

Analysis of De Novo *HOXA13* Polyalanine Expansions Supports Replication Slippage Without Repair in Their Generation

Kailey M. Owens,¹ Shane C. Quinonez,¹ Peedikayil E. Thomas,² Catherine E. Keegan,^{1,2} Nanci Lefebvre,³ Diane Roulston,³ Christine A. Larsen,⁴ H. Scott Stadler,⁴ and Jeffrey W. Innis^{1,2*}

¹Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan

²Department of Human Genetics, University of Michigan, Ann Arbor, Michigan

³Department of Pathology, Cytogenetics Laboratory, University of Michigan, Ann Arbor, Michigan

⁴Department of Molecular and Medical Genetics, Oregon Health Sciences University and Shriners Hospital for Children, Portland, Oregon

Manuscript Received: 3 October 2012; Manuscript Accepted: 12 December 2012

Polyalanine repeat expansion diseases are hypothesized to result from unequal chromosomal recombination, yet mechanistic studies are lacking. We identified two de novo cases of hand-foot-genital syndrome (HFGS) associated with polyalanine expansions in *HOXA13* that afforded rare opportunities to investigate the mechanism. The first patient with HFGS was heterozygous for a de novo nine codon polyalanine expansion. Haplotype investigation showed that the expansion arose on the maternally inherited chromosome but not through unequal crossing over between homologs, leaving unequal sister chromatid exchange during mitosis or meiosis or slipped mispairing as possible explanations. The asymptomatic father of the second patient with HFGS was mosaic for a six codon polyalanine expansion. Multiple tissue PCR and clonal analysis of paternal fibroblasts showed only expansion/WT and WT/WT clones, and haplotype data showed that two unaffected offspring inherited the same paternal allele without the expansion, supporting a postzygotic origin. Absence of the contracted allele in the mosaic father does not support sister chromatid exchange in the origin of the expansion. Mosaicism for *HOXA13* polyalanine expansions may be associated with a normal phenotype, making examination of parental DNA essential in apparently de novo HFGS cases to predict accurate recurrence risks. We could not find an example in the literature where unequal sister chromatid exchange has been proven for any polyalanine expansion, suggesting that the principal mechanism for polyalanine expansions (and contractions) is slipped mispairing without repair or that the true frequency of unequal sister chromatid exchange involving these repeats is low. © 2013 Wiley Periodicals, Inc.

Key words: hand-foot-genital syndrome; *HOXA13*; polyalanine expansion

INTRODUCTION

Hand-foot-genital syndrome (HFGS; OMIM 140000) is an autosomal dominant condition characterized by limb and urogenital

How to Cite this Article:

Owens KM, Quinonez SC, Thomas PE, Keegan CE, Lefebvre N, Roulston D, Larsen CA, Stadler HS, Innis JW. 2013. Analysis of de novo *HOXA13* polyalanine expansions supports replication slippage without repair in their generation.

Am J Med Genet Part A 161A:1019–1027.

defects, including bilateral shortening of the thumbs and great toes, vesicoureteral reflux, incomplete Müllerian fusion in females, and hypospadias in males [Innis, 2006]. The HFGS is caused by mutations in the *HOXA13* gene (OMIM 142959) [Mortlock and Innis, 1997; Goodman, 2000]. The *HOXA13* gene includes two exons; exon one contains three large polyalanine repeat tracts of 14, 12, and 18 residues (labeled I through III for simplicity), while exon two encodes the homeodomain. The majority of reported mutations in the *HOXA13* gene in patients with HFGS have been in-frame, meiotically and mitotically stable, short (7–14 residue) expansions within any one of the three large polyalanine tracts, while point mutations comprise nearly all of the remaining mutations [Innis, 2006].

At least nine distinct diseases are associated with short polyalanine expansions in other genes encoded by imperfect triplet

Grant sponsor: University of Michigan, Department of Human Genetics and the Department of Pediatrics.

None of the authors have any conflicts of interest related to this publication.

*Correspondence to:

Jeffrey W. Innis, 4909 Buhl Building, 1241 E. Catherine Street, Ann Arbor, MI 48109-5618. E-mail: innis@umich.edu

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 26 March 2013

DOI 10.1002/ajmg.a.35843

repeats [Brown and Brown, 2004], and recently *NIPAI* polyalanine expansions have been associated with earlier onset and shortened median survival in amyotrophic lateral sclerosis [Blauw et al., 2012]. While replication slippage and associated mechanisms [Chen et al., 2005; Pearson et al., 2005; McMurray, 2010] have been shown to cause the unstable contractions and expansions associated with classic trinucleotide repeat diseases, polyalanine expansions are hypothesized to be caused by unequal crossing-over [Warren, 1997; Brown and Brown, 2004]; however, this has not been proven in *HOXA13* polyalanine expansion mutations in patients with HFGS. Replication slippage or slipped mispairing [Strand et al., 1993; Mortlock et al., 1996; Tishkoff et al., 1997; Innis et al., 2004; Chen et al., 2005; Parodi et al., 2008; Trochet et al., 2007] mediated by misalignment of short repetitive sequences during replication is another mechanism capable of generating polyalanine expansion (or contraction) mutations. Unequal sister chromatid exchange or homologous recombination is not supported by the observations of (1) polyalanine tract expansions involving at least two duplications in single alleles of *HOXD13* and *FOXL2* [Brown and Brown, 2004], (2) sequence incompatibilities with recombination in *PHOX2B* [Trochet et al., 2007], and (3) lack of deletion products expected for unequal sister chromatid exchange in mosaic patients with polyalanine expansions in *PHOX2B* [Trochet et al., 2007; Parodi et al., 2008]. We identified two rare cases of apparently de novo HFGS associated with *HOXA13* polyalanine expansions allowing for experimental testing of these hypotheses.

