

Mutations in *MEOX1*, Encoding Mesenchyme Homeobox 1, Cause Klippel-Feil Anomaly

Jawahir Y. Mohamed,¹ Eissa Faqeih,² Abdulmonem Alsiddiky,³ Muneera J. Alshammari,^{1,4}
Niema A. Ibrahim,¹ and Fowzan S. Alkuraya^{1,5,*}

Klippel-Feil syndrome (KFS) is a segmentation malformation of the cervical spine; clinically, it manifests as a short neck with reduced mobility and a low posterior hairline. Several genes have been proposed as candidates for KFS when it is present with other associated anomalies, but the genetics of isolated KFS have been difficult to study because of the syndrome's mostly sporadic occurrence. We describe a multiplex consanguineous family in which isolated KFS maps to a single 17q21.31 locus that harbors a homozygous frame-shift deletion in *MEOX1*; this deletion results in complete instability of the transcript. Direct sequencing of this gene in two siblings from another consanguineous family affected by isolated KFS uncovered another homozygous truncating (nonsense) *MEOX1* mutation that also leads to complete degradation of the transcript. This gene encodes a transcription factor with a well-established and nonredundant role in somite development, and homozygous null alleles of *Meox1* in mice have a cervical skeletal defect that is remarkably similar to the one we observe in human individuals with *MEOX1* mutations. Our data strongly suggest that KFS is the human phenotypic equivalent of the sclerotome polarity defect that results from *Meox1* deficiency in mice.

The vertebral column is a major anatomical structure that distinguishes an entire subphylum of the animal kingdom. Its conspicuous segmented pattern as individual vertebrae joined together by various joints and ligaments provides many mechanical advantages that facilitate movements in various axes.¹ This segmentation is the result of a highly orchestrated and complex developmental process that is marked by the formation of somites, mesoderm-derived compartments that are mostly mesenchymal but are encapsulated by epithelium, and that proceeds in caudal-rostral direction.¹ Extensive patterning takes place during somitogenesis and involves polarization, differentiation, and segmentation, the end result of which is that individual somites divide into three different compartments: sclerotome, myotome, and dermatome. These compartments each give rise to a distinct group of tissues: vertebral bodies, vertebral muscles, and dorsal skin dermis, respectively.¹⁻³

Several somite segmentation defects are known to give rise to recognizable clinical phenotypes in humans. Those affecting the cervical spine are particularly disabling because of its high range of mobility. Klippel-Feil syndrome (KFS [MIM 214300]) is a prototypic segmentation defect in the cervical spine; it manifests clinically as a short neck with painless restriction in mobility and a low posterior hairline.⁴ Radiologically, KFS is characterized by a variable degree of neck vertebral fusion, which is often associated with additional skeletal defects such as Sprengel's deformity of the scapula (the scapula is positioned higher than normal) and omovertebral bone (extra bone forms between the medial aspect of the scapula and

the vertebral body).⁵ KFS affects 1:40,000 individuals and is often sporadic.⁶ However, familial occurrence and the observation of KFS in association with various chromosomal aberrations support a genetic origin of KFS, although this has been difficult to study.⁷ *PAX1* (MIM 167411), *GDF6* (MIM 601147), and *GDF3* (MIM 606522) play roles in somite development and have been the focus of sequencing projects as candidate genes. Several missense variants have been identified in these genes in a small percentage of KFS-affected individuals with various other manifestations.⁸⁻¹⁰ Interestingly, some of these presumed pathogenic variants are now listed in Exome Variant Server (EVS) as population variants with a frequency much higher than that of KFS, raising doubts about the original proposed link. The best available evidence for a Mendelian form of KFS comes from previously reported consanguineous multiplex pedigrees in which KFS appears to follow an autosomal-recessive mode of inheritance.¹¹ However, no mapping data are available for any of these families, so the gene or genes that underlie KFS in these families remain unknown. Here, we describe an autosomal-recessive form of KFS that segregates with truncation mutations in *MEOX1* (MIM 600147), encoding a transcription factor with a well-established role in somite development, in two multiplex consanguineous families.

