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Differential Effects of FGFR2 Mutations on Syndactyly and Cleft Palate in Apert Syndrome

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Summary

Apert syndrome is a distinctive human malformation characterized by craniosynostosis and severe syndactyly of the hands and feet. It is caused by specific missense substitutions involving adjacent amino acids (Ser252Trp or Pro253Arg) in the linker between the second and third extracellular immunoglobulin domains of fibroblast growth factor receptor 2 (FGFR2). We have developed a simple PCR assay for these mutations in genomic DNA, based on the creation of novel *Sfi*I and *Bst*UI restriction sites. Analysis of DNA from 70 unrelated patients with Apert syndrome showed that 45 had the Ser252Trp mutation and 25 had the Pro253Arg mutation. Phenotypic differences between these two groups of patients were investigated. Significant differences were found for severity of syndactyly and presence of cleft palate. The syndactyly was more severe with the Pro253Arg mutation, for both the hands and the feet. In contrast, cleft palate was significantly more common in the Ser252Trp patients. No convincing differences were found in the prevalence of other malformations associated with Apert syndrome. We conclude that, although the phenotype attributable to the two mutations is very similar, there are subtle differences. The opposite trends for severity of syndactyly and cleft palate in relation to the two mutations may relate to the varying patterns of temporal and tissue-specific expression of different fibroblast growth factors, the ligands for FGFR2.

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Introduction

Apert syndrome (acrocephalosyndactyly type I; ACS I) was first recognized at the beginning of this century (Apert 1906). The sexes are affected with equal severity, and rare instances of vertical transmission are consistent with autosomal dominant inheritance (Rollnick 1988; Lewanda et al. 1993), although most cases arise by new mutation, with a paternal age effect (Blank 1960; Erickson and Cohen 1974; Risch et al. 1987). The birth prevalence from pooled North American and European data has been estimated as 1/65,000 (Cohen et al. 1992).

The hallmarks of Apert syndrome are craniosynostosis (Cinalli et al. 1995) and severe, symmetrical syndactyly of the hands and feet, which can be graded according to severity (Cohen and Kreiborg 1995). A variety of other malformations occur at lower frequency. In one series, cleft soft palate or bifid uvula was found in 76% of 75 patients (Kreiborg and Cohen 1992), and fusions of the cervical vertebrae, especially at the C5–C6 level, were found in 68% of 68 patients (Kreiborg et al. 1992). From a series of 136 patients, minimum values have been estimated for the prevalence of cardiovascular defects (10%) and genitourinary abnormalities (9.6%), with gastrointestinal and respiratory anomalies (1.5% each) occurring at lower frequencies (Cohen and Kreiborg 1993*b*). Generalized dilution of skin and hair color occurs in some patients, which is less severe than in oculocutaneous albinism but can manifest with iris transillumination and photophobia (Margolis et al. 1977). Neurodevelopment is frequently affected: in a retrospective study of hospital records on 29 patients, IQ scores were >70 in 48%, 50–70 in 31%, 35–49 in 14%, and <35 in 7% (Patton et al. 1988). A recent study of sleeping intracranial pressure in 13 patients showed raised (>15 mmHg) or borderline raised (10–15 mmHg) pressures in 5 and 7 cases, respectively (Thompson et al. 1995); intracranial malformations (e.g., agenesis of the corpus callosum) may also contribute to learning difficulties (Cohen and Kreiborg 1990). It is not understood why all patients with Apert syndrome have craniosynostosis and syndactyly, yet the other malformations are variable. Before the genetic basis of the disorder was

elucidated, it was difficult to reconcile these diverse clinical features in terms of a single arrested developmental process or pathological mechanism.

We previously identified specific missense substitutions of fibroblast growth factor receptor 2 (FGFR2) involving adjacent amino acids in the linker between the second (IgII) and third (IgIII) immunoglobulin-like domains (either serine 252 to tryptophan [S252W] or proline 253 to arginine [P253R]), in all 40 unrelated cases of Apert syndrome studied (Wilkie et al. 1995*b*). Allelic mutations in the main part of the IgIII domain of FGFR2 have been identified in the Pfeiffer and Jackson-Weiss craniosynostosis syndromes, in which the limb malformations are milder than in Apert syndrome (Jabs et al. 1994; Lajeunie et al. 1995; Rutland et al. 1995; Schell et al. 1995), and in Crouzon syndrome, in which the limbs usually are normal (Jabs et al. 1994; Reardon et al. 1994; Gorry et al. 1995; Ma et al. 1995; Oldridge et al. 1995; Park et al. 1995*a;* Steinberger et al. 1995). Whereas these latter craniosynostosis syndromes result from a variety of different FGFR2 point mutations, with, in some cases, identical mutations giving rise to more than one syndrome, the mutations responsible for Apert syndrome are very specific. All these mutations in FGFR2, the murine homologue of which is expressed in both skull and limb at early stages of development (Peters et al. 1992; Orr-Urtreger et al. 1993), share the common feature of abnormal cranial suture morphogenesis, leading to craniosynostosis. However, despite their proximity within the extracellular region of the FGFR2 molecule, they exert different effects on development: the Apert mutations stand apart from the others because of the severe syndactyly and the higher frequency of additional malformations. The pathogenic mechanism of these differences is not known.

Although the two adjacent mutations that cause Apert syndrome give a characteristic phenotype, in our initial study the mutations showed subtle differences in the severity of syndactyly: the S252W substitution was associated with less severe syndactyly than was P253R (Wilkie et al. 1995*b*). Subsequently, Park et al. (1995*b*) found one or other of these substitutions in 34/35 unrelated Apert syndrome patients, but they concluded that, in terms of phenotypic features, there were no differences between the two mutations. To extend our understanding of the phenotype and to investigate possible genotype-phenotype correlations in detail, we have evaluated clinically an expanded series of 87 patients with Apert syndrome and have determined the genotype in 66 of these (64 of whom are unrelated) and in 6 additional patients. This analysis confirms the remarkably specific nature of the two Apert mutations, which account for all 70 unrelated patients in our series, and indicates that there are subtle but statistically significant differences in the phenotypes attributable to the two mutations.

