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

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Hox genes encode a highly conserved family of transcription factors with fundamental roles in body patterning during embryogenesis.1 Studies in mouse and chick have shown that the 5’ HoxD and HoxA genes are critical for vertebrate limb and urogenital tract development.2 In humans, mutations in HOXD13 and HOXA13 cause the rare dominantly inherited limb malformation syndromes synpolydactyly (SPD) (MIM 186000) and hand-foot-genital syndrome (HFGS, MIM 140000), respectively. SPD is characterised by syndactyly between the third and fourth fingers and between the fourth and fifth toes, with variable digit duplication in the syndactyly web. Most cases result from expansions of a polyalanine tract in the N-terminal region of HOXD13 but frameshifting deletions have been identified in three families with an atypical foot phenotype.3,4 HFGS is characterised by short thumbs and halluces, hypoplasias in males, Müllerian duct fusion defects in females, and urinary tract malformations in both sexes. Most cases result from nonsense mutations in HOXA13, but two polyalanine tract expansions and one missense mutation have also been described.5–7

Here we report two Belgian families, one with the first missense mutation to be identified in HOXD13 and the other with only the third polyalanine tract expansion to be identified in HOXA13. Remarkably, intermarriage between the two families has resulted in a girl heterozygous for both mutations, the first human HOXD13/HOXA13 double heterozygote to be reported. Her digital abnormalities are strikingly more severe than those in carriers of each individual mutation, indicating that the two mutations act synergistically.

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-
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 had retrograde ejaculation and his sister (III.2) had suffered recurrent urinary tract infections as a child.

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 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 HOXD13).

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 form a salt bridge with a specific phosphate group in the DNA backbone. 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 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.7 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.8 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,26 27 occurs in the third of the protein's three N-terminal polyalanine tracts. Similar pathological expansions have been identified in HOXD13, causing SPD,28 as well as in three non-homeodomain transcription factors, CBFA1, ZIC2, and FOXL2, causing cleidocranial dysplasia, holoprosencephaly, and blepharophimosis-ptosis-epicanthus inversus syndrome respectively.29 30 In 20 SPD families with different sized expansions, the penetrance and phenotypic severity were found to increase with increasing expansion size.3 In the three HFGS families, the phenotype is similar in severity, although III.2 in

Figure 2  Limb abnormalities in the proband. Photographs of the left hand (A, B) and right hand (D, E) at 6 months, showing syndactyly between the third and fourth fingers, with an extra nail in the syndactylous web on the left, and hypoplastic thumbs. Radiographs of the left hand at 2 days (C) and right hand at 6 months (F), showing duplication of the fourth proximal and distal phalanges, with osseous syndactyly between the duplicated proximal phalanges on the left, a rudimentary extra central metacarpal, hypoplastic first distal and proximal phalanges and first metacarpals, absent middle phalanges in the second, fourth, and fifth fingers, and hypoplastic middle phalanges in the third fingers. (G) Photograph of the feet at 18 months, showing short, medially deviated halluces and small second to fifth toes. (H) Radiograph of the left foot at 16 months, showing small first distal and proximal phalanges and first metatarsal, and absent middle phalanges in the second to fifth toes.
loss of function mutations in clinically indistinguishable from that produced by probable wild type HOXA13, although the phenotype they produce is exert a similar dominant negative effect over the remaining proteins.

The proband is heterozygous for both the R31W substitution Synergistic effects in the double heterozygote with the function of wild type Hoxd13 and other 5 synpolydactyly homolog ejaculation. Recent genetic complementation studies in the family 2 is the first reported HFGS patient with retrograde Limp abnormalities in the proband’s mother (IV.6, family phalanx. (B) Radiograph of the mother’s left foot at 27 1/2 years, showing mild shortening of the fifth middle phalanges in all her remaining digits (fig 2C, F, H). 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 HOXD13,7 leaving 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 Hoxd13. Thus, Hoxd13 mice have one rudimentary extra postaxial digit in the forelimbs, but Hoxa13/-/- mice have two extra central digits in the forelimbs. The proband’s HOXD13 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, 4A), and shortening or symphalangism of the middle phalanges of the second to fifth toes (figs 3B and 4B), whereas the proband has hypoplastic middle phalanges in the third and fifth toes.

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 HOXD13,7 leaving shortening of the phalanges and metapalanges. The proband’s HOXD13 mutation may well thus have contributed to her severe thumb hypoplasia.

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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+/− mice12,13 and those in humans heterozygous for probable loss of function mutations in HOXA13 and HOXD13.14 These different phenotypes in humans and mice may be explained by subtle differences in the functional effects exerted by the specific targeted and spontaneous mutations in question. They may also reflect differences in genetic background, differences in sensitivity to reduced Hox13 gene dosage, and/or differences in the roles played by HOXA13 and HOXD13 in the two species during early autopod development. Whatever the cause, their existence indicates that precise predictions of human mutant Hox phenotypes cannot readily be made from the corresponding mouse models.

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REFERENCES