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Craniosynostosis: genes and mechanisms

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Enlargement of the skull vault occurs by appositional growth at the fibrous joints between the bones, termed cranial sutures. Relatively little is known about the developmental biology of this process, but genetically determined disorders of premature cranial suture fusion (craniosynostosis) provide one route to the identification of some of the key molecules involved. Mutations of the *MSX2*, *FGFR1*, *FGFR2*, *FGFR3* and *TWIST* genes yield new insights, both into normal and abnormal cranial suture biogenesis and into problems of broad interest, such as the conservation of molecular pathways in development, and mechanisms of mutation and dominance.

INTRODUCTION

Craniosynostosis is important for two reasons. It provides a model system for studying the genetic and environmental factors in a pathway of developmental malformation; and it represents a significant medical problem, occurring in ~1 in 2500 individuals (1–4). The abnormal skull growth may be associated with raised intracranial pressure, impaired cerebral blood flow, airway obstruction, impaired vision and hearing, learning difficulties and adverse psychological effects (5–8). These remain significant problems despite important advances in surgical management over the past 20 years.

In humans, mineralisation of the cranial vault mostly occurs directly from membrane derived from paraxial mesoderm, proceeding outwards from several ossification centres from ~13 weeks of embryonic development (reviewed in 9). At ~18 weeks these mineralising bone fronts meet and sutures are induced along the lines of approximation. Subsequently, the skull enlarges by appositional growth at the suture with deposition of premineralised bone matrix (osteoid) along the suture margins. The major cranial sutures are shown in Figure 1A. Premature fusion of one or more of these sutures (craniosynostosis) prevents further growth along the margin; excessive growth at other sutures leads to skull distortion (reviewed in 10). The suture itself is anatomically a simple structure (Fig. 1B), comprising the two plates of bone separated by a narrow space containing immature, rapidly dividing osteogenic stem cells, a proportion of which are recruited to differentiate into osteoblasts and make new bone. Developmentally, the problem of craniosynostosis may be posed as follows (11): what causes the sutural tissue to fail in the execution of its proliferative and anti-differentiative functions?

Both genetic and environmental factors contribute to craniosynostosis. Abnormal mechanical forces (external pressure or deficiency in underlying brain growth) may be a predisposing cause in some cases (12). In others, a family history or associated anomalies suggest a genetically determined condition. Over 100 syndromes associated with craniosynostosis have been delineated (13,14): most of the common ones exhibit dominant inheritance. The clinical observation that many craniosynostosis syndromes are accompanied by limb abnormalities (see Box 1) suggests that aspects of craniofacial and limb development utilise common molecular pathways, an idea supported by experimental evidence (15). This insight has been an important contributor to recent success in the identification of genes mutated in craniosynostosis. All the genes identified to date were already known to be major players in the development of fruitflies and mice and were pinpointed using positional candidate approaches in relatively small families. The new findings from human syndromes reveal hitherto unsuspected aspects of the structure and biology of the mutated genes and their cognate proteins.

MUTATIONS IN *MSX2*, *FGFR1*, *FGFR2*, *FGFR3*, *FBN1* AND *TWIST* GENES

Table 1 catalogues all mutations that cause craniosynostosis as a primary clinical feature, a list currently comprising 64 different mutations of six genes in 474 independent patients. These range from unique missense mutations in the *MSX2* (muscle segment homeobox 2) and *FBN1* (fibrillin) genes described in single families, to 46 mutations in seven phenotypes for *FGFR2* (fibroblast growth factor receptor 2). Mutations of *FBN1* are more commonly associated with Marfan syndrome (reviewed in 64) and are not further discussed here. The *TWIST* gene is the most recent (January 1997) addition to the list; relatively few mutations have been published to date. Figure 2 illustrates the position of mutations in relation to functional motifs in MSX2, FGFR1, -2, -3 and TWIST.

Many interesting patterns may be discerned from scrutiny of Table 1 and Figure 2. FGFR mutations in particular present one of the most remarkable series in human genetics of genotype–phenotype correlations for allelic and non-allelic mutations. The following points are worth highlighting; their functional significance is addressed in the subsequent section.