MATERIAL AND METHODS

This study was approved by the University of Michigan Medical Institutional Review Board. Genomic DNA was extracted from leukocytes.

HOXA13 exons 1 and 2 were amplified by PCR using custom oligonucleotide primer pairs designed using Primer3 as previously described [Innis et al., 2004]. To obtain independent clones, PCR products were ligated into the p-GEM[®] T-Easy Vector system (Promega Corp., Fitchburg, WI), electroporated MegaX DH10 β T1R Electrocomp[™] cells (Invitrogen/Life Technologies Corp., Grand Island, NY), and blue-white colony selection was used.

We used SNPs upstream and downstream of *HOXA13* and inverse PCR to determine SNP phase on each chromosome. BstBI restriction enzyme sites located outside of the region of interest were utilized to cut maternal genomic DNA and re-ligate, followed by inverse PCR to appose upstream and downstream SNPs. The PCR products were gel-excised and cloned. Sequencing of 18 clones using custom primers produced the final haplotypes (Fig. 3).

Luciferase assays were performed in NG108-15 cells comparing *HOXA13* wt and *HOXA13*-G177R mutant proteins. The *HOXA13*-G177R mutant construct was generated by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), and the presence of this mutation was confirmed by DNA sequencing. NG108-15 cells (ATCC#HB-12317) were maintained in DMEM media (Gibco/Life Technologies Corp., Grand Valley, NY) supplemented with 10% FBS (Atlanta Biologicals, Norcross, GA), HAT (Invitrogen), and 1% penicillin/streptomycin. Cells (1×10^5) were seeded in 12-well plates and grown for 24 hr at 37°C with 5% CO₂. Transfections were performed using FuGENE6 transfection

reagent (Roche Applied Science, Indianapolis, IN), 0.1 μ g pRL-CMV Renilla, and 0.25 μ g pCAGGS-*HOXA13* wild type or mutant, along with 0.5 μ g of a pGL4.23 plasmid (Promega) encoding luciferase and containing an EphA7 *cis*-regulatory element previously shown to be regulated by *HOXA13* [Stadler et al., 2001]. Empty pGL4.23 and pCAGGS expression vectors were used as controls (red bars). The Dual-Glo Luciferase Assay system (Promega) was used to detect luciferase activity 24 hr posttransfection in OptiPlate-96F black plates using a Fusion Microplate Analyzer (Perkin Elmer, Waltham, MA). Six replicates of each transfection were performed and the transfection assay was repeated a total of three separate times. Results were normalized for transfection efficiency using a *Renilla* luciferase expression vector as described by the manufacturer (Promega). Because the pGL4.23 vector also contains several *HOXA13* binding sites, background activation of the empty pGL4.23 by *HOXA13* was also subtracted from the final luciferase levels after *Renilla* normalization.

CLINICAL REPORTS

Patient 1 is a female born to nonconsanguineous parents. No pregnancy or birth history was available. The patient had a history of normal developmental milestones. We first evaluated her at 17 years, 10 months of age. At that time, her height was 150.2 centimeters (~3rd centile), her weight was 45.7 kg (5–10th centile), and her head circumference was 54.0 cm (~35th centile). The patient's medical history was significant for genitourinary abnormalities including a small bladder, urinary leakage, and multiple urinary tract infections necessitating numerous Mitrofanoff and bladder augmentation procedures. Additional medical history was significant for back and heel pain, a right inguinal hernia status—postrepair, a left heminephrectomy, and scoliosis with no thoracolumbar vertebral segmentation anomaly. Clinical examination and radiographic findings showed a short distal phalanx of the thumbs bilaterally, micronychia of the thumbs bilaterally, limited flexion of the DIP joint and distal phalanges of the fourth fingers bilaterally, radial fifth finger clinodactyly bilaterally, short distal phalanges of all toes bilaterally, short middle phalanges of all toes bilaterally, fusion of the middle and distal phalanges of all toes bilaterally, hypoplasia of the cuneiform bilaterally with right-sided fusion with the metatarsal, and micronychia of the great toes bilaterally (Fig. 1A). Based on these historical and physical findings, the patient was diagnosed with HFGS. The patient's mother and maternal half-sister were also examined and were unaffected (Fig. 1B). The patient's father is deceased but reportedly did not have thumb, toe, or genitourinary abnormalities. A maternal half-brother and paternal half-brother were also both reportedly unaffected. Based on the family history and physical exams, we hypothesized that the first patient had de novo HFGS.

