*MUTATION IN BRIEF*

# A *HOXA13* Allele With a Missense Mutation in the Homeobox and a Dinucleotide Deletion in the Promoter Underlies Guttmacher Syndrome

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**Guttmacher syndrome, a dominantly inherited combination of distal limb and genital tract abnormalities, has several features in common with hand-foot-genital syndrome (HFGS), including hypoplastic first digits and hypospadias. The presence of features not seen in HFGS, however, including postaxial polydactyly of the hands and uniphalangeal 2nd toes with absent nails, suggests that it represents a distinct entity. HFGS is caused by mutations in the *HOXA13* gene. We have therefore re-investigated the original Guttmacher syndrome family, and have found that affected individuals are heterozygous for a novel missense mutation in the *HOXA13* homeobox (c.1112A>T; homeodomain residue Q50L), which arose on an allele already carrying a novel 2-bp deletion (-78-79delGC) in the gene's highly conserved promoter region. This deletion produces no detectable abnormalities on its own, but may contribute to the phenotype in the affected individuals. The missense mutation, which alters a key residue in the recognition helix of the homeodomain, is likely to perturb HOXA13's DNA-binding properties, resulting in both a loss and a specific gain of function.**


KEY WORDS: Hand-foot-genital syndrome; HFGS; HOXA13; Guttmacher syndrome; transcription factor; homeodomain

## INTRODUCTION

Guttmacher syndrome (MIM# 176305; Guttmacher, 1993) has a number of features in common with hand-foot-genital syndrome (HFGS; MIM# 140000), including hypoplastic thumbs and halluces, 5th finger clinobrachydactyly and hypospadias. However, two of its features, postaxial polydactyly of the hands and short or

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uniphalangeal 2nd toes with absent nails, have never been observed in HFGS patients. HFGS is now known to be caused by mutations in the *HOXA13* gene (Mortlock and Innis, 1997; Goodman et al., 2000; MIM#142959; GenBank sequences of human *HOXA13* [Accession Number U82827]). Therefore, we re-investigated the family described by Guttmacher (Fig. 1), to clarify the clinical features of relatives and to determine the molecular distinction underlying the two distinct phenotypes.

## METHODS

Two independent venous blood samples were obtained from the three affected individuals (II.2, III.3 and III.4; Fig. 1) and from four unaffected family members (I.2, II.1, II.3 and III.1) with their informed consent and the approval of the local ethical review board. Radiographs of the hands and feet were obtained from I.2 and II.3. Haplotype analysis was performed as described with short tandem repeat markers (Mortlock and Innis, 1997). We sequenced the *HOXA13* genes in II.2, III.3 and III.4. The entire coding portion of the gene and the region immediately upstream of the initiator methionine were amplified by PCR from genomic DNA (Goodman et al., 2000). Both strands of PCR products were cycle sequenced, either directly or after cloning into pCRScript (Stratagene), and analysed on an ABI377 automated sequencer by two independent labs.

## RESULTS

Given the similarity between Guttmacher syndrome and HFGS, as well as the postaxial polydactyly observed in the forelimbs of mice deficient for *Hoxd13* and *Hoxd12* (Dollé et al., 1993; Davis and Capecchi, 1996; Kondo et al., 1996), we first carried out haplotype analysis in the family using polymorphic markers in the vicinity of the *HOXA* and *HOXD* gene clusters. The results (Fig. 1 and data not shown) indicated that the affected father (II.2) had transmitted the same haplotype across the *HOXA* cluster region to his affected son and daughter, and the opposite haplotype to his unaffected daughter, consistent with linkage to *HOXA13*. Linkage was excluded across the *HOXD* cluster region. Previous routine karyotyping in II.2 had proved normal. To check for a submicroscopic deletion or rearrangement involving *HOXA13*, we carried out Southern blot analysis on II.2 and an unaffected control (II.1). No alteration was found in *HOXA13* restriction fragment lengths or gene dosage (data not shown).