(i) Most of the *FGFR* mutations are missense, with a smaller number of splice mutations or small insertions, deletions or indels, all of which remain in-frame. No nonsense or frameshift mutations have been described. This contrasts with the *TWIST* gene, for which the mutations mostly comprise nonsense changes and 21 bp duplications, with relatively fewer missense mutations.

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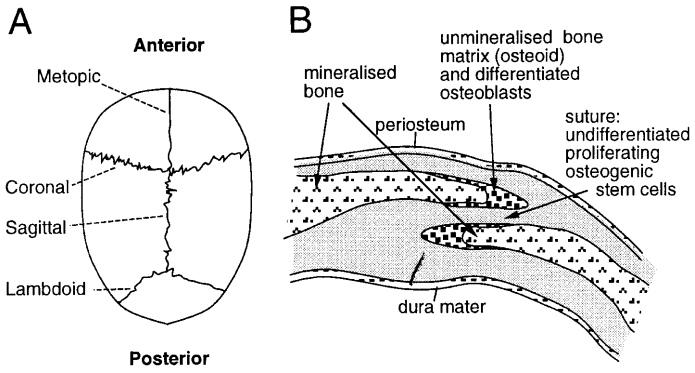

Figure 1. Normal cranial suture development. (**A**) View of child's skull from above, showing position of the major sutures. Coronal craniosynostosis leads to a short, broad skull; conversely, sagittal synostosis leads to a long, narrow skull. (**B**) Diagrammatic cross section through coronal suture. The skull bones overlap slightly. In craniosynostosis, the narrow space separating the bones is obliterated.

Box 1. Naming and recognition of craniosynostosis syndromes. Outside the field of clinical genetics, the basis for recognising specific syndromes carries considerable mystique. This is not always helped by disputes about definition and the use of multisyllabic eponymous terms. The naming of disorders has to be flexible, as it may need re-evaluation in the light of new clinical and molecular information. This is well illustrated by the craniosynostosis literature. Whereas some clinically defined disorders have turned out to correlate very closely with molecular pathogenesis (notably Apert syndrome), in other cases the distinction has become more blurred (for example, the Crouzon/Pfeiffer/Jackson–Weiss group). FGFR3 associated coronal craniosynostosis only became clearly defined once the specific mutation was identified (the 'labels' previously attached to patients who turn out to have this mutation included Pfeiffer, Saethre–Chotzen and Crouzon syndromes, as well as Adelaide-type, non-syndromic and brachydactyly associated craniosynostosis!): the term 'Muenke craniosynostosis' has been suggested by OMIM, and is used in this review. Boston craniosynostosis is a 'private' syndrome defined by the MSX2 mutation in the single family currently known: as the phenotype is variable and rather non-specific, it would be difficult to recognise clinically. This box summarises the disorders mentioned in this review.

Disorder	MIM#	First recognised	Main clinical features	Comments
Boston craniosynostosis	123101	1993	Supraorbital recession, not diagnostic	Single family with MSX2 mutation
Pfeiffer syndrome	101600	1964	Broad thumbs and great toes	Clinical boundary with Jackson–Weiss disputed
Apert syndrome	101200	1906	Bony syndactyly of hands and feet	
Crouzon syndrome	123500	1912	'Normal' limbs; subtype has acanthosis nigricans	Radiology reveals subtle limb abnormalities
Jackson–Weiss syndrome	123150	1976	Broad great toes, bony fusions in feet	Very variable within one enormous family
Beare–Stevenson syndrome	123790	1969	Cutis gyrata and acanthosis nigricans	
Muenke craniosynostosis	134394	1996	Difficult to diagnose clinically	Defined by Pro250Arg mutation in FGFR3
Shprintzen–Goldberg syndrome	182212	1982	Generalised connective tissue defect	Possibly heterogeneous
Saethre–Chotzen syndrome	101400	1931	2/3 syndactyly of hands; bifid great toes	Easily confused with Muenke craniosynostosis