Patient 2 is a male born to a 25-year-old mother and 27-year-old father who are nonconsanguineous. The patient was born at 39 weeks' gestation and weighed 4.1 kg (>90th centile) and was 51.4 cm long (75–90th centile). The pregnancy was uncomplicated. Shortly after birth, the patient was diagnosed with bacterial pneumonia and spent time in the neonatal intensive care unit. The patient met expected developmental milestones. We first evaluated him at 35 months of age. At that time, his height was 94.8

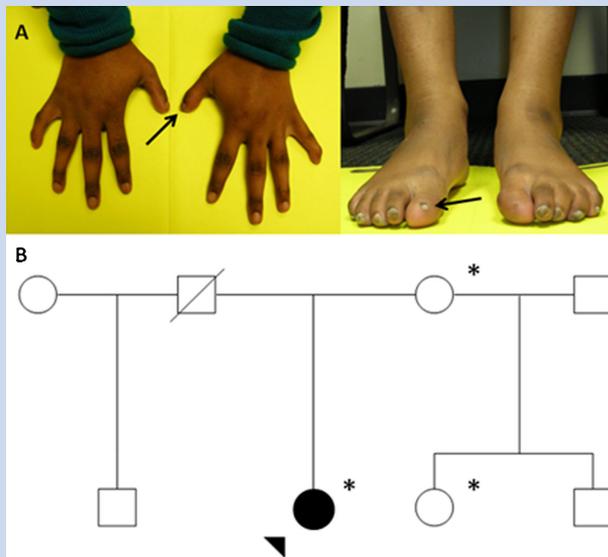


FIG. 1. Phenotype and pedigree of Patient 1. A: Limb phenotype in Patient 1 with hand-foot-genital syndrome (HFGS). Note the short thumbs and great toes with micronechia of the thumb and great toe bilaterally (arrows). B: Pedigree of the patient's family. Open squares or circles mean the individual is unaffected. An asterisk indicates individuals who were clinically examined.

centimeters (46th centile), his weight was 13.8 kg (40th centile), and his head circumference was 49.8 cm (54th centile). Physical examination revealed several bony abnormalities of the hands and feet, as well as partial cutaneous two-three-toe syndactyly bilaterally (Fig. 5C,D). The patient was also found to have mild glandular hypospadias with no history of urinary tract infections and occasional toe-walking. A skeletal survey showed the following abnormalities: short middle phalanx of all fingers, short proximal and distal phalanges of the thumbs, radial fifth finger clinodactyly bilaterally, short first metacarpals bilaterally, short distal phalanges of all toes bilaterally, short proximal phalanges of the great toes, absent ossification of the middle phalanges of toes 2 through 5 bilaterally, and short first metatarsals. Based on these findings, the patient was diagnosed with HFGS.

The patient's mother, father, brother, sister, maternal grandfather, and maternal grandmother were also examined and were unaffected. Based on the family history and physical exams, we hypothesized that the second patient also had de novo HFGS.

RESULTS

Patient 1

Amplification of an exon 1 segment including the large 18 residue polyalanine tract (III) resulted in one product compatible with wild type length (arrow, Fig. 2A) and a larger amplicon. Sequencing of independent clones from several independent amplifications showed one allele with a nine-alanine expansion in tract III (c.357_383 dup27, Fig. 2B) and, surprisingly, a second allele

with a point mutation, c.529G > C (p.Gly177Arg) (Genbank accession no. JN628255; Fig. 2C), showing the two sequence variations on separate homologs.

To determine if either mutation was inherited, maternal genomic DNA from two independent blood samples was analyzed for the presence of the nine-alanine expansion and the c.529G > C point mutation identified in Patient 1, which showed that the mother carried neither. While the mother could still be a low-level somatic or germline mosaic, neither mutated allele, nor any shorter *HOXA13* PCR products, were detected in repeated amplifications of leukocyte derived DNA. A SNP-based haplotype upstream and downstream of the *HOXA13* gene was constructed for Patient 1 and her mother to determine the parental origin of the polyalanine expansion. Patient 1's *HOXA13* SNP haplotype showed that the polyalanine expansion must have originated de novo on the maternal chromosome while the point mutation must have originated on the paternally inherited chromosome (Fig. 3).

To determine whether the polyalanine expansion occurred as a result of unequal crossing-over between homologs during meiosis, we used inverse PCR. Patient 1 was homozygous for the upstream SNPs rs7812039 and rs13243033, whereas her mother was heterozygous at these loci. Figure 3 also shows the results of inverse PCR with maternal DNA. The de novo polyalanine expansion did not arise by unequal crossing-over between maternal chromosome 7 homologs, and must have occurred as a result of either slipped mispairing or unequal sister chromatid exchange in maternal mitosis prior to gamete production or during meiosis II, or by either mechanism early on postzygotically. Although we did not examine for mosaicism in Patient 1, a deleted product by PCR analysis was never detected, suggesting that if the expansion had occurred postzygotically, unequal sister chromatid exchange was unlikely.

Given that homozygous loss of *Hoxa13* function is lethal [Mortlock et al., 1996; Warot et al., 1997; Shaut et al., 2008], and *HOXA13* polyalanine tract expansions lead to a loss of function [Innis et al., 2004], it is unlikely that Patient 1 would be compound heterozygous for two disease-causing mutations. However, the c.529G > C (p.Gly177Arg) point mutation found in Patient 1 has not been previously reported in dbSNP (build 135) or in the NHLBI Exome Sequencing Project (snp.gs.washington.edu/EVS/) and is therefore a very rare variant. Additionally, the glycine residue at position 177 is highly conserved in *HOXA13* among vertebrates [Mortlock et al., 2000; Lehoczy and Innis, 2008] and lies within a MEIS1 interacting domain [Williams et al., 2005]. Finally, arginine (basic, hydrophilic) is not a conservative substitution for glycine (small, neutral). Polyphen-2 analysis (genetics.bwh.harvard.edu/pph2/) showed this substitution to be "probably damaging" with a score of 0.992. Analysis of the c.529G > C point mutation using Genomic Evolutionary Rate Profiling (GERP) [Cooper et al., 2005] showed a rejected substitution score of 3.56, suggesting evolutionary selection against substitution at this nucleotide.