In all three affected individuals, two different sequence alterations were identified. The first (Fig. 1) is a deletion of a GC dinucleotide (-78-79delGC) in the promoter region of the gene, at positions -78 to -79 relative to the initiator methionine. This deletion occurs just upstream of the major transcription start site in mouse limb bud mRNA, which is located at position -62 (Post et al., 2000). As it destroys a *Hpa*II restriction site, its presence in the affected individuals was confirmed by PCR amplification and *Hpa*II digestion (data not shown). The second is an A-to-T transversion at position 1112 of the cDNA, which changes amino acid 371of the protein (residue 50 of the homeodomain) from glutamine (C**A**G) to leucine (C**T**G). This substitution (hereafter referred to as Q50L) does not alter a restriction site, but its presence was verified by cloning the PCR products and sequencing individual clones. Neither sequence alteration was identified in II.2's unaffected daughter (III.1) or wife (II.1).

To determine the origin of these changes, we examined the sequence of *HOXA13* in II.2's mother (I.1), father (I.2) and brother (II.3). I.1 is clinically unaffected, but one of her cousins (III.8 in the original pedigree drawing; not shown in Fig. 1) reportedly has unilateral preaxial polydactyly (Guttmacher, 1993). I.1 carries neither of the *HOXA13* sequence changes present in II.2, and the hand malformation in her cousin must therefore be unrelated. Interestingly, I.2 and II.3 carry the 2-bp deletion in the promoter, but not the amino acid substitution in the homeodomain. These results, as well as haplotype analysis in I.2 and II.3, show that the amino acid substitution in II.2 arose *de novo* on the allele already carrying the 2-bp deletion, which II.2 inherited from his father. The hands and feet of I.2 and II.3 are entirely normal on both clinical and radiological examination, and they do not have hypospadias or any other urogenital tract abnormality. In addition, II.3 has four daughters, none of whom has postaxial polydactyly, short thumbs or halluces, or 5th finger clinodactyly. The deletion does not appear to be a common polymorphism, however, since we did not identify it in 79 unaffected, unrelated controls of similar ethnic background.