Table 1. Mutations identified in craniosynostosis

Gene	Mutation[a]	Phenotype[b]	*n*[c]	Reference[d]
MSX2	Pro148His	B	1	17
FGFR1	Pro252Arg	P	10	18–20 (1)
FGFR2[e]	Tyr105Cys	C	1	21
	Ser252Trp	A	191	20,22–31 (39)
	Ser252Phe(CG→TT)	A	2	31,32
	Ser252Leu	N,C	1	32
	934CGC→TCT[SP→FS]	P	1	32
	Pro253Arg	A	93	20,22–26,29–31 (20)
	Ser267Pro	C	1	33
	982insTGG[insG]	C	1	20
	Cys278Phe	C,P	8	20,33
	1037del9[delHIQ]	C	1	33
	Gln289Pro	C,J	3	20,33,34
	Trp290Arg(T→C)	C	2	33
	Trp290Gly	C	2	35,36
	Trp290Cys(G→C)	P	1	37
	Trp290Cys(G→T)	P	1	(1)
	Lys292Glu	C	1	38
	1119–3T→G[f]	P	1	19
	1119–2A→G[f]	P,A	6	19,30,39 (1)
	1119–1G→C[f]	P	1	26
	Ala314Ser[f]	P	2	19
	Asp321Ala	P	1	39
	Tyr328Cys	C	1	40
	Asn331Ile	C	1	41
	1190ins6[insDA]	C	1	41
	Gly338Arg(G→C)	C	2	34 (1)
	Gly338Glu	C	2	21
	Tyr340His	C	5	26,40,42–44
	Thr341Pro	P	1	45
	Cys342Tyr	C,P	18	20,33,34,42–46 (1)
	Cys342Arg	P,C,J	14	19,20,26,35,43,45
	Cys342Phe	C	2	20,33
	Cys342Ser(G→C)	P,C	4	20,34,47 (1)
	Cys342Ser(T→A)	P,C	2	20,43
	Cys342Trp	C,P	5	26,35,42,44
	Ala344Ala(G→A)[f]	C,U	8	35,40,42,43,48–50 (2)
	Ala344Gly	J,C	2	34,40
	Ala344Pro	P	1	20
	Ser347Cys	C	4	26,33,40
	Ser351Cys	U	1	21
	Ser354Cys	C	5	33–35,43
	1245del9[delWLT]	C	1	41
	Val359Phe	P	1	20
	1263ins6[f]	P	1	20
	Ser372Cys	BS	1	51
	Tyr375Cys	BS	2	51
	Gly384Arg	U	1	21
FGFR3	Pro250Arg	M	33	52–56 (1)
	Ala391Glu	C-A	6	57,58
FBN1	Cys1223Tyr	SG	1	59,60
TWIST[g]	Tyr103stop(308insA)	S	1	61
	Tyr103stop(C→A)	S	1	62
	Glu104stop	S	1	(1)
	Gln119Pro	S	1	61
	Ser123stop	S	1	62
	Ser123Trp	S	1	(1)

Table 1. continued

Gene	Mutation[a]	Phenotype[b]	*n*[c]	Reference[d]
	Glu126stop	S	1	62
	Leu131Pro	S	1	62
	405ins21[insAALRKII]	S	1	61
	416ins21[insKIIPTLP]	S	4	61,62 (1)
	417ins21[insKIIPTLP]	S	1	62
	Asp141Tyr	S	1	(1)
	433del23	S	1	61

[a]Notation for mutations follows ref. 16. Square brackets show amino acid changes in single letter notation.
[b]Syndrome abbreviations: A, Apert; B, Boston craniosynostosis; BS, Beare–Stevenson; C, Crouzon; C-A, Crouzon/acanthosis nigricans; J, Jackson–Weiss; M, Muenke craniosynostosis; N, normal phenotype; P, Pfeiffer; S, Saethre–Chotzen; SG, Shprintzen–Goldberg; U, unclassified. Where more than one phenotype has been described, the most frequent is indicated first.
[c]Number of unrelated individuals.
[d]Figures in brackets indicate number of unpublished observations from the author's laboratory that are included in the total.
[e]Amino acid and DNA numbering from ref. 63.
[f]Effect on splicing proven or presumed.
[g]Amino acid and DNA numbering from ref. 61.

(ii) Many of the mutations are recurrent. In the case of *FGFR1*, *-2* and *-3*, some missense mutations occur much more frequently than others; in the case of the *TWIST* gene, 21 bp duplications (with three distinct molecular origins) have already been recorded six times.

