A novel mutation of HOXA13 in a family with hand-foot-genital syndrome and the role of polyalanine expansions in the spectrum of Müllerian fusion anomalies

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Abstract

Objective—To report a novel mutation found in a family with hand-foot-genital syndrome (HFGS). To characterize the genetic basis of true HFGS versus presence of non-HFGS-related uterovaginal septa.

Design—Case-control study.

Setting—Academic medical center.

Patient(s)—HFGS patients and family members; women with uterine or uterovaginal septa without other sequelae of HFGS.

Intervention(s)—Sequence analysis of HOXA13 in members of a family with HFGS (3 affected, 1 unaffected); sequence analysis of HOXA13 in biopsy samples obtained from 17 non-HFGS patients with idiopathic uterine or uterovaginal septa and in 11 normal controls.

Main Outcome Measure(s)—Presence or absence of mutations of HOXA13.

Result(s)—Affected members of a family with HFGS showed a novel expansion of the third polyalanine tract of HOXA13, inserting 10 alanines in-frame. None of the patients with idiopathic uterovaginal septa displayed mutations of HOXA13.

Conclusion(s)—The cause of uterovaginal septa without hand and foot symptoms differs from true HFGS. When patients present with septa, it is not necessary to subject them to x-rays of the distal limbs or to sequence analysis of HOXA13 unless they show clear signs of the other sequelae characteristic of true HFGS.

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Capsule

HOXA13 screening found no mutations in subjects with Müllerian fusion defects, but novel expansions in subjects with HFGS. This indicates that septa arise from a different genetic basis than HFGS.

Keywords

hand-foot-genital syndrome; HOXA13; polyalanine expansion; uterovaginal septum

Introduction

Hand-foot-genital syndrome (HFGS, sometimes referred to as hand-foot-uterus syndrome) is a rare congenital disease characterized by genitourinary defects and malformation of the distal limbs (1). Characteristic of the disorder are Müllerian fusion abnormalities such as presence of a partial or complete longitudinal uterovaginal septum. In both males and females, genitourinary defects such as hypospadias are common (2,3). Limb malformations typically include shortening of the digits and toes, medially-deviated great toes, pointed distal phalanxes in the thumbs and great toes, clinodactyly, and delayed ossification or fusion of the wrist bones (3).

HFGS has an autosomal dominant pattern of inheritance (4) and is fully penetrant. The disease phenotype has been linked to mutations of HOXA13 (5), the most distal member of the HOXA gene cluster and a transcription factor involved in distal limb and genitourinary tract development (6). Of the mutations previously described in HFGS patients, one family had a missense mutation, and all others are either gross deletions of HOXA13 or the entire HOXA cluster, nonsense mutations (5), or expansions of the polyalanine tracts located in exon 1 (7).

The HOXA13 protein contains three polyalanine tracts, all located in exon 1. Expansions of these polyalanine regions have been shown to be the most common cause of hand-foot-genital syndrome (7). Previous studies of HFGS families have identified expansions that add 6 (7,8), 8 (9,10), 9 (11), 11, 12 (10) and 14 (12) alanine residues to the polyalanine tracts in HOXA13. Disease-linked expansions have been reported in both the second and third polyalanine regions. Severity of disease has been shown to be directly proportional to the length of the polyalanine expansion (10).

While the deletions and nonsense mutations of HOXA13 appear to cause haploinsufficiency, polyalanine expansion of HOXA13 creates a dominant negative protein (12,13). This is consistent with the autosomal dominant pattern of inheritance in HFGS. Wild type HOXA13 and HOXD13 mislocalize when coexpressed with mutant HOXA13 that contains polyalanine expansions *in vitro*. The presence of additional alanine residues in the polyalanine tracts of HOXA13 has been shown to cause aggregations of both HOXA13 and HOXD13 in the cytoplasm, preventing these transcription factors from entering the nucleus. Additionally, the length of the polyalanine insertion is proportional to the amount of HOXA13 and HOXD13 protein sequestered in the cytoplasm; a longer polyalanine expansion in the mutant allele results in lower levels of normal HOXA13 and HOXD13 in the nucleus. (12)

While true HFGS is quite rare, Müllerian fusion anomalies have an estimated prevalence of 0.5–4% in the general public (14). Even in severe cases of HFGS, the deformities of the hands and feet can be subtle. This suggests that some cases of HFGS may have no obvious manifestations. The severity of HFGS has been shown to be proportional to the polyalanine repeat length, so other uterovaginal anomalies could potentially fall into this spectrum, perhaps harboring small polyalanine expansions. We hypothesized that uterovaginal septa represent a subtle form of HFGS, arising from the same genetic basis as true HFGS: mutations of HOXA13, especially expansions of the polyalanine regions that add one to five alanines.