Subjects and Methods

Ascertainment of Patients

Children and adults with Apert syndrome were ascertained through the three U.K. craniofacial units in Oxford, London, and Birmingham and by informal contacts with other surgeons and geneticists. Photographs or clinical details on four patients have been published by other authors (Narayan and Scott 1991; David et al. 1982; Henderson et al. 1995). Appropriate Ethics Committee approval was obtained. Initially the patients were invited to join the study by letter from a specialist surgeon or geneticist known to them. An information sheet was included with the letter, as was a consent form which the family were asked to complete and return. After consent was obtained, each patient and/or his or her parents were interviewed, and the clinical history and phenotype were assessed and recorded. Blood samples were taken for cytogenetic analysis, DNA extraction, and establishment of Epstein-Barr virus–transformed cell lines.

Clinical Measurements

For each patient, a detailed clinical examination was performed, which included clinical photographs and review of radiological tests where available. Aspects of the phenotype that were assessed are listed in table 1. An additive preoperative craniofacial severity score (range 0–5) was calculated from assessments, made independently by two clinical dysmorphologists, of the presence (score 1) or absence (score 0) of five facial features, comprising severe supraorbital ridging, marked maxillary hypoplasia, ocular proptosis, down-slanting palpebral fissures, and facial asymmetry (fig. 1). The ventricular size index was derived from preoperative computed-tomography scans, as a measure of the degree of ventriculomegaly: a value >35% indicates significant enlargement of the lateral cerebral ventricles (Lee and Rao 1987). Active hydrocephalus requiring insertion of a ventricular shunt was identified by evidence of progressive ventricular enlargement and/or raised intracranial pressure. A measure of the level of educational attainment was made on the basis of whether the child attended mainstream school or required special needs education because of developmental delay. The severity of syndactyly was assessed both clinically and radiologically (see below). All the clinical measurements and scores were made independently of the mutational analysis. Statistical comparison of number of affected cases with S252W and P253R mutations employed the *t*-test for normally distributed variables, the Mann-Whitney *U*-test for variables of unknown distribution, and the *G*-test of independence for discontinuous classes (Sokal and Rohlf 1981).

Table 1

Analysis of Genotype in Relation to Phenotype in Apert Syndrome

	S252W		P253R		No Genotype		Total				
	n	Mean ± SD[a]	*n*	Mean ± SD[a]	*n*	Mean ± SD[a]	*n*	Mean ± SD[a]	G[b]	df	*P*
Population:											
Male	16		12 (11[c])		9		37				
Female	26		12 (11[c])		12 (10[d])		50 (48[d])		.86	1	NS
Total	42		24 (22)		21		87				
Age at examination (years)		10.5 ± 8.6		12.5 ± 13.7		6.7 ± 6.4		10.1 ± 10.0			NS[e]
Median age (years)		9.65		5.6		3.7		7.0			
Maternal age (years)	38	30.40 ± 4.62	22	28.23 ± 5.45	16	27.26 ± 5.23	76	29.11 ± 5.11	*t* = 1.64	58	NS
Paternal age (years)	37	33.52 ± 5.81	21	32.01 ± 5.25	13	35.26 ± 7.16	71	33.39 ± 5.93	*t* = .99	56	NS
Birthweight (kg):[f]											
Male	15	3.66 ± .65	7	3.63 ± .63	7	3.47 ± .50	29	3.61 ± .60			NS
Female	19	3.31 ± .43	11	3.74 ± .45	8	3.63 ± .56	38	3.50 ± .49	*t* = 2.57	28	.02
Craniofacial score	42	2.43 ± 1.15	23	1.74 ± 1.14	21	2.05 ± 1.16	86	2.15 ± 1.17	3.69	2	NS
Cleft palate (including bifid uvula)	41	24 (58.5%)	23	4 (17.4%)	21	9 (42.9%)	85	37 (43.5%)	10.5	1	.002
Choanal stenosis	42	7 (16.7%)	23	1 (4.3%)	13	3 (23.1%)	78	11 (14.1%)	2.26	1	NS
Congenital heart defect	42	7 (16.7%)	23	0 (0%)	21	2 (9.5%)	86	9 (10.5%)	6.07	1	.02
Cervical spine fusion	19	15 (78.9%)	9	5 (55.5%)	15	9 (60.0%)	43	29 (67.4%)	1.46	1	NS
Hypopigmentation	42	13 (31.0%)	23	5 (21.7%)	21	5 (23.8%)	86	23 (26.7%)	.62	1	NS
Ventricular size index	21	32.5 ± 9.7	12	38.2 ± 6.0	13	37.4 ± 9.4	46	35.4 ± 9.0			NS[e]
Ventricular shunt	42	4 (9.5%)	23	1 (4.3%)	21	3 (14.3%)	86	8 (9.3%)			NS
CNS malformation:											
Corpus callosum	22	3	13	1	13	0	48	4			
Posterior fossa		1		2		2		5			
Other		1		0		0		1			
Total		5 (22.7%)		3 (23.1%)		2 (15.4%)		10 (20.8%)			NS
Special needs education	30	13 (43.3%)	13	4 (30.8%)	11	7 (63.6%)	54	24 (44.4%)	.58	1	NS
Hand morphological score	42	1.50 ± .59	24	2.04 ± .91	21	1.86 ± .85	87	1.73 ± .78	13.6	2	.002
Hand radiological score	18	1.33 ± .49	14	1.79 ± .43	12	1.58 ± .51	44	1.55 ± .50	6.5	1	.02
Foot morphological score	42	2.12 ± .59	24	2.79 ± .41	20	2.35 ± .49	86	2.36 ± .59	19.4	1	<.001

[a] Column shows either mean ± SD or no. (%) of cases with feature.
[b] Statistical comparison of S252W and P253R mutations is by *G*-test or *t*-test. NS = not significant.
[c] Excludes affected offspring of affected parent.
[d] Two individuals were already deceased at the time of the study.
[e] Mann-Whitney *U*-test.
[f] Excludes babies born at <37 wk gestation.