(iii) Allelic missense mutations of *FGFR2* and *FGFR3* have widely varying phenotypes. Many *FGFR3* mutations are characterised by short-limbed bone dysplasia of varying severity (hypochondroplasia, achondroplasia, thanatophoric dysplasia); craniosynostosis is rare in the first two of these disorders (reviewed in 67). Especially noteworthy is that mutations immediately adjacent to the P250R mutation in FGFR3 (R248C and S249C) cause thanatophoric dysplasia type I (Fig. 2).

(iv) Identical mutations of FGFR paralogs are observed in several regions of the molecule (Fig. 2). Pro→Arg mutations of the IgII–IgIII linker cause Pfeiffer syndrome in FGFR1, Apert syndrome in FGFR2 and Muenke craniosynostosis in FGFR3; Ser or Gly→Cys and Tyr→Cys mutations of the juxtamembrane region cause Beare–Stevenson syndrome in FGFR2 and thanatophoric dysplasia type I in FGFR3; Gly→Arg mutations in the transmembrane region (differing in position by two amino acids) cause unclassified craniosynostosis in FGFR2 and achondroplasia in FGFR3.

(v) Many of the other missense mutations of FGFR2 create or destroy a cysteine residue in one of the immunoglobulin-like domains. Most notably, C342 represents a mutation hotspot; all but one of the amino acid substitutions that can arise by mutating one nucleotide of the TGC codon have been observed (the exception is Cys→Gly).

(vi) Different substitutions of the same amino acid may give different phenotypes: in FGFR2, S252W and S252F cause Apert syndrome whereas the phenotype with S252L is usually normal; C342Y tends to give a Crouzon phenotype whereas C342R tends to give a Pfeiffer phenotype (there is, however, some overlap).

(vii) Identical substitutions may be associated with variable limb phenotypes: noteworthy examples include the variable phenotype associated with the A344G mutation in the original Jackson–Weiss pedigree, and the Apert-like phenotype observed in a single instance of the 1119–2A→G splice mutation (30), which is more usually associated with Pfeiffer syndrome.

Identification of these mutations has necessitated some reappraisal of the rather confusing clinical classification of the craniosynostosis disorders. Although a reasonable correlation between clinical description and mutation has emerged, a notable exception is the P250R mutation in FGFR3. The phenotype is rather non-specific and quite variable, as witnessed by the variety of labels previously attached to patients who have subsequently turned out to have this mutation (see Box 1). This is a good example of a disorder that is better classified by mutation rather than phenotype. Another area of contention is the distinctiveness, or lack of it, of the syndromic labels of Crouzon, Pfeiffer and Jackson–Weiss. Classically, these have been distinguished primarily on examination of the limb. Although the limbs in Crouzon syndrome are supposed to be normal, radiological examination often reveals subtle abnormalities (68). The frequently quoted assertion that the phenotypes breed true within families lacks careful documentation and has many counter-examples (20,26,38,48).

MOLECULAR MECHANISM OF CRANIOSYNOSTOSIS MUTATIONS

All the mutations described to date are dominantly acting and, hence, the abnormal gene products must exert their effects in the presence of wild-type protein. The mechanisms of action have been the subject of keen investigation and provide some excellent examples of mechanisms of dominance (69).

A good starting point is to consider the effects of heterozygous null mutation. The phenotypes of *Fgfr1*+/– and *Fgfr3*+/– mice, and *FGFR3*+/– humans (4p– syndrome), are very different from the craniosynostosis syndromes resulting from mutation of the corresponding genes (13,70–73). By contrast, possible localisation of Saethre–Chotzen syndrome to human 7p was originally investigated because the phenotype appeared similar to patients with 7p deletions (74), and murine *twist*+/– heterozygotes, although described as normal in the original report (75), exhibit subtle cranial and digital abnormalities strikingly reminiscent of the human disorder (62). These and other observations suggest that the human MSX2 and FGFR mutations involve gain of function, whereas the TWIST mutations are largely loss of function (haploinsufficiency). This is supported by