The index is a 10-year-old Saudi girl who was referred by orthopedics because of KFS. She was born to healthy first-cousin parents who had previously had a healthy male child (Figure 1). Respiratory distress was diagnosed shortly after her term delivery, but she only had a brief

¹Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 11211, Saudi Arabia; ²Department of Pediatrics, King Fahad Medical City, Riyadh 11525, Saudi Arabia; ³Department of Orthopedics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia; ⁴Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia; ⁵Department of Anatomy and Cell Biology, College of Medicine, Riyadh 11533, Saudi Arabia

*Correspondence: falkuraya@kfshrc.edu.sa

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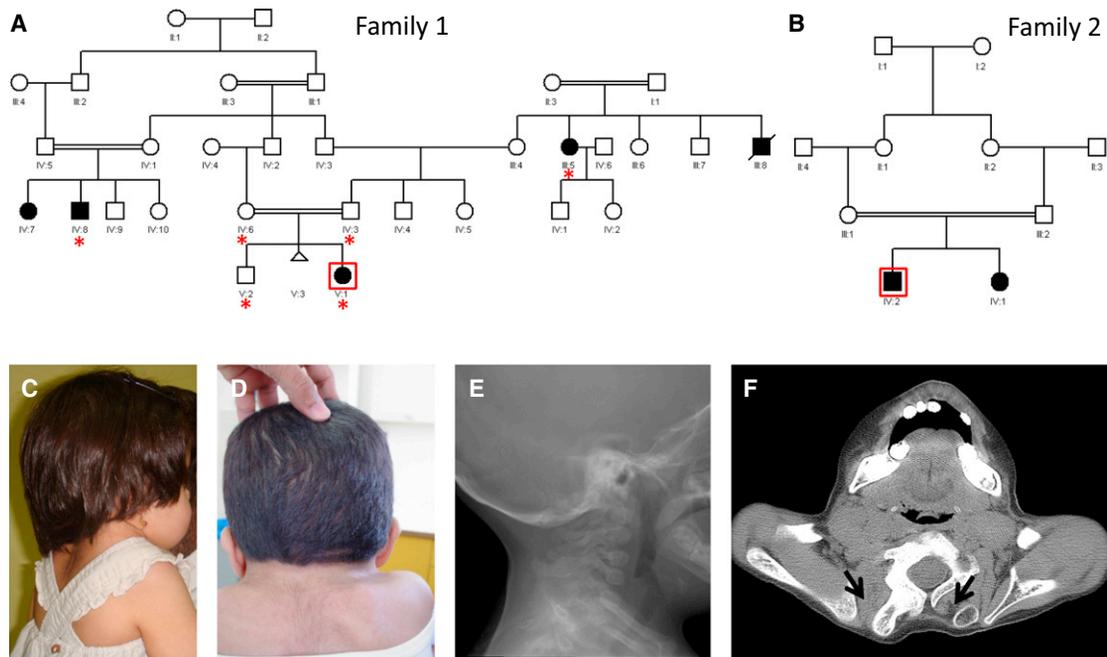


Figure 1. Identification of Two Families with Autosomal-Recessive KFS

(A) Pedigree of family 1 (red asterisks denote individuals whose DNA was available for analysis).

(B) Pedigree of family 2.

(C) Clinical photograph of the index individual in family 1; note a short neck and elevated scapulae.

(D) Clinical photograph of the index individual in family 2; note a short neck, a low posterior hair line, and elevated scapulae.

(E) Cervical-spine radiogram showing a segmentation defect.

(F) Computed-tomography image of the shoulder of the index individual in family 2. Note that the bilateral omovertebral bone (black arrow) is much more developed on the right, where it is fused with the right posterior segment of the C7 vertebral body.

requirement for supplemental oxygen. A short neck was noted on neonatal examination, and radiological examination revealed a cervical segmentation defect consistent with KFS. Her development has been normal, and there was no history suggestive of the involvement of other systems. Physical examination revealed normal weight and head circumference but borderline short stature (fifth centile for age). Her neck was short, and she had a low posterior hairline (Figure 1). She was unable to flex, extend, or rotate her neck or lift her right hand to touch the back of her head. The rest of the physical examination was normal. A full skeletal survey confirmed the C2-C3 fusion and Sprengel's deformity but revealed no additional skeletal defects (Figure 1). The family history and photographs revealed the presence of several similarly affected relatives (Figure 1).