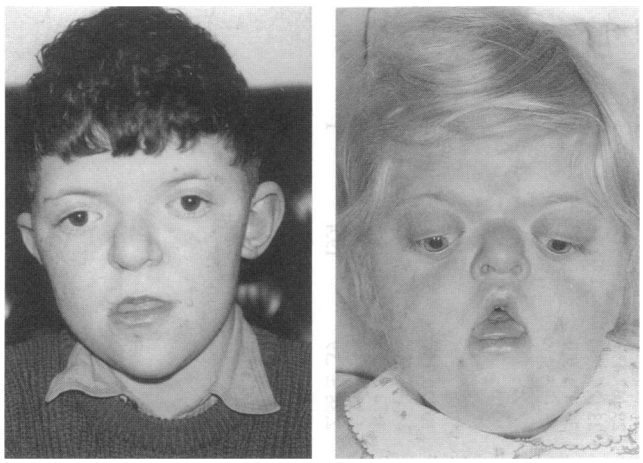

Figure 1 Craniofacial appearance in Apert syndrome. The craniofacial score (range 0–5) was calculated from the preoperative facial phenotype: the presence of five facial features, comprising severe supraorbital ridging, marked maxillary hypoplasia, ocular proptosis, down-slanting palpebral fissures, and facial asymmetry. *Left,* Mild facial phenotype (score 0). *Right,* Severe facial phenotype (score 5).

Definition of Syndactyly Scores

The hand and foot morphological scores were based on a previous classification (Upton 1991). In the Apert syndrome hand (fig. 2), the central three digits are always syndactylous: in the mildest form (hand morphological score 1), the thumb and part of the little finger

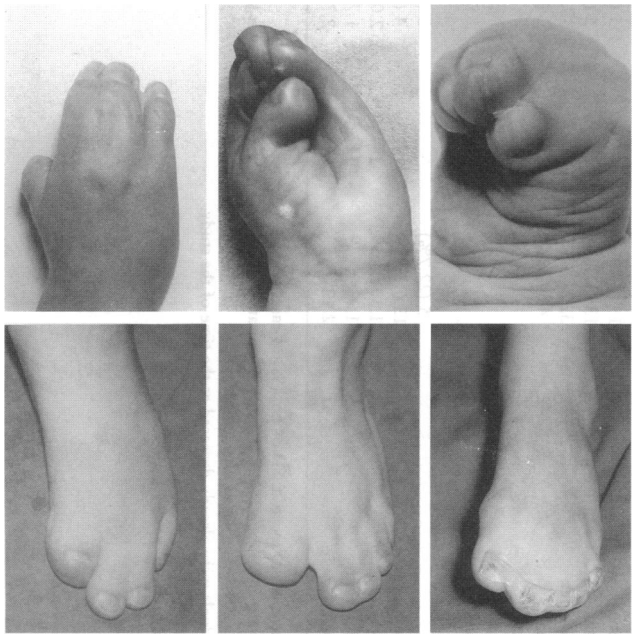

Figure 2 Range of severity of syndactyly in Apert syndrome, and classification of hands (*upper panels*) and feet (*lower panels*), by using a morphological score. *Left,* Score 1 (least severe). *Center,* Score 2 (intermediate). *Right,* Score 3 (most severe).

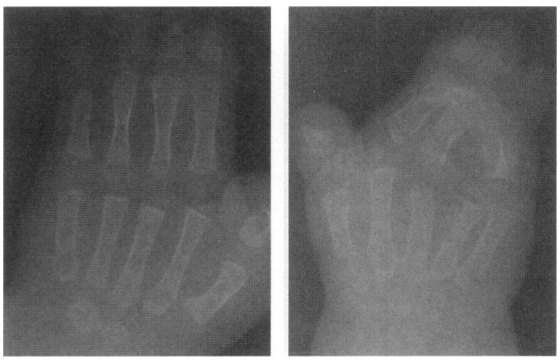

Figure 3 Radiological features of the hand in Apert syndrome. *Left,* Fusions between the proximal and middle phalanges, in the proximal-distal axis only (hand radiological score 1). *Right,* Severe fusions occurring mainly between the middle and distal phalanges, across the anterior-posterior axis of the hand (hand radiological score 2).

are separate from the syndactylous mass; in the second type (score 2), the little finger is not separate; and, in the third type (score 3), the thumb and all the fingers are included in the syndactyly. Similarly, syndactyly in the foot may involve three or fewer digits (foot morphological score 1), involve digits 2–5 with a separate big toe (score 2), or be continuous (score 3). The hand radiological score was assigned on the basis of examinations of preoperative hand x-rays, with the help of two paediatric radiologists. Two distinct patterns of bony malformation were observed: the presence of fusions in the proximal-distal axis only (score 1) or severe fusions occurring across the anterior-posterior axis of the hand (score 2) (fig. 3).

Genomic Analysis of Apert Mutations

To enable analysis of Apert mutations in genomic DNA, we used inverse PCR (Triglia et al. 1988) to obtain the DNA sequence of the 3′ end of the intron immediately upstream of the IIIa exon of FGFR2 (for explanation of exon nomenclature, see Wilkie et al. 1995*b*). Genomic DNA (10 μg) from a normal individual was digested with either *Nla*III or *Eae*I, diluted to ~6 μg/ml, and incubated with 20 U T4 DNA ligase at 16°C overnight. After heat inactivation, 500 μl was digested with *Bsa*AI and was phenol/chloroform extracted and ethanol precipitated. PCR was performed by use of the primers Inv 1 (5′-TCAAGGTTCTCAAGGTGAGGAC-3′) and Inv 2 (5′-GACCACTGTGGAGGCATTTG-3′), designed from previously published sequence (Dionne et al. 1990; Miki et al. 1992), yielding products of 217 bp and ~1.4 kb for the *Nla*III and *Eae*I digests, respectively. These were cloned into pCR-Script (Stratagene) and were DNA sequenced.

