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Familial Klippel-Feil Syndrome and Paracentric Inversion inv(8)(q22.2q23.3)

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Summary

Klippel-Feil Syndrome (KFS) is characterized by congenital vertebral fusion believed to result from faulty segmentation along the embryo's developing axis. KFS appears to be a heterogeneous disease often associated with craniofacial malformation. Here we provide the first evidence of a familial KFS gene locus on 8q, where an inv(8)(q22.2q23.3) has been found segregating with congenital vertebral fusion. The four-generation KF2-01 family present with a dominant form of the KFS where the sequence of vertebral fusion was confined to the cervical spine (always including the C2-3 fusion and reduced expression of the C4-5 and C6-7 fusions) in association with malformation of laryngeal cartilages and mild-to-severe vocal impairment.

Introduction

Congenital fusion of the vertebrae, commonly referred to as Klippel-Feil syndrome (KFS) (Klippel and Feil 1912; Feil 1919), appears to be a heterogenous disease primarily reported as sporadic cases (Gorlin 1990, pp. 896–99; Nguyen and Tyrrel 1993). KFS has been associated with a broad spectrum of developmental anomalies ranging from mild cosmetic deformity to severe disability and lethality (table 1). Diagnosis of congenital vertebral blocks of two or more fused vertebrae, principally within the neck (cervical) region of the spine, are usually apparent from lateral cervical radiographs, after completion of normal spinal ossification in the young child (McBride 1992). However, one apparent affected KFS family individual has been reported with typical associated abnormalities but no vertebral fusion (Sheffield 1982).

Approximately 1/42,000 people are afflicted with KFS (Brown et al. 1964), and, of the ~30 affected families reported in this century, most had three or fewer affected individuals with no clear mechanism of inheritance (Gunderson et al. 1967; Shaver et al. 1986). In addition to the KF2-01 family described here, five KFS families have been reported with a definite autosomal dominant mode of inheritance with fusion of the 2d and 3d cervical vertebrae (C2-3 fusion) reported in all fusion sequences and in association with hearing defects and facial dysmorphology (Pfeiffer et al. 1992), cleft palate (Sheffield 1982), mild basilar impression (Gunderson et al. 1967), and os odontoideum (Morgan et al. 1989). Also of interest is a sporadic case of KFS in a 6-year-old girl with a balanced de novo reciprocal translocation, ie., 46,XX,t(5,17) (q11.2;q23) (Ohashi et al. 1992).

The term "fusion" provides the best clinical description for KFS affected vertebrae; however, this term has generally been regarded as a misnomer in relation to KFS. This reflects the current view on the developmental etiology of KFS as the result of faulty segmentation of the somites from the segmental plates between the 3d and 7th wk of development. In humans, the segmental plates are laid down during the process of gastrulation, appearing on each side of the midline epithelium as the primitive streak regresses along the rostro-caudal axis of the embryo. As development proceeds, neurulation takes place in the midline, and the segmental plates come to flank the neural tube and notochord. Somites segment from the rostral end of each plate in an orderly rostro-caudal sequence (Stern and Keynes 1988*a*). The sclerotome from somite pairs (caudal of somite 4) migrates medially to surround the neural tube and notochord to eventually form the vertebrae (Stern and Keynes 1988*a*). Therefore, the complete fusion of vertebral bodies with a more rostral/cervical position has been considered indicative of an earlier segmental error of somitogenesis. In this report, we describe a highly informative familial occurrence of KFS in association with vocal impairment.

Material and Methods

Radiography

Radiographs were taken of anterior, posterior, and lateral views of the cervical and upper thoracic spine, with the neck flexed and extended (fig. 1).

