Severe digital abnormalities in a patient heterozygous for both a novel missense mutation in **HOXD13** and a polyalanine tract expansion in **HOXA13**

**P Debeer, C Bacchelli, P J Scambler, L De Smet, J-P Fryns, F R Goodman**

**CASE REPORTS**

**The proband**

The proband (fig 1) was born with severe bilateral hand abnormalities (fig 2A-F). She had complete cutaneous syndactyly between the third and fourth fingers, duplication of the distal and proximal phalanges of the fourth fingers, and a rudimentary extra central metacarpal. In addition, both thumbs were hypoplastic, with small distal and proximal phalanges and extremely small "angel wing" first metacarpals, and all her fingers were short, with small or absent middle phalanges. In the feet (fig 2G, H), she had short, medially deviated halluces, with small phalanges and first metatarsals. Her remaining toes were also short, with absent middle phalanges. From the age of 3 months, she suffered recurrent urinary tract infections, including one episode of pyelonephritis, but an ultrasound scan of her kidneys and bladder, a DMSA renal scan, and a voiding cystourethrogram showed no abnormalities. She had hypoplastic labia majora and a narrow vaginal introitus, but an ultrasound scan at 2½ years showed an apparently normal uterus.

**Family 1**

The only clinically obvious digital abnormality in the proband’s mother (IV.6, family 1, fig 1) was bilateral fifth fin-

g-ner clinodactyly (fig 3A). A radiograph of her feet, however (fig 3B), showed small spurs of bone in the first web spaces, repre-

senting partial duplication of the bases of the second metatarsals. In addition, the halluces and first metatarsals were unus-

ually broad, the middle phalanges of the second and third toes were hypoplastic, and the middle phalanges of the fourth and fifth toes were fused with the distal phalanges. She was discovered to be a member of a large four generation pedigree with dominantly inherited hand abnormalities (family 1, fig 1), part of which has been reported previously. 15 Fifteen of her relatives also had bilateral fifth finger clinodactyly. Three had in addition been born with mild unilateral SPD, radiologically documented in two cases (III.8 and IV.4) as cutaneous syndactyly between the third and fourth fingers, with a duplicated distal phalanx in the syndactylous web. 17 One obligate mutation carrier on pedigree analysis (II.2) had normal hands.

**Family 2**

The proband’s father (III.1, family 2, fig 1) also had bilateral fifth finger clinodactyly. In addition, he had small thumbs with hypoplastic thenar eminences and short first metacarpals, as well as bilateral bony fusion between the scaphoid and
RESULTS

Biosystems). Analysed on an ABI 377 automated sequencer (Applied Biosystems), amplified fragments were either cycle sequenced directly (Applied Biosystems Prism Dye Terminator Kit) or subcloned into pCRScript (Stratagene) before being cycle sequenced and sequenced. Amplified fragments were either cycle sequenced directly (Applied Biosystems Prism Dye Terminator Kit) or subcloned into pCRScript (Stratagene) before being cycle sequenced and analysed on an ABI 377 automated sequencer (Applied Biosystems).

METHODS

Venous blood samples for DNA extraction were obtained from the proband, both parents, 13 other members of family 1, and two other members of family 2, with their informed consent and the approval of the local research ethics committee. To search for mutations in HOXD13. In family 2, striped symbols represent people with hand and foot abnormalities typical of HFGS. A dagger above a pedigree symbol indicates that the person was found on DNA analysis to carry a polyalanine expansion in HOXA13.

Figure 1 Pedigree of the two families. A horizontal line above a pedigree symbol indicates that the person was examined clinically. In family 1, pale grey symbols represent subjects with bilateral fifth finger clinodactyly only. Dark grey symbols represent subjects who also had SPD in one hand. The dotted symbol indicates a subject who was clinically unaffected but an obligate mutation carrier on pedigree analysis. An asterisk above a pedigree symbol indicates that the person was found on DNA analysis to carry an R31W mutation in the homeodomain of HOXD13. In family 2, striped symbols represent people with hand and foot abnormalities typical of HFGS. A dagger above a pedigree symbol indicates that the person was found on DNA analysis to carry a polyalanine expansion in HOXA13.

trapezoid, and fibrous fusion between the scaphoid and trapezium (fig 3C). In the feet (fig 3D), he had small medially deviated halluces with short first metatarsals. The remaining toes were also short, with hypoplastic middle phalanges in the second toes, and fusion between the middle and distal phalanges in the third to fifth toes. Four other members of his family (family 2, fig 1) had similar hand and foot abnormalities. He also had retrograde ejaculation and his sister (III.2) had suffered recurrent urinary tract infections as a child.