To analyze the Apert mutations, PCR was performed by use of the intron primer 6/7AF (5′-GGTCTCTCA-TTCTCCCATCCC-3′), with the previously described

primer 5S (Wilkie et al. 1995*b*) and the buffer of Gyapay et al. (1994) in a volume of 25 μl. After a 4-min denaturation at 94°C, amplification was initiated by addition of primers and 0.4 U AmpliTaq (Perkin Elmer). Thirty-five cycles of annealing (1 min at 60.5°C) and denaturation (1 min at 94°C) were performed, the upward temperature shift being ramped at 1 °C/s, on a Hybaid OmniGene Temperature Cycler. There was a final extension step of 10 min. The 159-bp product was digested with either *Sfi*I or *Bst*UI (New England Biolabs) and was analyzed on 4% Metaphor (Flowgen) gels. Although Park et al. (1995*a*, 1995*b*) have also described a primer sequence (5′-TGACAGCC[C]TCTG[G]ACAACACAAC-3′) upstream of exon IIIa for genomic diagnosis of Apert mutations, there are two missing nucleotides (shown in square brackets), compared with the usual wild-type sequence, according to our data from nine independent chromosomes (D. M. Moloney and A. O. M. Wilkie, unpublished data), so amplification using this primer may be unreliable.

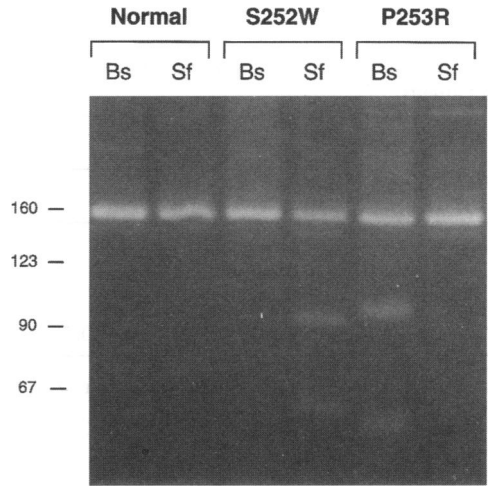

Figure 4 Mutational analysis by restriction-enzyme digestion of genomic DNA from patients with Apert syndrome. The 159-bp 6/7AF-5S PCR product was digested with *Bst*UI (Bs) or *Sfi*I (Sf) and was analyzed on a 4% Metaphor gel. The S252W mutation cuts, with *Sfi*I, into 96-bp + 63-bp fragments; the P253R mutation cuts, with *Bst*UI, into 100-bp + 59-bp fragments. Size markers are *Msp*I-cut pBR322.

Results

Mutational Analysis of 72 Patients with Apert Syndrome

Mutational analysis from genomic DNA was undertaken in 66 (64 unrelated) of the 87 patients in the clinical study, together with 6 additional unrelated patients on whom detailed phenotypic information was not available. The S252W and P253R mutations, which correspond at the DNA level to C→G transversions at positions 934 and 937, respectively, of the FGFR2 cDNA sequence (Wilkie et al. 1995*b*), create unique *Sfi*I and *Bst*UI restriction sites, respectively, in the 159-bp 6/7AF-5S PCR product and hence are easily determined (fig. 4). All 72 patients had one or other of these mutations: 45 (64%) had S252W, and 27 (25 unrelated or 36%) had P253R. These results confirm the remarkable specificity of the mutational basis of Apert syndrome.

Genotype-Phenotype Correlations

Eighty-seven patients with Apert syndrome (85 living and 2 deceased) were ascertained for the clinical study. Table 1 summarizes the demographic and phenotypic features, both of the group as a whole and as classified on the basis of genotype: S252W (n = 42), P253R (n = 24), and genotype not determined (n = 21). Each aspect of the phenotype was analyzed for possible correlations with the genotype.

Demographic characteristics.—The total number of males was 37, and the total number of females was 50, giving a male:female ratio of 1:1.35. The S252W and P253R groups did not differ significantly in either their sex ratio or average age at the time of the study. Although there were relatively more infants (age <1 year) and adults in the P253R group, this was not statistically significant. The average age of the parents at the birth of the affected individual did not differ significantly between the two mutations, for either mothers or fathers. Overall, both the mean maternal age (29.1 years) and the mean paternal age (33.4 years) exceeded the population mean and were consistent with the previously documented paternal age effect (Blank 1960; Erickson and Cohen 1974; Risch et al. 1987): an analysis of these data is presented elsewhere (Moloney et al., in press). The four Apert syndrome individuals who had reproduced all carried the rarer, P253R mutation, but Park et al. (1995*b*) described an S252W case with an affected child: larger numbers would be needed to determine whether there is any difference, in reproductive potential, between patients with the P253R mutation and patients with the S252W mutation.

Growth.—The average birthweight of babies born at or after 37 wk gestation was 3.61 kg for males and 3.50 kg for females, compared with normal values (from standard centile charts) of 3.50 kg and 3.40 kg, respectively. Possible reasons for the higher birthweight of Apert syndrome babies have been discussed elsewhere (Cohen and Kreiborg 1993*a*). For male birthweight, we found no significant difference between the S252W and P253R mutations, but female P253R babies were heavier (P = .02) than female S252W babies. However, this may be a chance association, since female P253R babies were also heavier than male babies who had the same mutation.