Therefore, to test the effect of this sequence variation on function, the p.Gly177Arg mutation was introduced into a plasmid containing the mouse *Hoxa13* cDNA through site-directed mutagenesis and then the p.Gly177Arg *HOXA13* transcriptional activation function was measured relative to WT *HOXA13* on the *EphA7* promoter in vitro. The *EphA7* promoter is regulated in vivo by *Hoxa13* [Stadler et al., 2001]. As seen in Figure 4, the p.Gly177Arg

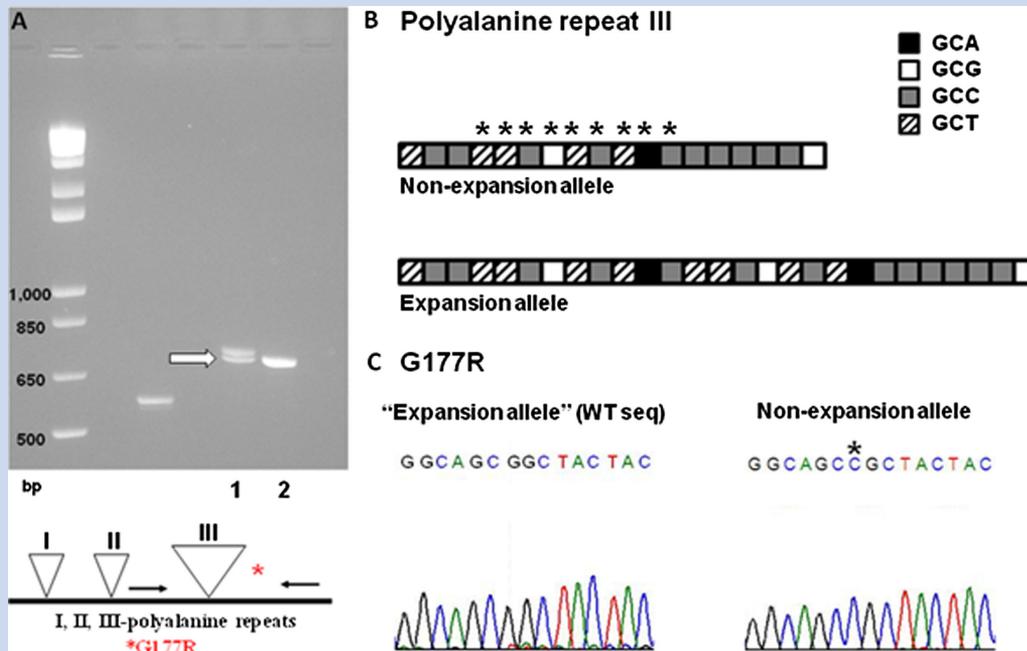


FIG. 2. Polyalanine expansion and rare variant in Patient 1. **A:** The PCR and clone sequencing analysis of *HOXA13* polyalanine tract III. Lane 1: Patient 1 PCR showing two products, one corresponding with the WT allele (arrow) and the other with the expansion. Lane 2: WT DNA. The PCR product visualized adjacent to the marker lane was a separate control reaction. **B:** Sequencing of cloned expansion products in the patient showed a 27 bp (nine-alanine) tandem duplication [c.357_383 dup27]. Imperfect GCN triplets encoding alanine residues are depicted as shown; * nine-alanine triplet codons duplicated in the expansion allele. **C:** Sequencing downstream of the polyalanine tract III showed a point mutation [c.529G > C, p.Gly177Arg; Genbank accession no. JN628255] in the allele not carrying the polyalanine expansion. The WT sequence is shown on the left and the asterisk indicates the mutated base in a PCR clone from the nonexpansion allele [c.529G > C].

mutation did not significantly affect transcriptional activation by *HOXA13* in this in vitro assay ($P = 0.631$). This result does not preclude an effect of this amino acid substitution on regulatory activity at other target loci in different cellular contexts, but it underscores the importance of functional analysis of sequence variants. Also, if Patient 1's father was heterozygous for the p.Gly177Arg variant, these results would be consistent with absence of paternal HFGS features.

Patient 2

The PCR analysis of large repeat tract III followed by cloning and sequencing showed Patient 2 to carry one 6-alanine expansion allele (c.358_375 dup18, inherited from his father) and a 10-alanine contraction allele (c.355_384 del30, inherited from his asymptomatic mother and her asymptomatic father). Close examination of Patient 2's father showed a normal extremity exam (Fig. 5A,B), suggesting either lack of penetrance or mosaicism. Paternal and maternal *HOXA13* PCR sequences matched Patient 2's two alleles (Fig. 5E,F). A SNP-based haplotype analysis upstream and downstream of *HOXA13* was performed to identify the paternal chromosome carrying the expanded alanine tract (Fig. 6). Both unaffected siblings carried the same paternal allele as Patient 2 without the expanded alanine tract, suggesting that the father was

mosaic for the expansion. The PCR sequencing on the paternal grandparents showed that neither were carriers, supporting the hypothesis that Patient 2's father sustained a postzygotic polyalanine expansion.

To prove mosaicism and to distinguish unequal sister chromatid exchange from slipped mispairing, a skin biopsy was obtained from the father to look for WT, expanded, and contracted polyalanine tract III alleles. One week later, after minimal expansion, the fibroblasts were harvested, and a sample was plated into ten 96-well plates at an average concentration of 0.8 cells per well. Many wells showed no growth, but genomic DNA from 58 wells was harvested and subjected to *HOXA13* PCR. The DNA from 11 clones failed to amplify. Thirty-four clones had both an expansion allele and a normal allele; 10 clones showed only amplification of the normal sized allele. Thus, 34/44 clones (77%) showed expansion and normal alleles, and 10/44 clones (23%) had normal alleles only, by PCR. A separate cloning experiment performed later after further expansion of the original culture, plated at higher density, detected no contracted allele among 159 samples. An additional paternal DNA sample derived from saliva showed only six-alanine expansion allele and normal allele products by PCR. No shorter six-alanine contraction allele product was observed in any genomic PCR with paternal DNA from any tissue or with any of the clonal fibroblast isolates.