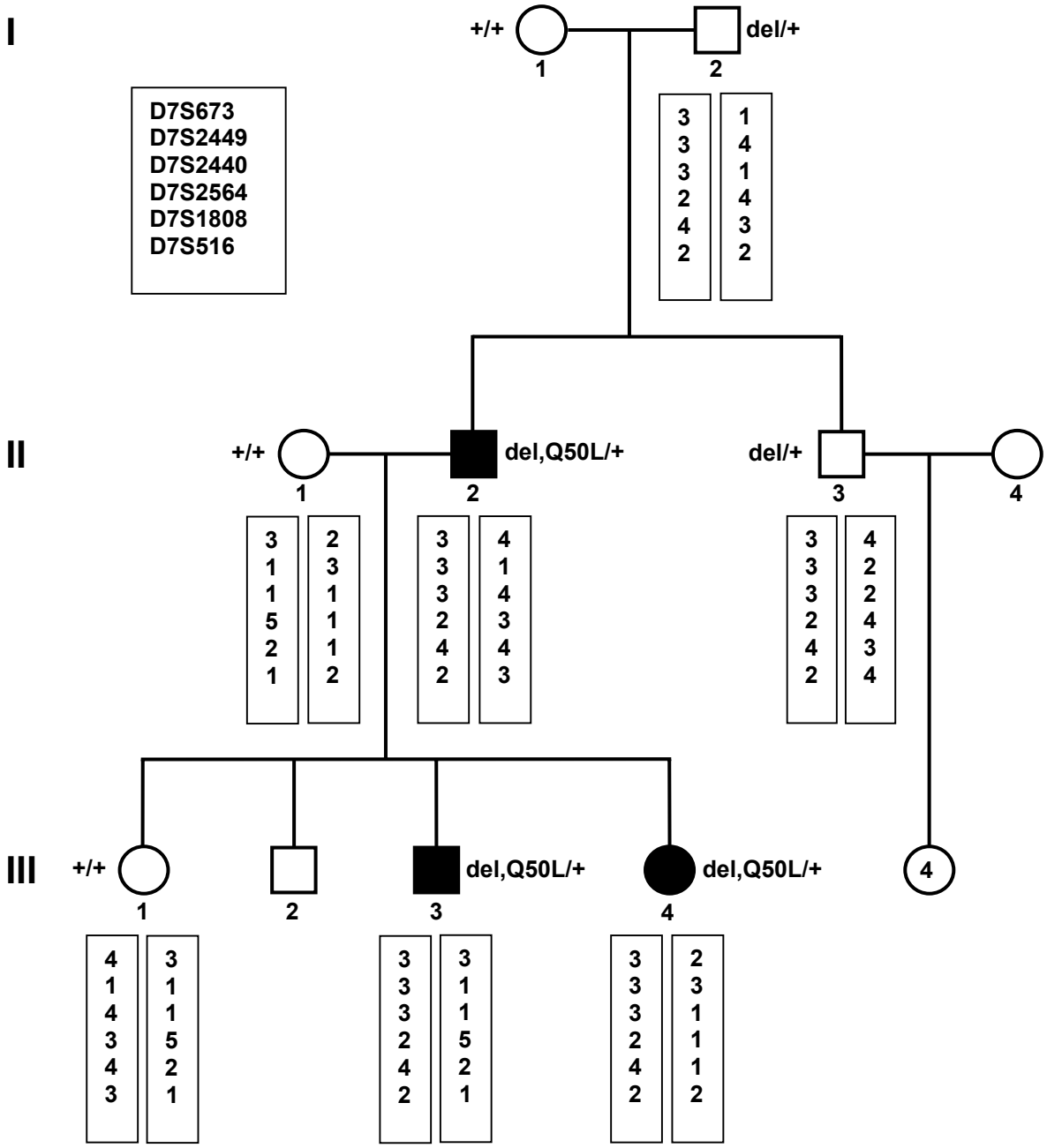


**Figure 1.** Pedigree drawing of the family (filled symbols represent affected individuals), showing results of haplotype and sequence analysis. D7S673, D7S2449, D7S2440, D7S2564, D7S1808 and D7S516 are polymorphic markers spanning a 3.3-cM interval on chromosome 7p14 (Dib et al., 1996). The *HOXA* gene cluster lies between D7S2440 and D7S2564, on the same 730 kb YAC clone as the two markers (Van Laer et al., 1997). In the genotypes beside the pedigree symbols, a plus sign indicates a normal *HOXA13* allele; del indicates the 2-bp deletion in the *HOXA13* promoter; and Q50L indicates the missense mutation in the HOXA13 homeodomain.

## DISCUSSION

What contribution is made by each sequence change to the Guttmacher syndrome phenotype? The 2-bp deletion in the promoter appears not to produce any clinical or radiological abnormalities on its own, but might compound the effects of the missense mutation in the homeobox by perturbing the transcriptional regulation of *HOXA13*. The region immediately upstream of the initiator methionine is highly conserved between humans and mice, and sequence alterations in this region could potentially disrupt normal control of the timing, domain or level of *HOXA13* expression. In mice, mutations outside the coding regions of *Hox* genes (e.g. *Ulnaless*) can cause developmental defects due to expression of normal HOX proteins at an abnormal time or in an abnormal location (Peichel et al., 1997; Hérault et al., 1997). Similar regulatory mutations may also underlie human phenotypes linked to the *HOXD* cluster (Ventruto et al., 1983; Fujimoto et al., 1998). The 2-bp deletion described here is the first potentially significant sequence alteration to be reported in a human *HOX* gene promoter region.

The missense mutation in the homeobox, by contrast, almost certainly plays an important part in the Guttmacher syndrome phenotype. Residue 50 lies in the recognition helix of the homeodomain, with its side chain projecting into the major groove of the DNA. All invertebrate and vertebrate HOX proteins have a glutamine at this position, but other homeodomain-containing proteins have a variety of different amino acids, including lysine, serine, histidine, cysteine, alanine, glycine and isoleucine (Gehring et al., 1994; Bürglin, 1997). We have never observed this HOXA13 Q50L substitution in >50 normal control individuals. Although early biochemical and genetic studies suggested that residue 50 might be a key determinant of the DNA-binding specificity of different homeodomains (Hanes and Brent, 1989; Treisman et al., 1989; Schier and Gehring, 1992), more recent structural studies indicate that its contribution to binding affinity and specificity depends on the identity of the amino acid occupying the position (Tucker-Kellogg et al., 1997; Fraenkel and Pabo, 1998; Grant et al., 2000). Glutamine, for instance, appears to make only water-mediated and van der Waals contacts with target DNA, and adopts multiple conformations in NMR and crystal structures, whereas lysine can form direct hydrogen bonds at certain sites. An engineered Q50A substitution in the engrailed homeodomain results in only a 2-fold reduction in DNA-binding affinity at a TAATTA engrailed consensus binding site, but a Q50K substitution changes binding site preference to TAATCC, with significantly increased affinity (Ades and Sauer, 1994). The only naturally occurring missense mutation previously reported at this position is Q50P in the HLXB9 homeodomain (Hagan et al., 2000), which is predicted to result in a complete loss of function, due to disruption of the alpha-helical structure of the recognition helix. The Q50L mutation reported here should not affect protein stability in this way, but is highly likely to perturb HOXA13's DNA-binding properties.