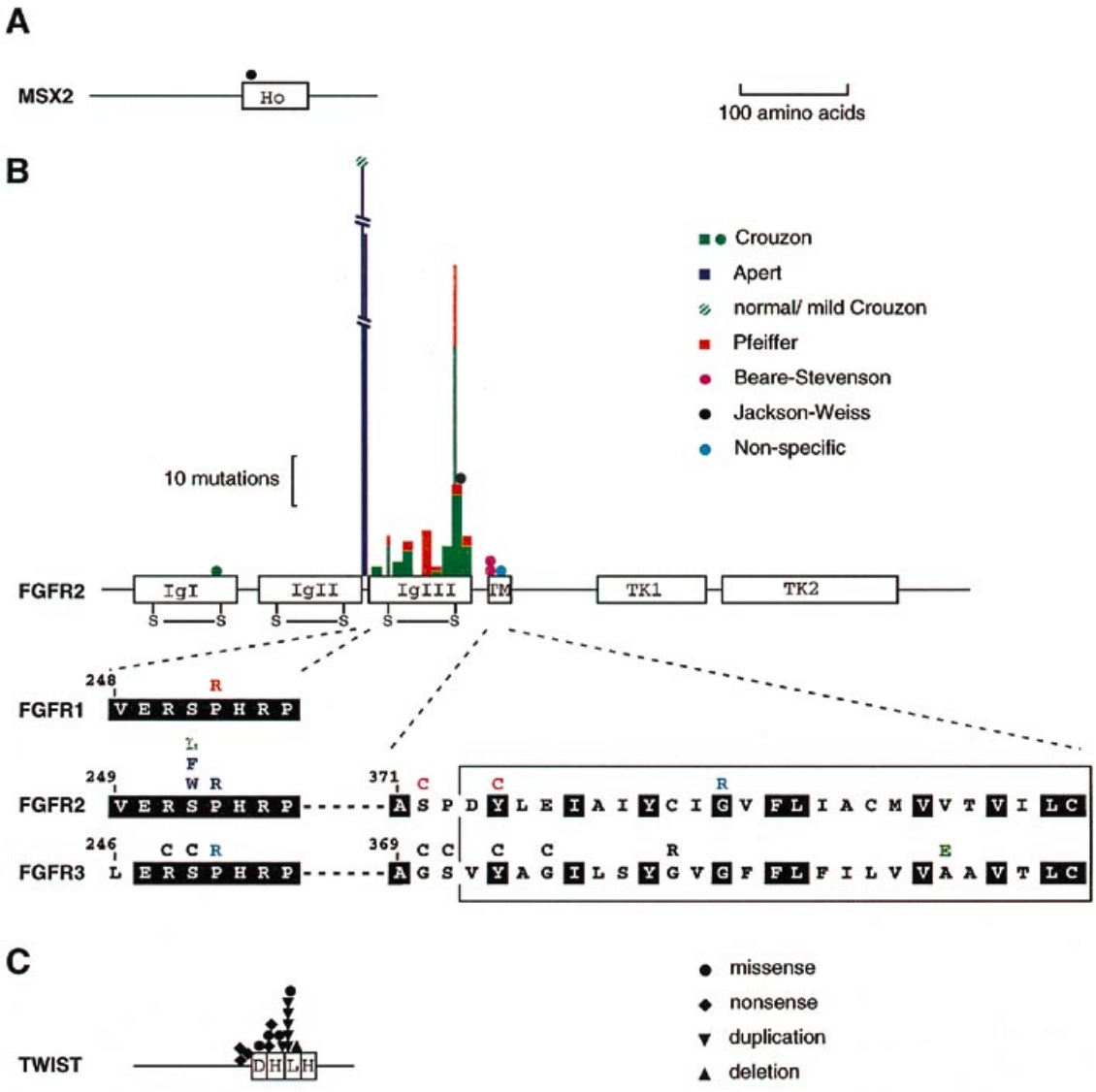

Figure 2. Structure of MSX2, FGFR2 and TWIST (drawn to scale) showing conserved motifs and mutations in craniosynostosis. (**A**) MSX2 is a transcription factor cloned by homology to the *Drosophila msh* gene and includes a highly conserved DNA-binding homeodomain (Ho). (**B**) FGFR2 is a transmembrane receptor tyrosine kinase. Ligand binds to extracellular immunoglobulin-like domains (IgI, IgII and IgIII), each of which contains a pair of disulphide linked cysteines. Ligand-induced dimerisation leads to autophosphorylation of the split tyrosine kinase (TK1 and TK2). The relative frequency of mutations in IgII–IgIII leading to Apert, Crouzon and Pfeiffer syndromes is indicated on the histogram. Coloured dots denote other rare but important mutations. Below, amino acid sequences of IgII–IgIII linker (FGFR1, -2 and -3) and transmembrane (TM) region (FGFR2 and -3) are given, with conserved residues highlighted in black. The rectangle surrounds the membrane-spanning region. Substitutions leading to craniosynostosis (coloured letters) or bone dysplasia (black letters) are shown above each sequence (in FGFR3, the mutations R248C, S249C, G370C, S371C and Y373C cause thanatophoric dysplasia type I; G375C and G380R cause achondroplasia). The double mutation S252F, P253S in FGFR2 is omitted for clarity. (**C**) TWIST is a transcription factor. Molecules dimerise via the helix–loop–helix (HLH) motif, binding DNA at the basic region D. For further information see refs 17 (MSX2), 65,66 (FGFR) and 61,62 (TWIST).

evidence of frequent nonsense mutations in the *TWIST* gene, but not in the *MSX2* or *FGFR* genes (Table 1 and Fig. 2). Several approaches have been taken to elucidate the gain of function mechanisms of the MSX2 and FGFR mutations. These are described below, and the conclusions summarised in Table 2.

MSX2

The P148H substitution occurs at position 7 of the highly conserved homeodomain, which is involved in both DNA and protein interactions. Although initial studies did not demonstrate any difference in the binding properties of the mutant protein to a target DNA sequence, more recent work has shown that the P148H substitution does confer enhanced DNA binding affinity with a reduced dissociation rate, without altering target specificity (76). Two groups have reported the production of mice carrying inserted *msx2* or *MSX2* transgenes, with markedly different effects on the phenotype (77,78). In one study, said to have achieved *msx2* overexpression of 2-fold or less (76), the mice were viable and a proportion developed premature cranial suture fusion, mimicking