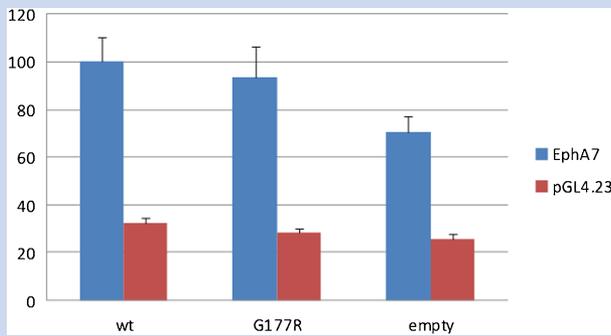


FIG. 4. In vitro transcriptional activation function of p.Gly177Arg HOXA13 on the *EphA7* promoter. Percent luciferase activities (relative to wt control) are indicated on the y-axis. HOXA13 wt or G177R (normalized to *Renilla* and empty vector controls) are plotted on the x-axis. Results are the mean \pm SD of three separate experiments.

DISCUSSION

Family 2 is the most informative regarding mechanism(s) for *HOXA13* polyalanine expansions. From four different sites (blood, skin, saliva, and gonads by inheritance) and from individual fibroblast clones of skin, only the expanded and normal allele sizes were observed, supporting somatic and gonadal mosaicism in the asymptomatic father of Patient 2. In addition, if the mechanism of the expansion was unequal crossing over between sister chromatids, there should have been three different paternal cell lines: a six-alanine expanded/WT, WT/WT and a six-alanine contracted/WT. That no six-alanine contracted product was observed indicates that either a cell line with a six-alanine contraction/WT genotype was selected against in vivo or in vitro or that the mutational mechanism is not unequal sister chromatid exchange. The former explanation seems very unlikely given that a 10-alanine contraction allele is a low frequency polymorphism in the normal population, and indeed survives quite well in Patient 2, his mother, and maternal grandfather, making it difficult to argue that heterozygous loss of 6-alanine residues would be deleterious. Thus, slipped mispairing appears most likely as the mechanism of the postzygotic expansion in the mosaic father. Unequal crossing-over between homologs [Warren, 1997; Brown and Brown, 2004], which is also theoretically possible as a mechanism of polyalanine expansion, was not supported by the data from either of the patients described here.

Arai et al. [2007] proposed that unequal sister chromatid exchange may have led to de novo polyalanine expansions in the *PHOX2B* gene in patients with congenital central hypoventilation syndrome (CCHS; OMIM 209880); however, no data proving this mechanism was presented. The authors proposed that this mechanism could account for the stability of the polyalanine expansions as well as the presence of normal contraction alleles. Contraction alleles, although quite rare, have also been observed as presumably normal variants in the *HOXA13* gene [Brown and Brown, 2004;

Innis et al., 2004; Innis, 2006] and other genes with polyalanine repeats. In Family 1, we observed no mosaicism for the polyalanine expansion (and the expected reciprocal deleted product) in either maternal or proband DNA. Failure to observe the deleted product does not eliminate the possibility of either unequal sister chromatid exchange or slipped mispairing occurring in the maternal germline.

However, our data in Family 2 strongly support slipped mispairing during replication as the mechanism for this instance of *HOXA13* polyalanine expansion. Slipped mispairing was previously proposed as a mechanism for the instability of repetitive DNA tracts in yeast mutants for mismatch repair genes [Strand et al., 1993]. Trochet et al. [2007] also presented a family where unequal sister chromatid exchange was not a suitable explanation for a *PHOX2B* polyalanine expansion in a mosaic (leukocyte DNA) parent due to failure to detect a deleted product, and they proposed that a mispairing event without repair was more likely. Parodi et al. [2008] also documented somatic mosaicism for *PHOX2B* expansions, and did not identify shorter products expected for unequal sister chromatid exchange in mosaic individuals. Additional polyalanine tract expansions involving at least two duplications in single alleles of *HOXD13* and *FOXL2* [Brown and Brown, 2004], also support slipped mispairing. By replication slippage, both expansions and contractions could occur, depending on whether template or nascent strand misalignment was involved [Strand et al., 1993; Chen et al., 2005; McMurray, 2010]. Finally, Cocquemot et al. [2009] suggested FosTES (Fork stalling and TEmplate Switching) [Lee et al., 2007] as the mechanism for polyalanine expansion in the *Hoxd13* gene in the mouse mutant *Dyc*, whose structure is quite different from typical *HOXA13* or *HOXD13* polyalanine expansions. The authors stated that polyalanine tracts contain microhomology, symmetry, and specific sequence motifs that commonly lead to FosTES.

In our report, we have proven that slipped mispairing during replication must have occurred in Family 2 and is possible in Family 1. Whereas paternal mosaicism in Family 2 indicates a postzygotic origin for the expansion, the timing of expansion in Family 1 is unclear. In addition, the 10-alanine contraction in Proband 2 was inherited from the maternal grandfather. Thus, not unlike polyalanine expansions, this contraction has been stable in this family through at least three generations. However, it would be intriguing to examine for mosaicism of contractions as well, since an expanded allele would be an expected product of unequal sister chromatid exchange.