The family pedigree was highly suggestive of an autosomal-recessive mode of inheritance, so we enrolled available surviving affected members and relevant unaffected relatives (denoted by red asterisks in Figure 1) in a King Faisal Specialist Hospital and Research Center (KFHSRC) IRB-approved protocol after obtaining written informed consent from the participants (RAC# 2080006). DNA was extracted from whole blood, and genotyping on an Axiom platform was then performed according to the Manufacturer's instructions (Affymetrix, Santa Clara, CA, USA). Autozygosity mapping was performed on the resulting genotypes with autoSNPa.¹² Interestingly, only

one run of homozygosity (ROH), chr17: 29,323,850–45,448,560 (hg18), was exclusively shared by the affected members and was subsequently confirmed by linkage analysis with easyLINKAGE¹³ (Figure 2). This interval contains 477 genes, but close examination for genes with relevant phenotypes in the mouse knockout highlighted *MEOX1* as the most compelling candidate given the mouse phenotype and its established role in somite formation, especially in the cervical spine (see below). Therefore, we proceeded with direct sequencing of *MEOX1* and uncovered a 1 bp deletion, c.94delG (RefSeq accession number NM_004527.3), that results in complete loss of the mutant transcript, most likely as a result of nonsense-mediated decay (NMD) (Figure 3). The mutation predicts frameshift and premature truncation of the resulting protein (p.Ala32Profs*165), so even if a mutant protein is made, it will completely lack the DNA-binding homeobox and is thus likely to be null (Figure 3). This mutation fully segregated with the phenotype and was absent in 210 Saudi controls and in the Exome Variant Server (EVS; see Web Resources) database.

In the course of this study, we were able to recruit another individual with typical KFS. He was diagnosed with Pierre-Robin sequence (MIM 261800) at birth (he had micrognathia and the typical U-shaped cleft palate, which was later repaired surgically). Congenital ptosis was diagnosed during follow up. His physical examination