the human disorder (77). In the other study, 13–22 copies of the *MSX2* transgene were integrated and the mice died around birth with severe craniofacial malformations, but with no evidence of craniosynostosis (78). No consistent differences in phenotype between mice carrying the normal and mutant versions of the transgene were observed in either study. The results suggest that normal craniofacial development is very sensitive to (wild-type) MSX2 dosage; the enhanced DNA binding affinity of the P148H protein may mimic the effect of a mild elevation in MSX2 dosage, sufficient to cause craniosynostosis but not the more severe malformations.

FGFR

The mechanism of FGFR craniosynostosis mutations has been reported in two experimental systems, *Xenopus* oocytes/embryos and HeLa cells. Blastomere injection of mRNA encoding the *Xenopus* FGFR2 mutation C332Y (corresponding to C342Y in the human), but not wild-type mRNA, caused elongation of animal pole ectoderm and induction of *Xbra*, a marker of mesodermal expression, mimicking the effects of exogenous fibroblast growth factor 1 (FGF1). Mutant FGFR2 protein, assayed after mRNA injection of oocytes, demonstrated greater binding to antiphosphotyrosine antibodies and higher kinase activity than wild-type, yet was unable to bind FGF1. Under non-reducing but not reducing conditions, a slower migrating form of the mutant protein was apparent, consistent with dimerisation. These data were interpreted as showing two distinct consequences of the Cys→Tyr mutation: (i) abolition of FGF1 binding due to disruption of the IgIII domain but also (ii) the mutated cysteine leaves an unpaired partner (C268 in *Xenopus*, C278 in human) which is free to bond covalently with another mutant molecule, resulting in ligand-independent constitutive activation (79). A prediction of this model is that homozygosity for the mutation would cause early embryonic lethality, due to abolition of FGF binding.

In a follow up paper, further FGFR2 mutations were studied in *Xenopus* (80). Injection of C268F mutant mRNA gave similar results to those for C332Y. Co-injection with mRNA encoding a dominant-negative FGFR1 molecule caused the mesoderm inducing effect to be competed out (the dominant-negative FGFR1 forms non-productive heterodimers, demonstrating that the mutant FGFR2 needs to dimerise for its action). A double mutant, C268F/C332Y (which lacks an unpaired cysteine), failed to induce elongation of animal pole ectoderm although it did exhibit increased phosphotyrosine levels.