We reported on the molecular basis of *Hypodactyly* in mice, a 50 base pair deletion in *Hoxa13*, and proposed that the deletion arose from slipped mispairing or unequal crossing over catalyzed by nearby 10 base pair GC-rich microhomology in the first exon of *Hoxa13* [Mortlock et al., 1996]. The location of human polyalanine expansions as well as the *Hypodactyly* deletion in *HOXA13* exon 1 associated with numerous GC-rich microhomologies supports the hypothesis that the structure predisposes to expansions, contractions, and deletions of several types depending on the nature of the mispairing event.

Finally, other than for *PHOX2B* [Parodi et al., 2008; Trochet et al., 2007], this is the third investigation of mosaic polyalanine expansion carriers documenting results consistent with slipped

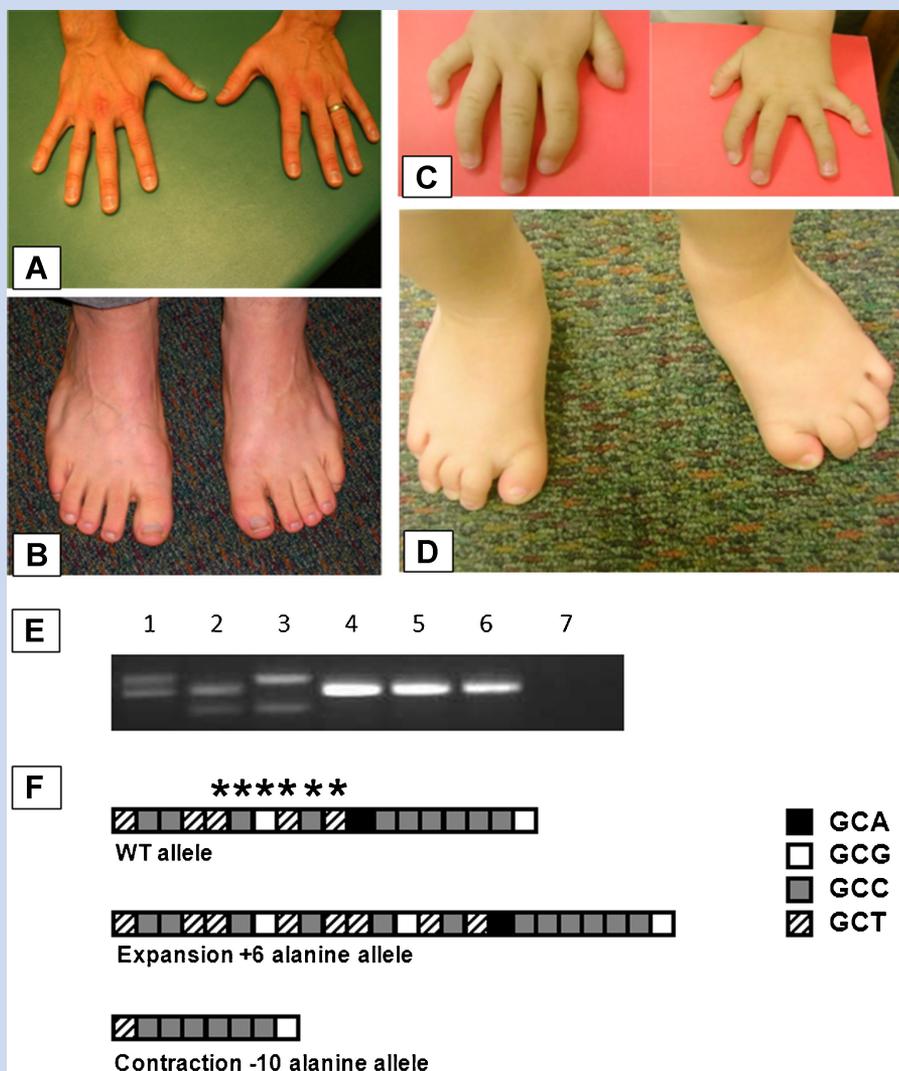


FIG. 5. Phenotype and polyalanine repeat size in Family 2. A,B: Hands and feet of asymptomatic father of Patient 2. C,D: Hands and feet of Patient 2 demonstrating typical HFGS skeletal features. E: *HOXA13* PCR products across large repeat III in Family 2. Lane 1, asymptomatic father (6-alanine expansion [c.358_375 dup18]/WT alleles); lane 2, asymptomatic mother (10-alanine contraction [c.355_384 del30]/WT); lane 3, Patient 2 (6-alanine expansion/10-alanine contraction); lanes 4,5, asymptomatic sister and brother (WT/WT); lane 6, normal control; lane 7, water control. F: Imperfect GCX triplets encoding alanine residues are depicted as shown; * six-alanine triplet codons duplicated in the expansion allele. The contraction allele sequence of triplet codons inherited from the maternal grandfather is shown.

mispairing. In contrast, despite the possibility of unequal sister chromatid exchange or homologous recombination generating polyalanine expansions, there has been no report of a proven occurrence. It will be important to identify mosaic individuals for other polyalanine expansion disorders, and possibly for contractions, and investigate particularly by clonal analysis whether replication slippage is the predominant mechanism or not.