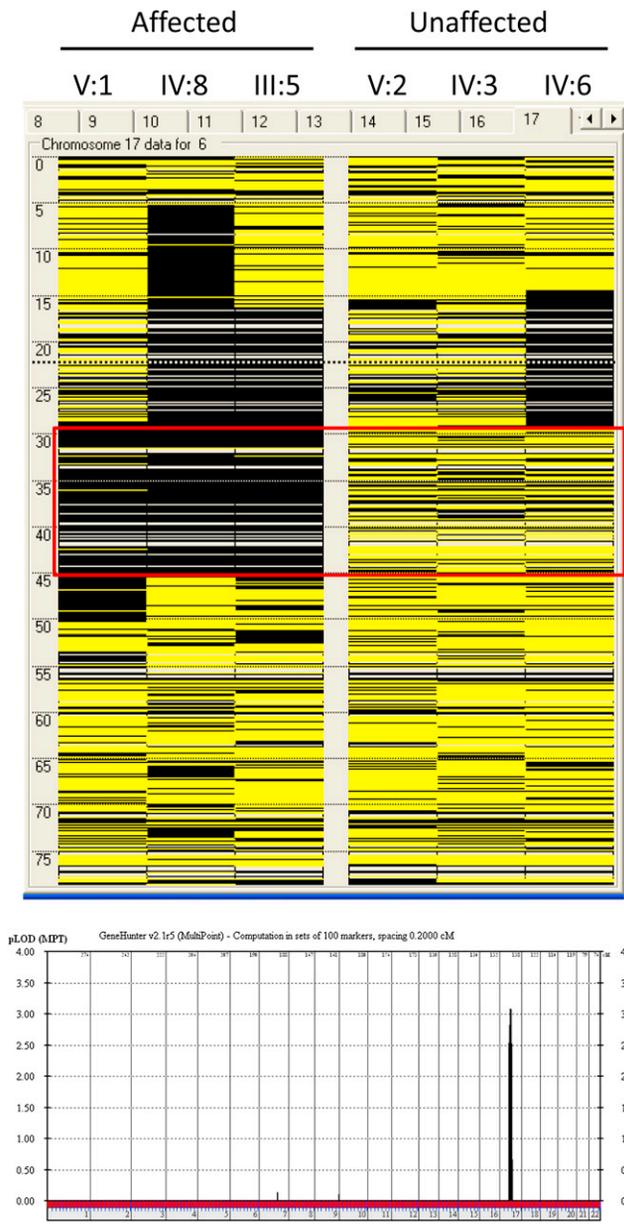


Figure 2. Identification of an Autosomal-Recessive KFS Locus
 Upper panel: Homozygosity mapping of family 1 via autoSNPa. Each column represents the homozygosity profile of one individual, and each row represents the call of an individual SNP on chromosome 17 (yellow, heterozygous; black, homozygous). Note the large run of homozygosity that is exclusively shared by the affected members (boxed in red) in family 1 (only three members were available for analysis).
 Lower panel: Linkage analysis confirmed the critical run of homozygosity, evidenced by the presence of a single significant linkage peak corresponding to the same interval.

revealed a short neck with severely limited mobility, a low posterior hairline, scoliosis, and ptosis. His skeletal survey revealed, in addition to a cervical segmentation defect, the presence of omovertebral bones, Sprengel's deformity, and dextroscoliotic deformity of the thoracic spine between T2 and T11 (Figure 1). His family history revealed that his parents are first cousins and that he has an affected sister.

Subsequent evaluation of the sister revealed identical physical findings except that the cleft palate was absent. We sequenced the entire coding region of *MEOX1* and uncovered a homozygous transition, c.664C>T, that created a premature stop codon, i.e., nonsense mutation p.Arg222* (Figure 3). Although this mutation affects the last exon and so is unlikely to be subject to NMD, RT-PCR with blood-derived RNA consistently showed complete absence of the mutant transcript (Figure 3). NMD caused by introduction of premature termination codon in the last exon has been described before but very rarely.^{14,15} Our results indicate that both mutations are null, which is consistent with the similarity in clinical phenotype between the two families.

Meox1 and its paralogue, *Meox2*, encode homeobox proteins that are localized to many mesodermal structures.^{16,17} Their expression patterns are both overlapping and distinct.^{16–19} The focus has mostly been on their roles in somite development, which also appear to be both overlapping and distinct. *Meox1* appears to have a non-redundant role in the development of the sclerotome whereas *Meox2* appears to have a nonredundant role in the development of the myotome such that in *Meox1/Meox2* double homozygous knockout mice there is severe deficiency of the sclerotome- and myotome-derived structures, whereas the presence of one copy of *Meox1* or *Meox2* rescues sclerotome or myotome development, respectively.²⁰ Two independent null alleles in *Meox1* have been described, and although heterozygotes are phenotypically normal, homozygotes have an identical segmentation defect of the cervical spine but normal muscle development. This pattern is remarkably similar to the phenotype we observe in individuals with *MEOX1*-truncating mutations.²¹

The mechanism by which *Meox1* controls normal somite development is incompletely understood. One proposed mechanism is that *Meox1* maintains the appropriate expression of downstream genes known to regulate somite development; namely, these genes are *Bapx1*, *Tbx18*, and *Uncx*.^{21,22} This proposal is supported by the fact that *Meox1* physically binds the promoter of these genes and that its deficiency perturbs their expression domain. One conspicuous effect of *Meox1* deficiency is the diminution of the normal caudal/rostral polarity of somites at very early stages of development, and although differential cell proliferation has been proposed as a mechanism, the exact mechanism remains unknown.²¹ Interestingly, the authors who describe the distinct skeletal profile in *Meox1*^{-/-} mice speculated that the equivalent human phenotype might be a spondylocostal dysostosis syndrome, and our data strongly support the idea that KFS is indeed the human phenotypic equivalent. Of note, a feedback loop between *Meox1* and *Hoxa2* has been demonstrated in the development of the second branchial arch, and although *Meox1* was proposed to have a redundant role in this process, we note that the Pierre-Robin sequence seen here in one of the two human