Laryngeal Examination and Voice Analysis

Laryngeal examination was carried out with both a mirror and a nasolaryngoscope (Olympus T3, 4.7 mm

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Table 1

Anomalies Reported in Association with Klippel-Feil Syndrome

System	Manifestation
Musculoskeletal	Vertebral fusion, scoliosis (60%), spina bifida (occulta, aperta, and cystica), os odontoidium, torticollis, cervical ribs, digital hypoplasia, basilar impression, and sprengles shoulder (30%) often associated with short neck, neck webbing, low posterior hairline; myelocele, myeloencephalococele, syrgohydromyelia, and Chiari malformation
Craniofacial	Cleft palate (10%), jaw duplication, micrognathia, otolaryngeal abnormalities including conductive deafness and microtia, bifid uvula, ocular abnormalities, and facial and thyroid asymmetry
Hindbrain and Neurological	Extraocular muscle palsies, paraesthesias and pain in lower limbs, sensorineural deafness, partial or complete absence of cranial nerve nuclei or tracts, respiratory apnea, sleep disturbances and bimanual synkinesis, mental retardation, and spasticity
Urogenital	Urogenital anomalies (30%) including renal agenises; renal, pelvic, and ureteral duplication; and absent vagina
Cardiovascular	Congenital heart disease (14%), e.g., ventricular septal defects

diameter). Judgments on vocal impairment were made relative to proband III-5 (Clarke et al. 1994).

Chromosome and FISH Analysis

Cytogenetic studies were performed on cultures from peripheral blood lymphocytes. For high-resolution studies, ethidium bromide (final concentration 1 μg/ml) was added to the cultures, 2 h prior to harvest.

With FISH studies, slides were denatured in 70% formamide/2 × SSC at 70°C for 5 min and dehydrated in an ethanol series. Hybridization with a biotin-labeled chromosome 8–specific painting probe (Cambio, denatured at 70°C for 7 min) involved an overnight incubation at 42°C. Detection was with fluorescein-labeled avidin and chromosomes were counterstained with propidium iodide.

Results

KF2-01 Family Study

A total of 71% of living KF2-01 family members radiographed had fused vertebrae (fig. 1). Of the entire KF2-01 family (living and deceased), 74% appeared affected, with 64% of males and 84% of females (fig. 1). Medical and family histories assessed relative to KFS provided no evidence of consanguineous marriages.

Fusion Profile

All affected individuals tested via spinal radiography exhibited vertebral fusion(s), always including fusion of the 2d and 3d cervical vertebrae, to form a continuous C2-3 block vertebra (fig. 2). The vertebral bodies, transverse processes, superior articular processes, pedicles, and spines were completely fused in the C2-3 fusion and in most other vertebral fusions (C4-5 and C6-7). There was no sign of the intervertebral space between vertebral bodies of C2-3 fusions. Except for the C2-3 fusion in male proband 111-7, all fused vertebrae appeared to have maintained their normal antero-posterior length. Spinal processes were often misaligned, but they were never fused. Such vertebral block formation was often associated with the thinning of adjacent vertebral interspaces (fig. 2*c*). However, thinning of adjacent interspaces was never classified in this study as fusion. All affected suffered from torticollis, however, with the possible exception of deceased child IV-7 (see below); abnormal vertebral configurations did not result in any