WEBSITE RESOURCES

OMIM: <http://www.ncbi.nlm.nih.gov/>

GERP calculation: <http://gvs.gs.washington.edu/SeattleSeq/Annotation/>

Polyphen-2: <http://genetics.bwh.harvard.edu/pph2/>

NHLBI Exome Sequencing Project: <http://snp.gs.washington.edu/EVS/>

UCSC Genome Browser: <http://genome.ucsc.edu/dbSNP135:>
<http://www.ncbi.nlm.nih.gov/snp/>

SIFT: <http://sift.jcvi.org/>

Primer3: <http://frodo.wi.mit.edu/primer3/>

GeneReviews: <http://www.ncbi.nlm.nih.gov/sites/GeneTests/review?db=GeneTests>

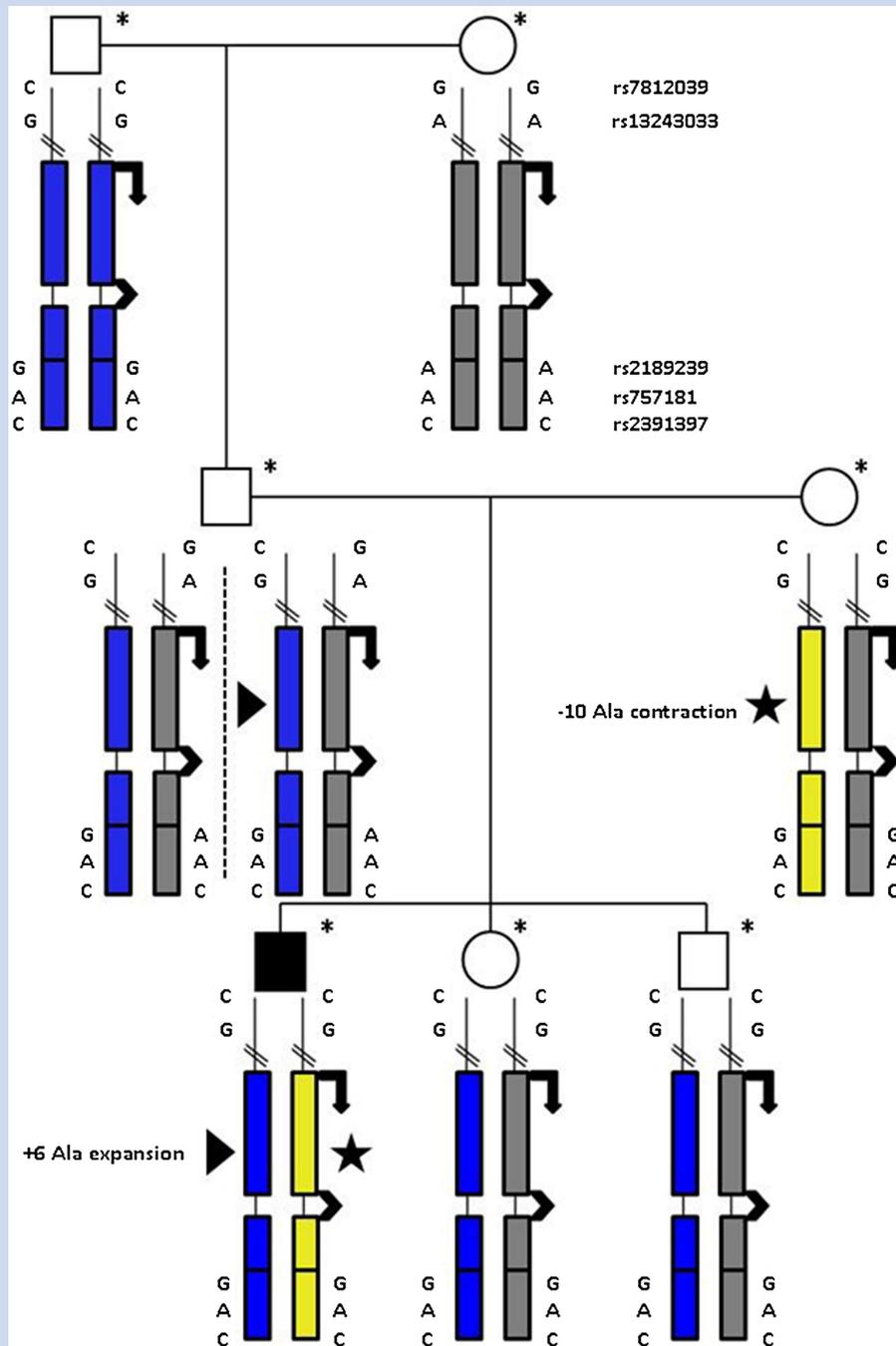


FIG. 6. Pedigree and haplotypes of Family 2. Patient 2 with HFCS, filled in square. Dotted line: father of proband is mosaic for *HOXA13* polyalanine expansion/WT and WT/WT allele-bearing cells. Patient 2 inherited a 10-alanine contraction [instead of 18 alanines, this allele has 8-alanine residues in tract III] from his asymptomatic mother. All individuals with an * were examined. Asymptomatic siblings of Patient 2 inherited the same chromosome haplotype from their father, without the polyalanine expansion observed in Patient 2.

ACKNOWLEDGMENTS

The authors thank the patients and their families for their research participation. We thank Dr. Tom Glover for helpful comments.

This research was funded by the University of Michigan, Department of Human Genetics and the Department of Pediatrics. Dr. Innis is the Morton S. and Henrietta K. Sellner Professor of Human Genetics.