Materials and Methods

Study Participants

Three members of a family with HFGS (3 affected, 1 unaffected) were recruited under an approved Human Investigation Committee (HIC) protocol. Informed consent was obtained from all subjects, and blood samples were collected for DNA extraction. The family members underwent clinical examinations and x-ray imaging of the distal limbs.

Seventeen patients who had undergone surgery to remove idiopathic (non-HFGS-related) uterine or uterovaginal septa were identified retrospectively. Samples from biopsies taken at the time of septum removal were requested from surgical pathology archives under an approved HIC protocol.

Control DNA was obtained from 11 healthy volunteers with no history of reproductive problems.

DNA extraction

Total genomic DNA was extracted from blood samples using a DNeasy Blood and Tissue Kit (QIAGEN).

For idiopathic septum samples, three 15 micron sections were cut from each paraffin block. Paraffin was removed using xylene and ethanol washes. DNA was then extracted from the formalin-fixed paraffin-embedded tissue using a DNeasy Blood and Tissue Kit.

PCR and Sequencing

The entire HOXA13 gene, including the promoter region, exons and the intron, were divided into seven overlapping sections, and primers were designed using Primer3. Each section of the genomic DNA was amplified for every sample by polymerase chain reaction (PCR) using Hot Star Taq Polymerase (QIAGEN). For the paraffin-embedded samples, DMSO was added to the PCR reaction mixture at 5% v/v in order to improve amplification. PCR products were run on a 1.5% agarose gel, and then gel purified using a QIAquick Gel Extraction Kit (QIAGEN). Purified DNA was submitted to the Yale core facility for fluorescent electrophoretic sequencing using Applied Biosystems 3730 capillary instruments. Results were analyzed using 4Peaks (Mekentosj) and BLAST (NCBI).

Molecular Cloning

In order to separate the two alleles found in the polyalanine region for sequencing, direct cloning of PCR products was performed using a TOPO TA Cloning Kit for Sequencing (Invitrogen). Samples from PCR reactions amplifying the polyalanine regions for the HFGS family members were inserted into the pCR4-TOPO vector according to manufacturer's instructions. The ligated constructs were transformed into TOP-10 chemically competent cells (Invitrogen) by incubating the E. coli cells with the cloning reaction on ice for 30 minutes, followed by a 30 second heat shock at 42°C. The cells were then incubated for 1 hour at 37°C with added SOC media, and then were spread on LB plus ampicillin selective plates and incubated overnight at 37°C. Colonies were picked, grown overnight in LB plus ampicillin selective media, and plasmids were purified using a QIAprep Spin Miniprep Kit (QIAGEN). The HOXA13 polyalanine region inserted into the plasmid was PCR amplified using T3 and T7 primers sites located in the plasmid. The PCR products were run on a 1.5% agarose gel, gel purified, and submitted for sequencing as described above.

Results

The subjects who underwent surgery to remove isolated, non-HFGS-related septa represented a range of severity. Twelve women had uterine septa only, while five subjects had complete longitudinal uterine and vaginal septa.

No HOXA13 mutations were found in any of the samples from patients with idiopathic septa. A novel polymorphism was found in the 3'-untranslated region of HOXA13 in all subjects, but was also found in normal controls. The polymorphism consists of a deletion and a transversion, changing the sequence beginning at base 3158 from AAAAAAAAAAACCCT to AAAAAAAAACCCCT.

A family presented with classic signs of HFGS. The daughter, son, father, and paternal grandfather all appeared to be affected, while the mother and paternal grandmother were normal. The daughter, a 19-year-old female, presented with a complete longitudinal uterine and vaginal septum, hypospadias, and an imperforate hymen. The affected men all had hypospadias. Radiographic findings in the daughter and father showed bilateral shortening of the thumbs and halluces, bilateral shortening of both hand and foot phalanges and lack of normal tufting, bilateral pointing of the distal phalanges of the thumbs, and bilateral fusions of the wrist bones and distal interphalangeal joints. The son and paternal grandfather displayed similar abnormalities of the hands and feet. Two of the paternal grandfather's three daughters from another marriage were reported as having reproductive tract abnormalities and unspecified hand and foot morphologies.