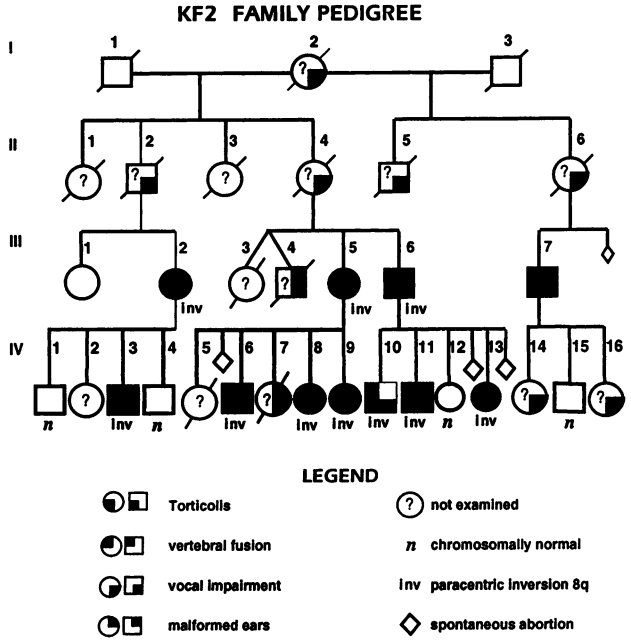

Figure 1 Family pedigree of the KF2-01 kindred. Fourteen of the 19 living family members were assessed via spinal radiographs. Ten of 14 had at least one vertebral fusion (always C2-3) and the inversion on chromosome 8. The inversion always segregated with the KFS phenotype. Deceased individuals classified as affected were either obligate carriers of this dominant disease or voice impaired. IV-7 was deaf and mentally retarded, with scoliosis. The pedigree is complete except for the three unaffected children of unaffected female III-1.

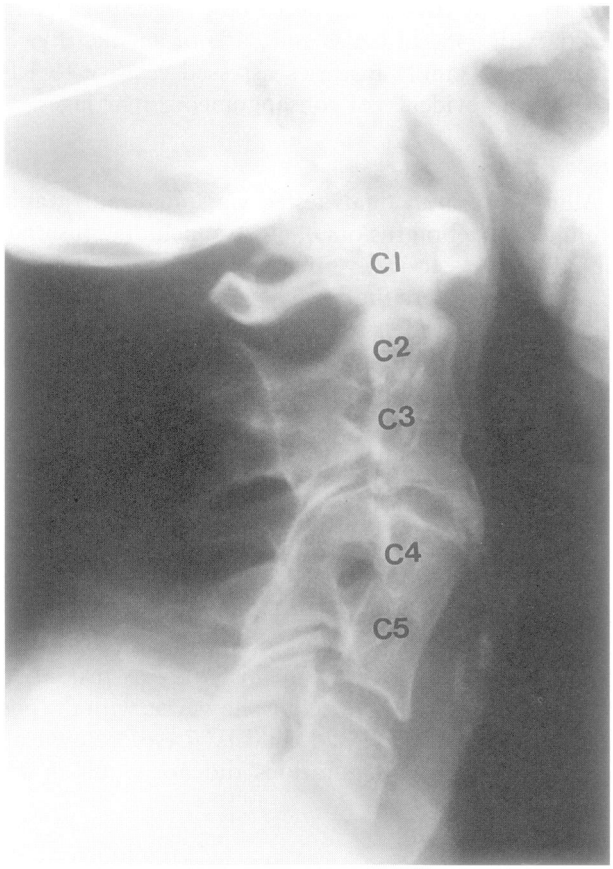

Figure 2 Vertebral fusion. Lateral cervical radiograph of IV-10 showing the C2-3 and C4-5 fusion.

notable spinal instability or pronounced neurological deficit.

Fusion frequency appeared to correlate with voice impairment, in that all those with two or more fusions were vocally impaired, in addition to male proband IV-3 who had the single C2-3 fusion (table 2). C2-3 fusion was obvious in child IV-13 at 12 mo.

Voice Impairment

Ninety percent of all affected individuals (76% of the living affected) were obviously vocally impaired (fig. 1) and were given a comparative ranking (table 2) relative to an extensive speech analysis carried out previously on proband III-6 (Clarke et al. 1994). Those individuals with severe voice impairment were essentially aphonic and communicated with a soft, hoarse whisper that was relatively ineffective, except in very quiet background-noise conditions. Even those affected individuals with a voice judged to be mildly impaired still had a soft and hoarse voice. The most severe cases of voice impairment were among males, and reports suggest deterioration of voice as they approached puberty. All individuals without vertebral fusion were judged to have a completely normal voice. The affected status of deceased individuals II-5 and III-4 was reliant on their voice impairment.

The previous clinical case report by Clarke et al. (1994), which reviewed aphonic proband III-6, determined that severe voice impairment was directly related to malformed laryngeal cartilages (fig. 3) and that it was not the result of a common dysplasia that could account for both cervical fusion and laryngeal abnormalities. Laryngeal examination also revealed absent right vocal cord movement and restricted left vocal cord motion. The vocal cords consequently failed to adduct to the midline for phonation, and vocal cord vibration was absent (Clarke et al. 1994). This, however, did not fully account for the similar voice defect so prevalent in the family (table 2). Individual IV-10, the son of proband III-6, also had severe voice impairment in association with an infantile epiglottis; however, laryngoscopy revealed that the proband's vocal cords did adduct to the midline but that movement was usually slowed.