In an alternative approach, the ability of human FGFR2 mutants to induce focus formation when transfected into NIH 3T3 cells was measured (81). Full length FGFR2 (normal or mutant) was inactive in this assay, so chimeric molecules containing the transmembrane and/or tyrosine kinase portions of the NEU receptor were constructed. Broadly similar results to the *Xenopus* experiments, including evidence of mutant receptor cross-linking, were obtained for the mutations Y340H, C342Y and S354C. The findings for Y340H suggest that intermolecular cross-linking between cysteines may occur even when the cysteines remain as a pair: this may be due to unfolding of the domain leading to exposure of the buried cysteines (11,82).

The mechanism of the transmembrane mutation G384R in FGFR2 has not been studied directly, but several groups have investigated the similar (Fig. 2) G380R achondroplasia mutation in FGFR3. A variety of evidence suggests that this mutation shows weak ligand independent activation (reviewed in 67). The basic side chain Arg is presumed to form a transient hydrogen bond with the transmembrane helix of a partner molecule, rendering the receptor monomers slightly ‘sticky’. The Crouzon/acanthosis nigricans mutation A391E in FGFR3 might also form intermolecular hydrogen bonds, but the qualitative differences in phenotype with achondroplasia show that there must be differences in how this is executed. One suggestion is that the A391E/FGFR3 mutant might act through heterodimerisation with a normal FGFR2 molecule (67).

The mechanism of the paralogous Pro→Arg mutations in the IgII–IgIII linker of FGFR1, -2 and -3 appears to be distinct. Evidence from the naturally occurring mutations shown in Figure 2 suggests that it results from a highly specific interaction (summarised in 32). It is of interest that in *Drosophila* DFR2 and *Caenorhabditis* egl-15, an arginine occurs naturally at the corresponding position of these FGFR homologs (83), indicating that in a different biological context the presence of Arg is consistent with normal receptor function. We have speculated that substitution to bulky residues in this linker region might alter the relative orientation of the IgII and IgIII domains and hence mimic or accentuate the effects of ligand binding (11). Tentative evidence for this was found in the *Xenopus* system, in which greater binding of FGF1 and FGF2 to translated receptor was consistently observed after injection of *FGFR1* mRNA encoding the P160R mutation, compared with wild-type. No elongation of animal pole ectoderm or increased tyrosine kinase activity was however observed (80). In a different approach, surface plasmon resonance analysis has been used to investigate in real time the binding of FGFs to normal and mutant FGFR2 constructs. Reduced dissociation of FGF2 from FGFR2 constructs containing the Apert mutations S252W or P253R, compared to wild-type, was observed (J.Anderson, H.D.Burns, P.Enriquez-Harris, A.O.M.Wilkie and J.K. Heath, manuscript in preparation).

Table 2. Proposed mechanisms of dominance in craniosynostosis mutations

Mechanism	Example
Haploinsufficiency	TWIST
Structural disruption	FBN1
Reduced dissociation of ligand	P148H in MSX2 (DNA binding)
	S252W and P253R in FGFR2 (FGF2 binding)
Covalent cross-linking of Cys	C278F, Y340H, C342Y, S354C in FGFR2
Transmembrane hydrogen bonding	?G384R in FGFR2, A391E in FGFR3

In summary, a variety of evidence points to the activating nature (constitutive or prolonged signalling) of FGFR2 mutations and this is largely corroborated by work on FGFR3 mutations in bone dysplasia (reviewed in 67). However, one study of FGF2-induced calcium signalling in fibroblasts from patients with achondroplasia and thanatophoric dysplasia gave results apparently inconsistent with other findings. Cells heterozygous for either R248C or S371C, or homozygous for the G380R mutation in FGFR3 (Fig. 2) exhibited a defective response to FGF2, leading the authors to suggest that the *in vivo* effect of these mutations was actually dominant-negative (84). A possible resolution of this paradox is discussed in the next section.

CRANIAL SUTURE MORPHOGENESIS AND CRANIOSYNOSTOSIS

The modern techniques of developmental biology have only recently been focused on the cranial sutures, following the discovery of craniosynostosis mutations in humans. There is still very little known about the molecular and cellular factors controlling the balance between proliferation and differentiation in these structures. It has been demonstrated that *Msx2* (17) and *Fgfr2* (85), as well as *transforming growth factor* β *1–3* (86,87) are expressed in mouse or rat sutures. In the case of *Fgfr2*, the domain of RNA expression coincides with active cell proliferation, but is mutually exclusive with *osteopontin*, an early marker of bone differentiation (85). This suggests that *Fgfr2* is a marker of proliferative, uncommitted cells of the suture, and is switched off as a cause or consequence of osteogenic differentiation.