Growth was analyzed by determining the age-equiva-

Table 2

Relationship of Hand and Foot Morphological Scores in Apert Syndrome Patients

Foot Score	Hand Score 1	2	3	Total (%)
1	3	1	1	5 (5.8)
2	26	16	2	44 (51.2)
3	11	11	15	37 (43.0)
Total (%)	40 [41[a]] (47.1)	28 (32.2)	18 (20.7)	

[a] In one patient with a hand score of 1, a foot score was not obtained.

lent centile for height at examination and then plotting the centile distribution for each type of mutation (data not shown). The median height for S252W fell in the 25–50th centile, and that for P253R fell in the 50–75th centile. Cohen and Kreiborg (1993*a*) have drawn attention to the complex relationship that exists between stature and age in Apert syndrome; hence, rigorous statistical analysis was not attempted. However, between the two mutations, no gross difference in patients' height was apparent.

Craniofacial phenotype.—The severity of craniofacial phenotype varies markedly between Apert syndrome patients (fig. 1), and we quantified this by using a five-point preoperative severity score (see Subjects and Methods). The mean score was higher for the S252W group than for the P253R group, and, although this difference was not significant, it is of interest that, of those patients genotyped, all three very mildly affected patients (score 0) had the P253R mutation, whereas the two most severely affected patients (score 5) both had the S252W mutation. Convincing evidence that the S252W mutation is associated with a more severe craniofacial phenotype came from the analysis of cleft soft palate (including bifid uvula), which had an overall prevalence of 43.5%. This occurred in 24/41 of the S252W patients but in only 4/23 of the P253R patients ($P = .002$). Choanal stenosis, a relatively uncommon (prevalence 14.1%) malformation in neonates with Apert syndrome, was also more frequent in the S252W group, although not significantly so (table 1).

Cardiac malformations.—Congenital heart defects were found in nine (10.5%) individuals: six had small ventricular septal defects, and there were single cases each of atrial septal defect, coarctation of the aorta, and dextrocardia. All seven cases that were genotyped carried the S252W mutation ($P = .02$). In the series reported by Park et al. (1995*b*), cardiac defects were found in 4/25 S252W patients and in 2/9 P253R patients: when the two series are combined, the difference in prevalence of heart defects, between the mutations, is not significant ($G_1 = 2.1$).

CNS.—CNS malformations (including agenesis or hypogenesis of the corpus callosum, posterior fossa abnormalities, and other defects), assessed from preoperative CT scans, were present in 20.8% of those scanned. This may overestimate the overall frequency of CNS malformations, because symptomatic individuals were more likely to have been scanned. Active hydrocephalus requiring a ventricular shunt was present in 9.3% of patients, and significant ventriculomegaly was present in 48.5% of patients. To assess intellectual attainment, attendance at schools for learning difficulties was used as an approximate guide: 44.4% of 54 children >5 years old required special education. None of the CNS outcome measures differed significantly between the two mutational types.

Limb phenotype.—The limb phenotype was graded according to severity (see Subjects and Methods): a morphological score was recorded for both the hands and feet (fig. 2), and a radiological score (fig. 3) was recorded for the hands only (few foot x-rays were available, because the feet are rarely treated surgically). The relationship of hand and foot morphological scores of all patients in the study is shown in table 2. There was a significant tendency for severe hand scores to be associated with severe foot scores ($G_2 = 16.3$, $P < .001$). For the hand radiological scores, a similar number of patients had each of the two scores (20 had score 1, and 24 had score 2).

A comparison of the mean morphological and radiological limb scores for each class of mutation is shown in table 1. When both morphological and radiological criteria for the hands, as well as morphological criteria alone for the feet, were considered, there was a significant tendency (P values ranging between .02 and <.001) for the P253R mutation to be associated with more severe syndactyly than was S252W. Figure 5 illustrates the difference in distribution of hand and foot morpho-

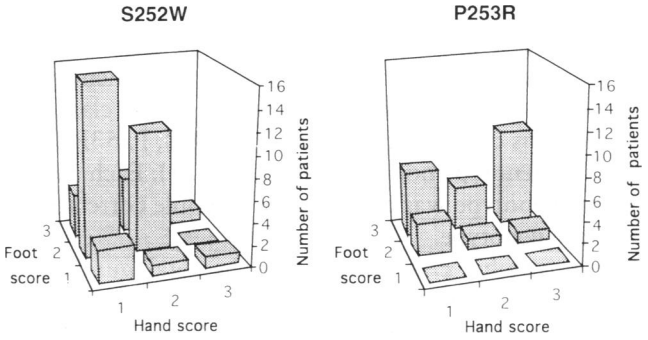

Figure 5 Distribution of hand and foot morphological scores in patients with the S252W mutation (*left*) and the P253R mutation (*right*).

logical scores in individual patients with one or the other of the two mutations.

Miscellaneous malformations.—Cervical spine fusion was frequent, being observed in 29 (67.4%) of 43 patients for whom radiographs were available. Pigmentary dilution of the skin was present in 23 (26.7%) of 86 patients. Urogenital malformations (three cases of inguinal hernia, one case of ectopic ureters, and one case of undescended testes) were unusual. None of these features was significantly associated with genotype.

Discussion

Narrow Mutational Spectrum in Apert Syndrome

The detection of either the S252W mutation or the P253R mutation in FGFR2 in all 40 unrelated patients in our original study of Apert syndrome (Wilkie et al. 1995*b*) suggested that this complex malformation arises from a very limited mutational spectrum. The present work provides further evidence for this, one or other of these mutations being present in all 30 additional patients genotyped. When our results are combined with those of Park et al. (1995*b*), of a total of 105 unrelated Apert syndrome patients, 70 have the S252W mutation, 34 have the P253R mutation, and in 1 patient the mutation was not identified. It is clear that the mutational spectrum of Apert syndrome is very narrow, which contrasts with the greater diversity of mutations of the IIIa and IIIc domains of FGFR2 that are responsible for Crouzon and Pfeiffer syndromes (reviewed by Muenke and Schell 1995; Wilkie et al. 1995*a*).

An understanding of the biological basis of this exquisite mutational specificity will require a combination of genetic, embryological, biochemical, and, ultimately, structural approaches. As a baseline, we wished to document whether the two common Apert mutations were associated with any phenotypic differences that would need to be incorporated into models of their pathophysiologic mechanism.