Deceased individuals classified as affected either were obligate carriers of this dominant disease or were reported as severely vocally impaired (II-5 and III-4) or both (I-2, II-2, II-4, and II-6). Two living, vocally impaired individuals (IV-14 and IV-16) that were unavailable for radiography were also assumed to have KFS.

Other Anomalies

All affected members in the KF2-01 family had microtia, and a number also had a previous history of mild conductive hearing impairment or had low-set and/or underdeveloped ears (fig. 3*d*) and bilaterally restricted supination and elbow flexion of the forearms (table 2). While vertebral fusion was not confirmed for the deceased child IV-7 (14 yr of age), she presented with KFS-

Table 2

Anomalous Features of the KF2-01 Family

Anomalies	Comment
Vertebral fusion	71% of living affected (fig. 1); 100% of affected with C2-3 fusion; other fusions present were C4-5 and C6-7 with one C2-3 and C4-7 sequence
Laryngeal	90% of affected with vocal impairment; aphonic hoarse weak whisper linked with abnormal laryngeal cartilages or aberrant vocal cord movement
Ears	100% of affected had microtia; ears occasionally small, low set and underdeveloped, anteverted; some progressive conductive deafness and oto-sclerosis
Restriction of arms	Over 50% of affected with moderate bilateral restricted supination and flexion movements; stiffness in legs
Other	Microstomia, scoliosis, and mental retardation; one case of midline teeth misalignment