Sequence analysis showed a novel mutation of HOXA13 in this family. The mutation is a 30-nucleotide insertion beginning at base 269 that expands the third polyalanine tract by 10 alanine residues. Affected members are heterozygous for the mutation, but it was not found in the unaffected mother or in control DNA.

Discussion

Hand-foot-genital syndrome was the second human disease shown to be linked to mutations in the homeobox (HOX) genes; the first was synpolydactyly, caused by a mutation of HOXD13 (15). HOX genes act to establish the anterior-posterior body plan and regulate tissue differentiation in all animal species. The HOX genes encode transcription factors, regulating target genes which in turn determine tissue identity. Arranged in four distinct clusters (HOXA-D), the linear organization of each cluster of HOX genes dictates the spatial and temporal pattern of gene expression. The 3'-most end of the each cluster is expressed both earliest in development and most anteriorly along the body axis; likewise, genes at the 5'-end of the cluster are expressed later and more posteriorly. (6,16,17)

HOXA13 is located at the 5'-most end of the HOXA cluster, making its expression both late and caudal. Normally, HOXA13 is involved in the development of the most distal end of the Müllerian duct, which becomes the vagina. Thus, HOXA13 mutations are obvious candidates for Müllerian fusion defects such as uterovaginal septa. (6,18)

However, while our results did link a new case of HFGS to a novel mutation of HOXA13, sequence analysis of patients with non-HFGS septa did not show a connection to mutations in HOXA13. None of the patients with uterovaginal septa and unknown skeletal morphology displayed any mutations of HOXA13, including polyalanine expansions. Without the characteristic hand and foot symptoms, idiopathic septa appear to have a genetic basis that differs from that of true HFGS. An isolated uterovaginal septum seems to be a distinct condition rather than a mild form of HFGS.

Clinically, these findings suggest that the presence of a uterovaginal septum alone does not warrant radiographic examination of the distal extremities or HOXA13 mutation analysis. When a septum is discovered in a patient, the hands and feet should be visually examined for clinodactyly and shortening of the fingers and toes. While our sample size was limited, our data suggest that if no such deviations are found, further analysis for HFGS appears unnecessary.

Acknowledgments

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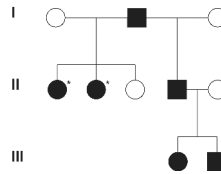
Figure 1.
Pedigree of the HFGS family. Filled symbols indicate affected individuals. Asterisk indicates that genitourinary anomalies were reliably reported, but no confirmed hand and foot data could be obtained. HFGS shows an autosomal dominant pattern of inheritance.
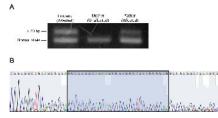
Figure 2.
(A) PCR amplification of the polyalanine region in affected and unaffected HFGS family members. Unaffected individuals are shown to be homozygous for the wild-type allele. HFGS-affected kindred are shown to be heterozygous, possessing one wild-type allele and another allele that contains a +30 bp gross insertion. None of the septum samples displayed the dual alleles characteristic of a polyalanine expansion. (B) Novel mutation of HOXA13. The 30 inserted nucleotides found in affected members of the HFGS family are highlighted and boxed. This mutation expands the third polyalanine tract in HOXA13 by 10 residues.

Table 1

Primer sequences used in PCR and sequencing.

1 – TATA & Promoter	*Forward:* TACCTGACCTGTCCCCACTGCT
	Reverse: CCCCTTCCATGTTCTTGTTG
2 – Polyalanine Region	*Forward:* CGACGAGCTCAACAAGAACA
	Reverse: TAGTAGCCGCTGCCGAAGTA
3 – Coding	*Forward:* TACTTCGGCAGCGGCTACTA
	Reverse: AGTACATTTGGCCGTTCCAG
4 – Intron	*Forward:* CTGGAACGGCCAAATGTACT
	Reverse: ACCCGCTGTACAATCCTGTC
5 – Homeodomain	*Forward:* GACAGGATTGTACAGCGGGT
	Reverse: GGGGTTGACGTTTGACATTT
6 – Coding & End	*Forward:* CCAGCCCGTTAAATGTCAAA
	Reverse: TTCTCCAGCAAATGTTTAGCAA
7 – 3'-UTR	*Forward:* TTGAATTGGCATTTCCCAAT
	Reverse: TCCCTTTTCTTAAAAATTTGGTAGC