Significance of Genotype-Phenotype Correlations

Variability in the clinical manifestations of Apert syndrome is well documented. To what extent can phenotypic differences be accounted for in terms of the two alternative mutations? Table 1, which summarizes our observations, shows that, although in most respects the phenotypes associated with the S252W and P253R mutations were not statistically distinguishable, there were apparently significant differences ($P \leq .05$) in female birthweight, in frequency of posterior cleft palate and of congenital heart disease, and in all three measures of severity of syndactyly. Caution is, however, required in the interpretation of significance values in this context, because multiple comparisons were undertaken. Park et al. (1995*b*) used the Bonferroni correction (in which the $P = .05$ value is divided by the number of comparisons made), and, employing a P value of $<.002$, concluded that there were no significant phenotypic differences between the two mutations. For our analysis, the equivalent P value would be .0025. Use of the Bonferroni correction is likely to be excessively cautious, because it is normally applied to differences in outcome from multiple independent treatments, whereas in this instance we are comparing multiple outcomes (which are *not necessarily independent*) from just two "treatments." Nevertheless, our principal conclusions bear up to testing by this stringent statistical criterion.

When the $P < .0025$ figure is used to compare the phenotypes of the two mutations, the findings for female birthweight and congenital heart disease may be coincidental, but the differences for cleft palate and syndactyly are likely to be real. Our conclusion for cleft palate is strengthened by the data of Park et al. (1995*b*): when the two studies are combined, 38/66 S252W patients had cleft palate, compared with only 5/32 P253R patients ($G_1 = 16.4$, $P < .001$). Particularly surprising is that, whereas the S252W mutation is more frequently associated with cleft palate (which probably reflects a generalized disturbance in craniofacial morphogenesis, see below), the P253R mutation is associated with more severe syndactyly. It is therefore oversimplistic to consider one mutation as more severe than the other: rather, they may have differential effects on different developmental systems during organogenesis.

The discrepancy between our findings for the limb and those from Park et al. (1995*b*) is probably explained by differences in the classification of syndactyly. Comparison with three previously published series (table 3) suggests a consistent bias toward severe scores in the series of Park et al. (1995*b*). In the case of the feet, these authors assigned 33/36 patients to the same score, precluding the detection of significant differences between the mutations. By contrast, the distributions of syndactyly scores in our study appear more comparable with those of Blauth and von Törne (1978), Upton (1991), and Cohen and Kreiborg (1995), and our finding of a greater severity of syndactyly with the P253R mutation, for both the hands (morphological and radiological criteria) and feet (morphological criteria only), confirms and extends our previous observations (Wilkie et al. 1995*b*). The trends that we have identified are found in both younger and older groups of patients and do not differ significantly between the sexes (data not shown). Ultimately, genotype-phenotype correlations from further independent series will be necessary to clarify these relationships further.

Although Apert syndrome is now considered to be a single nosologic entity (termed "ACS I"), during past decades a separate disorder (Apert-Crouzon disease, or Vogt cephalodactyly, or ACS II) has been recognized,

Table 3

Review of Hand and Foot Syndactyly Scores in Apert Syndrome

	Hand Syndactyly				Foot Syndactyly			
		% with Hand Score of				% with Foot Score of		
Study	No. of Patients	1	2	3	No. of Patients	1	2	3
Blauth and von Törne (1978)	...				80	13	30	57
Upton (1991)	68	41	35	24	...			
Cohen and Kreiborg (1995)	44	45	39	16	37	27	19	54
Park et al. (1995*b*)	36	3	56	41	36	0	8	92
Present study	87	47	32	21	86	6	51	43

on the basis of reports of patients with a severe "Crouzonoid" craniofacial phenotype but relatively mild syndactyly (Vogt 1933; Nager and de Reynier 1948; Temtamy and McKusick 1969). Although none of the patients in the present study exactly match this variant phenotype, the features described are those that we have found to be associated with the S252W mutation rather than with the P253R mutation. It is tempting to speculate that this abortive classification may, in fact, have correctly anticipated the existence of two "subgroups" of Apert syndrome.

Specificity of Apert Mutations

Notwithstanding the subtle phenotypic differences that we have identified between S252W and P253R mutations, their pathological effects are remarkably similar, so that confident prediction of genotype from phenotype in the individual case is not always possible. However, we disagree with the statement of Park et al. (1995*b*, p. 327) that "the lack of phenotypic differences in the two genotypic subgroups of patients with Apert syndrome is not unexpected, considering that the mutations themselves are adjacent and are in the same functional domain." In fact, nine other missense substitutions and one nonsense substitution of the codons for the serine-proline dipeptide are theoretically possible (and substitution of serine to leucine might be expected to be more common than substitution of serine to tryptophan; Wilkie et al. 1995*b*); but none has yet been observed, either in Apert syndrome or in any other disorder. Furthermore, cases of Pfeiffer syndrome due to FGFR1 mutation (Muenke et al. 1994) all have a proline-to-arginine substitution that corresponds precisely to the P253R mutation, whereas a serine-to-tryptophan substitution could not occur in FGFR1 because a different codon (TCC in FGFR1, vs. TCG in FGFR2) is used to encode the serine. This implies that the specificity of the Apert mutations depends not only on their position in the IgII-III linker, but also on the particular structural effects of the two substitutions observed. Modeling studies of the IgII-III domain structure (Wilkie et al. 1995*a*) suggest that these mutations would alter the relative orientation of the two Ig domains and/or the local conformation of the ligand-binding site and, hence, mimic—or, more probably, accentuate—binding of fibroblast growth factors (FGFs). However, it is unlikely that these structural effects would be precisely identical for the two mutations, and this could explain the subtle phenotypic differences that we have observed. Biochemical and crystallographic analysis of normal and mutant FGFR2—and of its ligand interactions—will be required for an exploration of these differences.