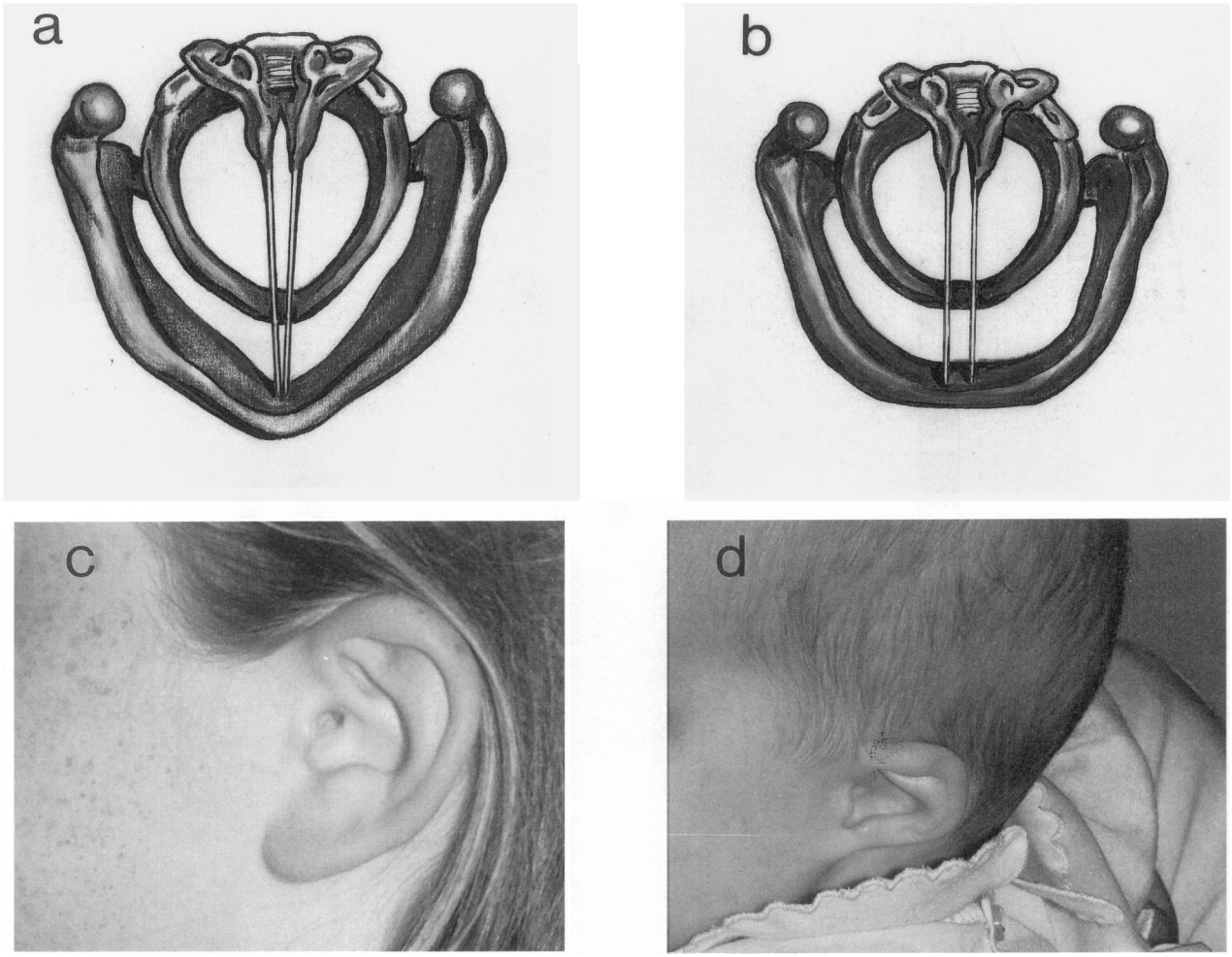

Figure 3 Otolaryngeal abnormalities. *a*, Schematic representation of normal laryngeal cartilages. *b*, Abnormal laryngeal cartilage morphology of proband III. Note the near parallel vocal cords. Surgical intervention and subsequent laryngoscopy and magnetic resonance imaging of proband III-1 revealed a very small glottic airway due to an extreme degree of posterior displacement of an infantile epiglottis and supraglottic stenosis (Clarke et al. 1994). The anterior commissure or keel of the thyroid cartilage was absent or flattened. There was no sign of edema of the vocal cords or other pathological features. Vocal cords failed to adduct to the midline in the normal "V" configuration. There was no movement of the right vocal cord, either for speech or on deep inspiration. *c*, Normal ears of unaffected female IV-12. *d*, Microtia and low-set ears of her affected sister, IV-13.

associated anomalies, including scoliosis, congenital deafness, and profound mental retardation (tables 1 and 2). This child also presented with extrapyramidal disease in association with microcephaly and somatic stunting and with minimal right facial paralysis, Cheyne-Stoke respirations, and bradycardia and involuntary movements (possibly myoclonic jerks) of the upper limbs and face, particularly the fingers, hands, and tongue, consistent with a severe bilateral dysfunction of the basal ganglia. In contrast, all other members of the family appeared to have normal control of movement. Child IV-13 had a mild degree of midline misalignment of the upper teeth.

KFS Assigned to 8q

Cytogenetic analysis in all KF2-01 affected individuals tested revealed an abnormal chromosome 8 (fig. 4). An inversion of the 8q22.2→q23.3 long-arm segment was detected on high-resolution studies, with affected individuals having the karyotype 46,XY or 46,XX, inv(8)(q22.2q23.3) (fig. 4*a*). The inversion always segregated with the KFS phenotype (fig. 1). FISH, using chromosome 8–specific painting probes (Cambio), confirmed the aberration to be confined to chromosome 8 (results not shown).