Several features of Guttmacher syndrome suggest that the Q50L mutation leads to a loss of normal HOXA13 activity. The combination of hypoplastic thumbs and halluces, 5$^{th}$ finger clinobrachydactyly and hypospadias also occurs in patients with typical HFGS caused by different nonsense mutations in *HOXA13* (Mortlock and Innis, 1997; Goodman et al., 2000), which probably result in functional haploinsufficiency for *HOXA13*. A similar clinical picture has been observed in a patient hemizygous for a chromosomal deletion that removes the entire *HOXA* gene cluster, including *HOXA13* (Devriendt et al., 1999). Deficiency of the 1$^{st}$ digits is likewise a prominent feature in mice homozygous for targeted loss-of-function mutations in *Hoxa13* (Fromental-Ramain et al., 1996), as well as in heterozygous *Hypodactyly* mice, which carry a spontaneous frameshifting deletion in *Hoxa13* (Mortlock et al., 1996). Loss of HOXA13 activity in Guttmacher syndrome probably results from altered DNA binding by the HOXA13$^{Q50L}$ mutant protein at normal HOXA13 target sites, or at least a subset of these sites.

Two features distinguish Guttmacher syndrome from HFGS: postaxial polydactyly of the hands and uniphalangeal 2$^{nd}$ toes with absent nails. These features do not occur in any of the patients or mouse mutants mentioned above, and cannot therefore be attributed to loss of HOXA13 activity. Their presence suggests that the Q50L mutation also produces a specific gain of function. This could result from altered DNA binding or transcriptional activity by the HOXA13$^{Q50L}$ mutant protein at a subset of normal HOXA13 target sites and/or at novel target sites, especially sites normally bound by other homeodomain proteins. Interestingly, 6%, 30% and 50% of *Hoxd12*$^{+/-}$, *Hoxd13*$^{+/-}$ and *Hoxd13*$^{-/-}$ mice respectively have a rudimentary extra postaxial digit in the forelimbs, as do 80-86% of *Hoxd12*$^{+/-}$/*Hoxd13*$^{+/-}$ *trans*-heterozygotes (Dollé et al., 1993; Davis and Capecchi, 1996; Kondo et al., 1996). Moreover, compound *Hoxd13*$^{-/-}$/*Hoxa13*$^{+/-}$ mutants have as many as seven digits in the forelimbs, as do mice homozygous for a deletion that removes *Hoxd11*, *Hoxd12* and *Hoxd13* (Fromental-Ramain et al., 1996; Zákány et al., 1997). Similarly, patients heterozygous for specific mutations in *HOXD13* have postaxial polydactyly of the feet (Muragaki et al., 1996; Goodman et al., 1998), and patients homozygous for a polyalanine tract expansion in HOXD13 have up to eight rudimentary digits in the hands (Akarsu et al., 1995).

Based on the foregoing, it is attractive to speculate that the postaxial polydactyly in Guttmacher syndrome might result from functional interference by the $HOXA13^{Q50L}$ mutant protein with one or more of the posterior HOXD proteins expressed in the developing forelimb autopod. However, it is also conceivable that genetic background affects the expression of the unique features of Guttmacher syndrome in the context of this missense mutation. It will be interesting to explore the effects of this mutation further, once authentic target genes and binding sites have been identified.

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