 1994;3:2153-2158.
- 53 Ruta M, Howk R, Ricca G, Drohan W, Zabelhansky M, Laureys G, Barton DE, Francke U, Schlessinger J, Givol D: A novel protein kinase gene whose expression is modulated during endothelial cell differentiation. *Oncogene* 1988;3:9-15.
- 54 Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, Pulleyn LJ, Rutland P, Reardon W, Malcolm S, Winter RM: A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. *Nat Genet* 1994;8:269-274.
- 55 Lajeune E, Ma HW, Bonaventura J, Munnich A, Le Merrer M: FGFR2 mutations in Pfeiffer syndrome. *Nat Genet* 1995;9:108.
- 56 Rutland P, Pulleyn LJ, Reardon W, Baraister M, Hayward R, Jones B, Malcolm S, Winter RM, Oldridge M, Slaney SF, Poole MD, Wilkie AOM: Identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes. *Nat Genet* 1995;9:173-176.
- 57 Schell U, Hehr A, Feldman GJ, Robin NH, Zackai EH, de Die-Smulders C, Viskochil DH, Steward JM, Wolff G, Ohashi H, Price RA, Cohen MM, Muenke M: Mutations in FGFR1 and FGFR2 cause familial and sporadic Pfeiffer syndrome. *Hum Mol Genet* 1995;4:323-328.
- 58 Cohen MM, Kreiborg S Jr, Lammer EJ, Cordero JE, Mastroiacovo P, Erickson JD, Roeper P, Martinez-Frias ML: Birth prevalence study of the Apert syndrome. *Am J Med Genet* 1992;42:655-659.
- 59 Apert ME: De l'acrocephalosyndactylie. *Bull Mém Soc Méd Hôp Paris* 1906;23:1310-1330.

- 60 Wilkie AOM, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, Hayward RD, David DJ, Pulleyn LJ, Rutland P, Malcolm S, Winter RM, Reardon W: Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. *Nat Genet* 1995;9:165-172.
- 61 Saethre M: Ein Beitrag zum Turmschädelproblem (Pathogenese, Erbllichkeit und Symptomalogie). *Dtsch Z Nervenheilkd* 1931,119:533-555.
- 62 Chotzen F: Eine eigenartige familiäre Entwicklungsstörung (Akrocephalosyndaktylie, Dystosis craniofacialis und Hypertelorismus). *Monatsschr Kinderheilkd* 1932;55:97-122.
- 63 Brueton LA, van Herwerden L, Chotai KA, Winter RM: The mapping of a gene for craniosynostosis: Evidence for linkage of the Saethre-Chotzen syndrome to distal chromosome 7p. *J Med Genet* 1992;29:681-685.
- 64 Lewanda AF, Cohen MM, Jackson CE, Taylor EW, Li X, Belooof M, Day D, Clarren SK, Ortiz R, Garcia C, Hauselman E, Figueroa LA, Wulfsberg E, Wilson M, Warman ML, Padwa BL, Whiteman DAH, Mulliken JB, Jabs EW: Genetic heterogeneity among craniosynostosis syndromes: Mapping the Saethre-Chotzen syndrome locus between D7S513 and D7S516 and exclusion of Jackson-Weiss and Crouzon syndrome loci from 7p. *Genomics* 1994;19:115-119.
- 65 Lewanda AF, Green ED, Weissenbach J, Jerald H, Taylor E, Summar ML, Phillis JA III, Cohen M, Feingold M, Mouradian W, Clarren SK, Jabs EW: Evidence that the Saethre-Chotzen syndrome locus lies between D7S664 and D7S507, by genetic analysis and detection of a microdeletion in a patient. *Am J Hum Genet* 1994;55:1195-1201.
- 66 van Herwerden L, Rose SP, Reardon W, Brueton LA, Weissenbach J, Malcolm S, Winter RM: Evidence for locus heterogeneity in acrocephalosyndactyly: A refined localization for the Saethre-Chotzen syndrome locus on distal chromosome 7p- and exclusion of Jackson-Weiss syndrome from craniosynostosis loci on 7p and 5q. *Am J Hum Genet* 1994;54:669-674.
- 67 Hollway GE, Phillips HA, Ades LC, Haan EA, Mulley JC: Localization of craniosynostosis Adelaide type to 4p16. *Hum Mol Genet* 1995;4:681-683.
- 68 Satokata I, Maas R: Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 1994;6:348-356.
- 69 Rousseau F, Bonaventure J, Legeai-Mallet L, Pelet A, Rozet JM, Maroteaux P, Le Merrer M, Munnich A: Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature* 1994;371:252-254.
- 70 Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, Winokur ST, Wasmuth JJ: Mutations in the transdomain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell* 1994;78:335-342.