Discussion

KFS appears to be a heterogenous developmental syndrome with variable expression. Vertebral fusion and associated craniofacial anomalies are recurring clinical features (see table 1) in dominant, recessive, and sporadic forms of the disease. While apparently affected individuals with cleft palate from some KFS familes (e.g.,

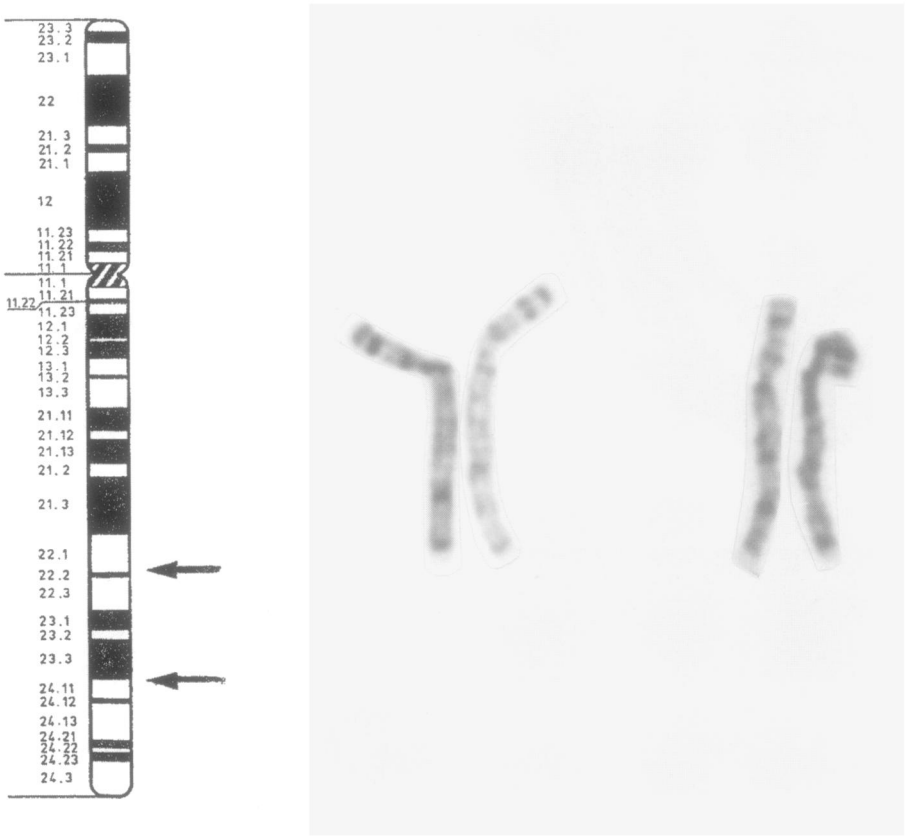

Figure 4 *a*, Idiogram of breakpoints of inversion on chromosome 8. *b*, Chromosomes 8 in patients III-5 (*right*) and IV-6 (*left*). The inverted 8 is on the right of each chromosome pair.

the KF1-01 family) have presented with no vertebral fusion (R. A. Clarke, J. H. Keearsley, and D. D. Walsh, unpublished data), here all affected members of the KF2-01 family exhibited typical Klippel-Feil anomaly of cervical vertebrae always including the C2-3 fusion. Most (90%) of the affected individuals also presented with vocal impairment due to malformation of the larynx (Clark et al. 1994).

The radiological diagnosis of fused cervical vertebrae includes a variety of entities such as postinfectious, posttraumatic, and postsurgical fusion; fibrodysplasia progressiva (Connor and Smith 1982); juvenile rheumatoid arthritis (Martel et al. 1962); and ossification of the posterior longitudinal ligament (Ratanasiri and Thiramont 1991) of the cervical spine. However, the unique characteristics of each of these entities clearly distinguish them from familial KFS while causing some concern in the diagnosis of sporadic KFS, especially in older individuals. Alcohol fetal syndrome also contributes to cervical bony fusion, increasing the incidence of sporadic KFS (Lawry 1977).

Here we have reported the first KFS family with vocal impairment. KFS has often previously been associated with conductive hearing impairment or other craniofacial abnormalities, including cleft palate. Other case studies have also reported the presence of abnormal epiglottis and associated difficulties in intubation. However, one common theme in the development of the craniofacial region is that all the cartilages involved therein have their embryonic origin in the segmented branchial region of the embryo.