REFERENCES

- Arai H, Otagiri T, Sasaki A, Hashimoto T, Umetsu K, Tokunaga K, Hayasaka K. 2007. De novo polyalanine expansion of PHOX2B in congenital central hypoventilation syndrome: Unequal sister chromatid exchange during paternal gametogenesis. *J Hum Genet* 52:921–925.
- Blauw HM, van Rheenen W, Koppers M, Van Damme P, Waibel S, Lemmens R, van Vught PWJ, Meyer T, Schulte C, Gasser T, Cuppen E, Pasterkamp RJ, Robberecht W, Ludolph AC, Veldink JH, van den Berg LH. 2012. NIPA1 polyalanine repeat expansions are associated with amyotrophic lateral sclerosis. *Hum Mol Genet* 21:2497–2502.
- Brown LY, Brown SA. 2004. Alanine tracts: The expanding story of human illness and trinucleotide repeats. *Trend Genet* 20:51–58.
- Chen J-M, Chuzhanova N, Stenson PD, Ferec C, Cooper DN. 2005. Meta-analysis of gross insertions causing human genetic disease: Novel mutational mechanisms and the role of replication slippage. *Hum Mutat* 25:207–221.
- Cocquempot O, Brault V, Babinet C, Herault Y. 2009. Fork stalling and template switching as a mechanism for polyalanine tract expansion affecting the *DYC* mutant of *Hoxd13*, a new murine model of synpolydactyly. *Genetics* 183:23–30.
- Cooper GM, Stone EA, Asimenos G, Comparative NISC Program Sequencing, Green ED, Batzoglou S, Sidow A. 2005. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res* 15:901–913.
- Goodman FR, Fryns J-P, Mortlock DP, Innis JW, Holmes LB, Donnenfeld AE, Feingold M, Beemer FA, Hennekam RCM, Scambler PJ. 2000. Novel *HOXA13* mutations and the phenotypic spectrum of hand-foot-genital syndrome. *Am J Hum Gen* 67:197–202.
- Innis JW. Hand-foot-genital syndrome. In: Pagon RA, Bird TC, Dolan CR, Stephens K, editors. *GeneReviews* [Internet]. Seattle, WA: University of Washington Seattle; 2006.
- Innis JW, Mortlock D, Chen Z, Ludwig M, Williams ME, Williams TM, Doyle CD, Shao Z, Glynn M, Mikulic D, Lehmann K, Mundlos S, Utsch B. 2004. Polyalanine expansion in *HOX A13*: Three new affected families and the molecular consequences in a mouse model. *Hum Mol Genet* 1322:2841–2851.
- Lee JA, Carvalho CMB, Lupski JR. 2007. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* 131:1235–1247.
- Lehoczy J, Innis JW. 2008. Expanded *HOXA13* polyalanine tracts in a monotreme. *Evol Dev* 10:433–438.
- McMurray CT. 2010. Mechanisms of trinucleotide repeat instability during human development. *Nat Rev Genet* 11:786–799.
- Mortlock DP, Innis JW. 1997. Mutation of *HOXA13* in hand-foot-genital syndrome. *Nat Genet* 15:179–180.
- Mortlock DP, Post LC, Innis JW. 1996. The molecular basis of *Hypodactyly (Hd)*: A deletion in *Hoxa13* leads to arrest of digital arch formation. *Nat Genet* 13:284–289.
- Mortlock DP, Sateesh P, Innis JW. 2000. Evolution of N-terminal sequences of the vertebrate *HOXA13* protein. *Mamm Genome* 11:151–158.
- Parodi S, Bachetti T, Lantieri F, Di Duca M, Santamaria G, Ottonello G, Matera I, Ravazzolo R, Ceccherini I. 2008. Parental origin and somatic mosaicism of PHOX2B mutations in congenital central hypoventilation syndrome. *Hum Mutat* 29:206–215.
- Pearson CE, Edamura KN, Cleary JD. 2005. Repeat instability: Mechanisms of dynamic mutations. *Nat Rev Genet* 6:729–742.
- Shaut CAE, Keene DR, Sorensen LK, Li DY, Stadler HS. 2008. *HOXA13* is essential for placental vascular patterning and labyrinth endothelial specification. *PLoS Genet* 4:e1000073.
- Stadler HS, Higgins KM, Capecchi MR. 2001. Loss of Eph-receptor expression correlates with loss of cell adhesion and chondrogenic capacity in *Hoxa13* mutant limbs. *Development* 128:4177–4188.
- Strand M, Prolia TA, Liskay RM, Petes TD. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 365:274–276.
- Tishkoff DX, Filosi N, Gaida GM, Kolodner RD. 1997. A novel mutation avoidance mechanism dependent on *S. cerevisiae* *RAD27* is distinct from DNA mismatch repair. *Cell* 88:253–263.
- Trochet D, de Pontual L, Keren B, Munnich A, Vekemans M, Stanislas L, Amiel J. 2007. Polyalanine expansions might not result from unequal crossing-over. *Hum Mutat* 28:1043–1044.
- Warot X, Fromental-Ramain C, Fraulob V, Chambon P, Dollé P. 1997. Gene dosage-dependent effects of the *Hoxa-13* and *Hoxd-13* mutations on morphogenesis of the terminal parts of the digestive and urogenital tracts. *Development* 124:4781–4791.
- Warren ST. 1997. Polyalanine expansion in synpolydactyly might result from unequal crossing-over of *HOXD13*. *Science* 275:408–409.
- Williams TM, Williams ME, Innis JW. 2005. Range of *HOX/TALE* super-class associations and protein domain requirements for *HOXA13-MEIS* interaction. *Dev Biol* 277:457–471.