This conclusion raises a paradox. If FGFR2 is associated with the undifferentiated state, why do activating mutations apparently cause differentiation, leading to craniosynostosis? Recently, use of *ex-utero* surgical techniques on fetal mice has shed new light on this question. Implantation of FGF2-soaked beads over the coronal suture disrupted the normal suture and resulted in the ectopic expression of *osteopontin*. *Fgfr*2 expression was absent from the immediate area underlying the bead, but was apparent as a ring surrounding its margin (85). This suggests that excessive FGF2 signalling (i) does result in osteogenic differentiation, and (ii) is associated with down-regulation of FGFR2 in the experimental system. The former conclusion is reminiscent of recent work on thanatophoric dysplasia type II, indicating that different intensities of FGFR3 signalling have qualitatively distinct cellular consequences (88,89), whilst the latter explains the finding that cranial sutures from patients with Crouzon syndrome contain a lower proportion of cells positive for FGFR2 antibody than sutures from control individuals (90). A reciprocal relationship between FGF production and FGFR expression has been observed previously (91,92).

Under this model, the behaviour of cells carrying FGFR2 mutations may be viewed as a subtle balance between two opposing forces: the intrinsically activating nature of the mutations, and the tendency for activation to cause down-regulation. This balance may change with time, place and cellular identity. This view would accommodate the paradoxical 'dominant-negative' effect of FGFR3 mutations on FGF2-induced calcium signalling in fibroblasts, described above (84). More speculatively, it may also begin to explain the mystery of why these mutations are neither lethal in early embryogenesis, nor associated with marked predisposition to neoplasia in later life.

How do TWIST and MSX2 link up with FGFR in biogenesis of the suture? Nothing is known about this at present, but there is a growing consensus that certain developmental pathways are conserved between *Drosophila* and vertebrates (93) and suture biogenesis could represent a further example. *twist* is well established as a critical gene for mesoderm induction in *Drosophila*, and later functions as a myogenic switch (94). Expression of a fibroblast growth factor receptor, *DFR1*, depends on *twist* (95,96) and null mutants for *DFR1* (*heartless*) are defective in muscle formation and show abnormal directional cell migration (97,98). Mesodermal expression of the *msh* gene is turned on later in myogenesis and is abolished in *twist* mutants (99,100). Certainly, the idea that aspects of mesoderm formation in *Drosophila*, and development of the cranial suture (a mesodermal structure) involve conserved pathways represents a reasonable working hypothesis, which has the benefits of harnessing the power of *Drosophila* genetics to the study of the human.

MUTATION HOTSPOTS IN *FGFR*s AND *TWIST*?

One further problem remains. It is clear that the spectra of mutations observed in the *TWIST* and *FGFR* genes are highly non-random. Although relatively few mutations have been described in *TWIST*, 21 bp duplications (with three distinct molecular origins) comprise about one third. This is interesting, but the explanation can be accommodated within conventional molecular biology, as a repeat unit with 21 bp periodicity is present in this region (62). Something more remarkable seems to be happening with the *FGFR* mutations in craniosynostosis and bone dysplasia. It is unlikely to be coincidental that the three highest germline point mutation rates described in the human (elevated ~1000-fold over background) all concern FGFRs: G380R in FGFR3 (101), P250R in FGFR3 (54) and S252W in FGFR2 (25). Increased paternal age associated with achondroplasia and Apert syndrome has long been suspected (reviewed in 102), and an exclusively paternal origin of mutation was shown in studies of 57 Apert syndrome (25) and 10 achondroplasia patients (103). This implicates spermatogenesis as being specifically involved in the elevated mutation rate, but the mechanism is not known. Conventional explanations, e.g., gene conversion appear unlikely given the diverse pattern of mutations observed. Circumstantial evidence for an alternative hypothesis, that the mutation rate is not elevated per se but that germ cells carrying the mutation have a selective advantage, is discussed elsewhere (32).

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