This is the first report to assign a KFS gene locus (8q22.2 or 8q23.3). In this family the paracentric inversion on 8q always segregates with vertebral fusions, indicating a location for a KFS gene (SGM1) at one of the breakpoints of the inverted segment. Indeed, the majority (22/23) of apparently balanced rearrangements that have been studied at the gene level and reviewed recently by Tommerup (1993) had breakpoints within the candidate gene locus. The adverse effects of breaks and rejoinings in such "balanced" rearrangements in producing phenotypic anomalies can involve possibilities such

as (1) submicroscopic deletions at the breakpoints, (2) gene mutation, or (3) gene disruption, with loss of gene function because of a "position effect."

To date, no obvious candidate disease genes have been mapped within the chromosome 8q breakpoints of the inverted segment, though band 8q24.1 appears critical to the Langer-Gideon syndrome and exostosis (Wood et al. 1993; Ludecke et al. 1995). The branchio-oculo facial syndrome, which shows some overlap of otolaryngeal anomalies with associated speech problems, has been localized proximal at 8q11.2-q12 (Wood et al. 1993). However, the genes that directly control the metameric pattern formation of the vertebrate spine have yet to be identified (Stern and Keynes 1988*b*; Cordes and Barsh 1994).

Faulty segmentation in association with malformation of vertebral and otolaryngeal structures, and KFS facial dysmorphology generally, is consistent with maldevelopment early in embryogenesis (Tucker and Tucker 1975; Hunt and Krumlauf 1991; Kessel and Gruss 1991; Ang and Rossant 1993), possibly prior to any visible signs of structural segmentation in the embryo. In addition, consistencies within the KF2-01 abnormal phenotype, including the C2-3 vertebral fusion (evident in 100% of positive spinal radiographs) and voice impairment (90% of affected family), indicated that the extremely high segregation ratio of affected within the family (74%) was not due to genetic heterogeneity but rather was due to the mutation of a single dominant gene/locus (SGM1) (Wood et al. 1993). This high segregation ratio suggests complete penetrance of the dominant KF2-01 mutant phenotype always segregating with the chromosome 8 inversion.

Vertebral formation is not uniform along the rostrocaudal axis (Meinhardt 1986). Somitogenesis/segmentation progresses caudally, and ossification of vertebral bodies progresses rostrally along the developing cervical spine (Morgan et al. 1989; Couly et al. 1993). A lack of uniformity that appears likely to relate to differences in developmental gene expression (Deutsch et al. 1988; Hunt et al. 1991; McGinnis and Krumlauf 1992; Cordes and Barsh 1994). This lends support to the notion that the affected SGM1 gene, with its associated vertebral anomalies restricted to the cervical spine, may also have restricted, regionalized influences on axial development, as do many of the homeotic genes.

During development in vertebrates, the lower face and throat arise from the branchial arches and pharyngeal pouches. Cartilaginous and connective tissues of the head and neck are derived from multipotential neural crest cells that migrate ventrally from the segmented rhombomeres of the hindbrain into the adjacent branchial arches (Hunt et al. 1991; Hunt and Krumlauf 1991; McGinnis and Krumlauf 1992). In the KF2-01 family, the morphological deformation of vertebral and laryngeal structures, low-set ears, microtia, microstomia, bilateral restriction of the upper limbs, and the periodic respirations and myoclonic jerks of proband IV-7 could all conceivably be linked to faulty segmental development axial structures of the embryo.

Until now, discussion about KFS etiology has been subdued in the literature because of the lack of informative phenotypes. The general lack of large or informative familial KFS profiles such as the KF2-01 family described here, in addition to the complication of potential KFS heterogeneity, has rendered comparative KFS analyses of limited significance. Even the hypothesis that the complete fusion of vertebral bodies being indicative of an early segmental error of somitogenesis has yet to be tested. We are currently undertaking developmental studies in KFS infants to determine the status of the spine in the earliest weeks of postnatal development. Positional cloning of the SGM1 gene from KF2-01 chromosome 8 breakpoints should represent a major definitive step in delineating the different types of KFS and elucidating the developmental biochemistry of the syndrome.

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