Embryological Interpretation

Congenital abnormalities due to single pathological mutations have traditionally been regarded as straightforward translations of genotype into phenotype, in contrast to "multifactorial" causes, in which the phenotype depends on complex interactions between the environmental and genetic factors (Carter 1969). As this study illustrates, the phenotype resulting from individual mutations is not necessarily uniform, suggesting that environmental and genetic (maternal as well as fetal) factors influence the final outcome. The Apert syndrome phenotype is particularly interesting in this context, being due to one of two possible mutations.

An important embryological point is that the cleft palate in Apert syndrome is likely to be secondary to other orofacial defects that are established prior to palate formation. This morphogenetic association is well documented in human and experimental animal studies: several transgenic mouse models with disruptions of genes expressed in craniofacial structures not including the palate show cleft palate as part of the abnormal craniofacial phenotype (reviewed by Ferguson 1994). Hence we may interpret the higher prevalence of cleft palate in the S252W group as a manifestation of a more generalized disturbance of craniofacial morphogenesis associated with this mutation.

The differential effect of the Apert mutations on cra-

niofacial morphology and syndactyly has implications for understanding the role of FGFR2 in development. Several distinct FGFs bind to each FGFR (Johnson and Williams 1993; Mason 1994), and both FGFs and FGFRs are widely (but specifically) expressed during organogenesis, so that productive signaling may depend on the coincident expression of particular FGFs and FGFR isoforms in particular developing sites. Clearly, FGFR2 signaling is involved in both digital and craniofacial skeletogenesis: the observation that the S252W mutation is more frequently associated with cleft palate whereas the P253R mutation results in more severe syndactyly suggests that the mechanisms of signaling are not identical in the developing limb and skull—and that a subtle difference, in conformation, between the two mutated forms of FGFR2 protein can tip the balance slightly toward greater abnormality of signaling in one or the other site.

Some insight into the different downstream effects of normal FGFR2 signaling at these sites may be gained from studies of expression in mouse embryos, which show that RNA transcripts of Fgfr2 are present in the undifferentiated interdigital mesenchyme of the limb and at the undifferentiated periphery of the developing bone of the skull vault (Iseki et al., in press; S. Iseki and G. M. Morriss-Kay, unpublished data). The subsequent normal differentiation pathways of the interdigital and skull-vault mesenchymes are different. Interdigital mesenchyme forms connective and muscular tissue, with some apoptosis, and does not share in the endochondral ossification fate of the condensed mesenchyme of the digits themselves. In contrast, the skull-vault mesenchyme is invaded by osteogenic tissue and is ultimately completely ossified. Further studies of FGF/FGFR2 expression patterns may elucidate how these different developmental processes relate to the differential effects of the Apert mutations.

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References

Apert E (1906) De l'acrocéphalosyndactylie. Bull Soc Med Hop (Paris) 23:1310–1330

Blank CE (1960) Apert's syndrome (a type of acrocephalosyndactyly)—observations on a British series of thirty-nine cases. Ann Hum Genet 24:151–164

Blauth W, von Törne O (1978) Der 'Apert-Fuss.' Z Orthop Ihre Grenzgeb 116:1–6

Carter CO (1969) Genetics of common disorders. Br Med Bull 25:52–57

Cinalli G, Renier D, Sebag G, Sainte-Rose C, Arnaud E, Pierre-Kahn A (1995) Chronic tonsillar herniation in Crouzon's and Apert's syndromes: the role of premature synostosis of the lambdoid suture. J Neurosurg 83:575–582

Cohen MM Jr, Kreiborg S (1990) The central nervous system in the Apert syndrome. Am J Med Genet 35:36–45

——— (1993*a*) Growth pattern in the Apert syndrome. Am J Med Genet 47:617–623

——— (1993*b*) Visceral anomalies in the Apert syndrome. Am J Med Genet 45:758–760

——— (1995) Hands and feet in the Apert syndrome. Am J Med Genet 57:82–96

Cohen MM Jr, Kreiborg S, Lammer EJ, Cordero JF, Mastroiacovo P, Erickson JD, Roeper P, et al (1992) Birth prevalence study of the Apert syndrome. Am J Med Genet 42:655–659

David DJ, Poswillo D, Simpson D (eds) (1982) The craniosynostoses: causes, natural history and management. Springer, Berlin

Dionne CA, Crumley G, Bellot F, Kaplow JM, Searfoss G, Ruta M, Burgess WH, et al (1990) Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. EMBO J 9:2685–2692

Erickson JD, Cohen MM Jr (1974) A study of parental age effects on the occurrence of fresh mutations for the Apert syndrome. Ann Hum Genet 38:89–96

Ferguson MWJ (1994) Craniofacial malformations: towards a molecular understanding. Nat Genet 6:329–330

Gorry MC, Preston RA, White GJ, Zhang Y, Singhal VK, Losken HW, Parker MG, et al (1995) Crouzon syndrome: mutations in two spliceoforms of *FGFR2* and a common point mutation shared with Jackson-Weiss syndrome. Hum Mol Genet 4:1387–1390

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–339

Henderson CA, Knaggs H, Clark A, Highet AS, Cunliffe WJ (1995) Apert's syndrome and androgen receptor staining of the basal cells of sebaceous glands. Br J Dermatol 132:139–143

Iseki S, Wilkie AOM, Morriss-Kay GM. Expression of the *Fgfr2* gene during mouse limb and skull development. J Anat (in press)

Jabs EW, Li X, Scott AF, Meyers G, Chen W, Eccles M, Mao J-i, et al (1994) Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. Nat Genet 8:275–279

Johnson DE, Williams LT (1993) Structural and functional diversity in the FGF receptor multigene family. In: Vande

Woude GF, Klein G (eds) Advances in cancer research. Academic Press, San Diego, pp 1–41

Kreiborg S, Barr M Jr, Cohen MM Jr (1992) Cervical spine in the Apert syndrome. Am J Med Genet 43:704–708