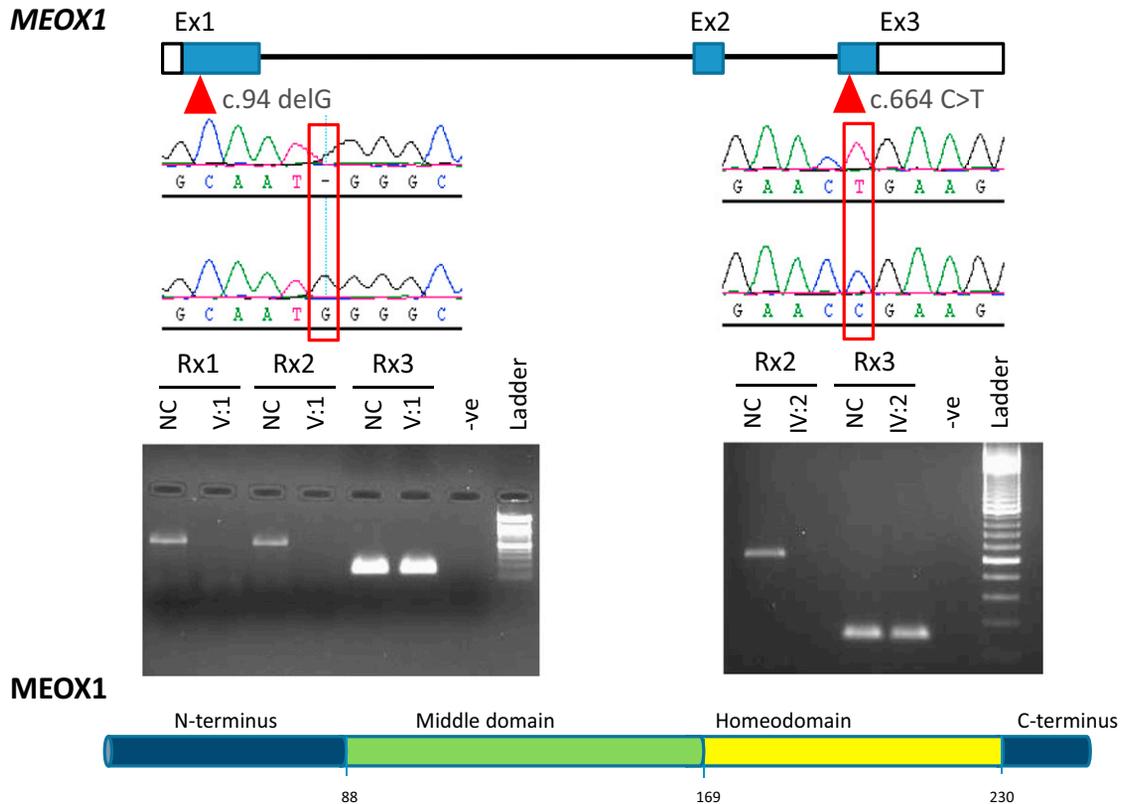


Figure 3. Identification of Two Null Alleles in *MEOX1*

The location of the two mutations is shown above the schematic depiction of *MEOX1* along with sequence chromatograms of the affected individual and normal control. An RT-PCR experiment for the mutation in family 1 and family 2 is also shown; Rxn1 refers to one segment of exon 1, Rxn2 refers to another segment of exon 1, and Rxn3 is the *GAPDH* control. Note the complete absence of the mutant transcript in the affected individuals compared to the control. The source of RNA was lymphoblasts from individual V:1 and whole blood from individual IV:2.

individuals with a *MEOX1* mutation suggests that this role may not be completely redundant in humans.²³

In summary, we show that homozygous truncating mutations in the gene encoding mesenchyme homeobox 1 cause an autosomal-recessive form of KFS. The mode of inheritance, the predilection of the segmentation defect to the cervical spine, and the sparing of myotome-derived structures are remarkably conserved features between humans and mice deficient in this gene. It remains to be seen what the contribution of *MEOX1* is, if any, to sporadic forms of KFS.

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Web Resources

The URLs for data presented herein are as follows:

Exome Variant Server (EVS), <http://evs.gs.washington.edu/EVS/>
 Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

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