RESULTS

Biosystems). Analysed on an ABI 377 automated sequencer (Applied Biosystems). Amplified fragments were either cycle sequenced directly (Applied Biosystems Prism Dye Terminator Kit) or subcloned into pCRScript (Stratagene) before being cycle sequenced and analysed on an ABI 377 automated sequencer (Applied Biosystems).

METHODS

Venous blood samples for DNA extraction were obtained from the proband, both parents, 13 other members of family 1, and two other members of family 2, with their informed consent and the approval of the local research ethics committee. To search for mutations in HOXD13 (GenBank accession numbers AF005219 and AF005220) and HOXA13 (GenBank accession number U82827), the entire coding region of each gene was amplified by PCR in four segments, as described previously. Amplified fragments were either cycle sequenced directly (Applied Biosystems Prism Dye Terminator Kit) or subcloned into pCRScript (Stratagene) before being cycle sequenced and analysed on an ABI 377 automated sequencer (Applied Biosystems).

RESULTS

Direct sequencing of HOXD13 in the proband showed a heterozygous C to T transition at position 892 of the coding sequence, which converts amino acid 298 (residue 31 of the homeodomain) from arginine to tryptophan. The same change was identified in the proband’s mother and in 12 other affected members of family 1 (fig 1) but not in the proband’s father or in 50 unrelated unaffected controls.

PCR amplification of the 5’ portion of exon 1 of HOXA13 in the proband yielded not only a product of the expected size, but also a second larger product, suggesting that she carried a small insertion. Cloning and sequencing of these products showed that she was heterozygous for a 27 bp in frame insertion after base 387 of the coding sequence, within the third of three imperfect trinucleotide repeats encoding polyalanine tracts. This insertion appears to have arisen by duplication of repeats 7-15, and increases the length of the tract from 18 to 27 residues. The same expansion was identified in her father and in two other affected members of family 2 (fig 1), indicating that it has remained stable in size in this family for at least three generations, but not in the proband’s mother.

Direct sequencing of the rest of the coding region of HOXA13 in the proband also showed a heterozygous A to G transition at position 1002. This base change was present in her father, and in II.2 and III.2 from family 2, but not in 50 unrelated unaffected controls. It does not alter an amino acid and is probably just a rare polymorphism (the first to be reported in HOXA13).

DISCUSSION

R31W substitution in HOXD13

Homeodomains are highly conserved DNA binding motifs consisting of a flexible N-terminal arm followed by three α-helices. Residue 31 of the HOXD13 homeodomain lies in the N-terminal part of helix II (fig 4). A comparison of 346 homeodomain proteins shows that 81%, including all invertebrate and vertebrate Hox proteins, have an arginine at this position, while most of the rest have lysine. In the antennapedia, engrailed, and HOXB1 homeodomains, R31 has been found to activate transcription in transfection assays by 88%. R31 is also predicted to form a salt bridge with a highly conserved glutamate residue at position 42 in helix III. This R31P missense mutation in MSXI, identified in a family with selective tooth agenesis, results in complete loss of protein activity both in vitro and in vivo. An R31H missense mutation in PTHX2, identified in a family with irisgoniodysgenesis syndrome, reduces the protein’s ability to activate transcription in transfection assays by 88%. A similar R31H missense mutation in MSX2, identified in a family with enlarged parietal foramina, reduces the protein’s capacity to bind DNA in gel shift assays by 85%, and results in a phenotype identical to that produced by deletion of the entire MSX2 gene. Histidine is a basic residue, like arginine, but its weaker positive charge and short ring structure probably impair its ability to make the salt bridges normally formed by R31. Tryptophan, by contrast, is a non-polar and bulky residue. The R31W missense mutation in HOXD13...
reported here is thus highly likely to destabilise the homeodomain-DNA complex, resulting in complete (or virtually complete) loss of function.

The phenotype produced by this mutation is remarkably mild. Only three of the 17 mutation carriers in family 1 had SPD in the hands, and in all three this was unilateral only, while none had SPD in the feet. Thirteen had just bilateral fifth finger clinodactyly, raising the possibility that some patients with dominantly inherited isolated fifth finger clinodactyly (brachydactyly type A3, MIM 112700) may harbour mutations in \(HOXD13\). Three other probable loss of function mutations in \(HOXD13\) also cause SPD at low penetrance only. In an Italian family with a frameshifting deletion in exon 1, only three of the 10 mutation carriers had SPD in the hands and one had SPD in the feet, while in a Scottish family with a frameshifting deletion in exon 2, only two of the 10 mutation carriers had SPD in the hands and seven had SPD in the feet. In an Italian family with a different frameshifting deletion in exon 2, neither of the two mutation carriers had SPD in the hands or feet. Mutation carriers in all three families shared a distinctive set of foot abnormalities, however, comprising partial duplication of the second metatarsals, broad halluces, and hypoplasia or symphalangism of the middle phalanges. Exactly the same abnormalities are produced by the missense mutation reported here (fig 3B), confirming previous suggestions that functional haploinsufficiency for \(HOXD13\) causes a characteristic foot phenotype subtly different from that caused by polyalanine tract expansions.25