Kreiborg S, Cohen MM Jr (1992) The oral manifestations of Apert syndrome. J Craniofac Genet Dev Biol 12:41–48

Lajeunie E, Ma HW, Bonaventure J, Munnich A, Le Merrer M, Renier D (1995) *FGFR2* mutations in Pfeiffer syndrome. Nat Genet 9:108

Lee SH, Rao KCVG (eds) (1987) Cranial computed tomography and MRI. McGraw Hill, New York

Lewanda AF, Cohen MM Jr, Hood J, Morsey S, Walters M, Kennedy JL Jr, Jabs EW (1993) Cytogenetic survey of Apert syndrome: reevaluation of a translocation (2;9)(p11.2; q34.2) in a patient suggests the breakpoints are not related to the disorder. Am J Dis Child 147:1306–1308

Ma HW, Lajeunie E, Le Merrer M, de Parseval N, Serville F, Weissenbach J, Munnich A, et al (1995) No evidence of genetic heterogeneity in Crouzon craniofacial dysostosis. Hum Genet 96:731–735

Margolis S, Siegel IM, Choy A, Breinin GM (1977) Oculocutaneous albinism associated with Apert's syndrome. Am J Ophthalmol 84:830–839

Mason IJ (1994) The ins and outs of fibroblast growth factors. Cell 78:547–552

Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AM-L, Aaronson SA (1992) Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci USA 89:246–250

Moloney DM, Slaney SF, Oldridge M, Wall SA, Sahlin P, Stenman G, Wilkie AOM. Exclusive paternal origin of new mutations in Apert syndrome. Nat Genet (in press)

Muenke M, Schell U (1995) Fibroblast-growth-factor receptor mutations in human skeletal disorders. Trends Genet 11: 308–313

Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, Pulleyn LJ, et al (1994) A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nat Genet 8:269–274

Nager FR, de Reynier JP (1948) Das Gehörorgan bei den angeborenen Kopfmissbildungen. Pract Oto-rhino-laryngol 10 Suppl 2:1–128

Narayan H, Scott IV (1991) Prenatal ultrasound diagnosis of Apert's syndrome. Prenat Diagn 10:187–192

Oldridge M, Wilkie AOM, Slaney SF, Poole MD, Pulleyn LJ, Rutland P, Hockley AD, et al (1995) Mutations in the third immunoglobulin domain of the fibroblast growth factor receptor-2 gene in Crouzon syndrome. Hum Mol Genet 4: 1077–1082

Orr-Urtreger A, Bedford MT, Burakova T, Arman E, Zimmer Y, Yayon A, Givol D, et al (1993) Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2). Dev Biol 158:475–486

Park W-J, Meyers GA, Li X, Theda C, Day D, Orlow SJ, Jones MC, et al (1995*a*) Novel *FGFR2* mutations in Crouzon and Jackson-Weiss syndromes show allelic heterogeneity and phenotypic variability. Hum Mol Genet 4:1229–1233

Park W-J, Theda C, Maestri NE, Meyers GA, Fryburg JS, Dufresne C, Cohen MM Jr, et al (1995*b*) Analysis of phenotypic features and FGFR2 mutations in Apert syndrome. Am J Hum Genet 57:321–328

Patton MA, Goodship J, Hayward R, Lansdown R (1988) Intellectual development in Apert's syndrome: a long term follow up of 29 patients. J Med Genet 25:164–167

Peters KG, Werner S, Chen G, Williams LT (1992) Two FGF receptor genes are differentially expressed in epithelial and mesenchymal tissues during limb formation and organogenesis in the mouse. Development 114:233–243

Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S (1994) Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. Nat Genet 8:98–103

Risch N, Reich EW, Wishnick MM, McCarthy JG (1987) Spontaneous mutation and parental age in humans. Am J Hum Genet 41:218–248

Rollnick BR (1988) Male transmission of Apert syndrome. Clin Genet 33:87–90

Rutland P, Pulleyn LJ, Reardon W, Baraitser M, Hayward R, Jones B, Malcolm S, et al (1995) Identical mutations in the *FGFR2* gene cause both Pfeiffer and Crouzon syndrome phenotypes. Nat Genet 9:173–176

Schell U, Hehr A, Feldman GJ, Robin NH, Zackai EH, de Die-Smulders C, Viskochil DH, et al (1995) Mutations in *FGFR1* and *FGFR2* cause familial and sporadic Pfeiffer syndrome. Hum Mol Genet 4:323–328

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research, 2d ed. WH Freeman, San Francisco

Steinberger D, Mulliken JB, Müller U (1995) Predisposition for cysteine substitutions in the immunoglobulin-like chain of FGFR2 in Crouzon syndrome. Hum Genet 96:113–115

Temtamy S, McKusick VA (1969) Synopsis of hand malformations with particular emphasis on genetic factors. Birth Defects 3:125–184

Thompson DNP, Harkness W, Jones B, Gonsalez S, Andar U, Hayward R (1995) Subdural intracranial pressure monitoring in craniosynostosis: its role in surgical management. Childs Nerv Syst 11:269–275

Triglia T, Peterson MG, Kemp DJ (1988) A procedure for *in vitro* amplification of DNA segments that lie outside the boundaries of known sequences. Nucleic Acids Res 16:8186

Upton J (1991) Classification and pathologic anatomy of limb anomalies. Clin Plast Surg 18:321–355

Vogt A (1933) Dyskephalie (Dysostosis craniofacialis, Maladie de Crouzon 1912) und eine neuartige Kombination dieser Krankheit mit Syndaktylie der 4 Extremitäten (Dyskephalodaktylie). Klin Monatsbl Augenheilkd 90:441-454

Wilkie AOM, Morriss-Kay GM, Jones EY, Heath JK (1995*a*) Functions of fibroblast growth factors and their receptors. Curr Biol 5:500–507

Wilkie AOM, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, Hayward RD, et al (1995*b*) Apert syndrome results from localized mutations of *FGFR2* and is allelic with Crouzon syndrome. Nat Genet 9:165–172