Polyalanine tract expansion in HOXA13

The nine alanine expansion in \(HOXA13\) in family 2, like the six and eight alanine expansions reported previously,10 11 occurs in the third of the protein’s three N-terminal polyalanine tracts. Similar pathological expansions have been identified in \(HOXD13\), causing SPD,25 as well as in three non-homeodomain transcription factors, CBFA1, ZIC2, and FOXL2, causing cleidocranial dysplasia, holoprosencephaly, and blepharophimosis-ptosis-epicanthus inversus syndrome respectively.26–28 In 20 SPD families with different sized expansions, the penetrance and phenotypic severity were found to increase with increasing expansion size.1 In the three HFGS families, the phenotype is similar in severity, although III.2 in
The proband is heterozygous for both the R31W substitution with the function of wild type Hoxd13 and other 5′ HOXD13 mutations in Hoxd13, have shown that the mutant protein interferes with the function of wild type Hoxd13 and other 5′ HOXD13 proteins. The expansions in HOXA13 that cause HFGS may exert a similar dominant negative effect over the remaining wild type HOXA13, although the phenotype they produce is clinically indistinguishable from that produced by probable loss of function mutations in HOXA13, as well as by hemizygosity for the entire HOXA13 gene.

**Synergistic effects in the double heterozygote**

The proband is heterozygous for both the R31W substitution in HOXD13 and the polyalanine tract expansion in HOXA13. Several features of her phenotype suggest that the two mutations act synergistically. First, the HOXA13 mutation on its own produces only mild shortening of the thumbs (fig 3C) and the HOXD13 mutation on its own does not affect the thumbs at all (fig 3A), whereas the proband has severely hypoplastic thumbs (fig 2A-F). Studies in mice have shown that Hoxa13 is expressed more strongly than Hoxd13 in the digit 1 primordium, and digit 1 development is particularly affected by loss of Hoxa13 function in both mice and humans. Nevertheless, abnormalities of digit 1 do occur in Hoxd13 mice, as well as in humans heterozygous for specific mutations in HOXD13, including shortening of the phalanges and metapalanges. The proband’s HOXD13 mutation may well thus have contributed to her severe thumb hypoplasia.

Second, the HOXD13 mutation on its own produces only unilateral SPD in the hands, with duplication of just the distal phalanges, in three out of 17 mutation carriers in family 1, whereas the proband has bilateral SPD in the hands, with duplication extending as far proximally as the metacarpals. The HOXA13 mutation on its own does not cause central polydactyly, nor does any other known HOXA13 mutation in either humans or mice. A specific missense mutation in the HOXA13 homeodomain has recently been found to underlie Guttmacher syndrome (MIM 176305), however, in which postaxial polydactyly occurs. Moreover, loss of one copy of Hoxa13 causes increased digit number in mice already lacking both copies of Hoxa13. Thus, Hoxa13 mice have one rudimentary extra postaxial digit in the forelimbs, but Hoxa13+/− mice have two extra central digits in the forelimbs. The proband’s HOXA13 mutation may therefore have contributed to her severe central polydactyly.

Third, each mutation on its own produces shortening of the middle phalanges of the fifth fingers (figs 3A and 4A), and shortening or symphalangism of the middle phalanges of the second to fifth toes (figs 4B and 4D), whereas the proband has hypoplastic middle phalanges in the third to fifth toes.
Human/mouse differences

An analysis of the phenotype of Hoxa13\+/-/Hoxd13\+/- doubly heterozygous mice also suggests a degree of functional overlap between Hoxa13 and Hoxd13. Interestingly, however, the specific abnormalities in these mice differ significantly from those in the proband. Like her, they have shortening of digit 1, soft tissue syndactyly between digits 3 and 4 in the forelimbs, and hypoplasia/aplasia of the middle phalanges in digits 2 and 5. However, unlike her, they also have fusion of the phalanges of digit 1 in the forelimbs, soft tissue syndactyly between digits 2, 3, and 4 in the hindlimbs, and, in about half, an extra postaxial digit in the forelimbs. Unlike her, moreover, they do not exhibit central polydactyly in the forelimbs or absence of virtually all the middle phalanges.

Significant differences also exist between the limb abnormalities in Hoxa13\+/- and Hoxd13\+/- mice\(^{12,13}\) and those in humans heterozygous for probable loss of function mutations in HOXA13 and HOXD13.\(^{14,15}\) These different phenotypes in humans and mice may be explained by subtle differences in the functional effects exerted by the specific targeted and paralog groups.

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