Clinica Chimica Acta 413 (2012) 1049–1052

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

A novel non-synonymous mutation in the homeodomain of HOXD13 causes synpolydactyly in a Chinese family

Binbin Wang [a,b,1], Baoqiang Xu [c,1], Zhi Cheng [a,b], Xueya Zhou [d], Jing Wang [a,b], Guang Yang [c], Longfei Cheng [a,b], Jun Yang [c,*], Xu Ma [a,b,e,**]

[a] Graduate school, Peking Union Medical College, Beijing, China
[b] National Research Institute for Family Planning, Beijing, 100081, China
[c] Department of Hand Surgery, China-Japan Union Hospital of Ji Lin University, 126 Xiantai Street, Changchun 130033, China
[d] MOE Key Laboratory of Bioinformatics and Bioinformatics Division, TNLIST and Department of Automation, Tsinghua University, Beijing 100084, People's Republic of China
[e] World Health Organization Collaborating Centre for Research in Human Reproduction, Beijing, China

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 16 February 2012
Accepted 16 February 2012
Available online 22 February 2012

Keywords:
HOXD13
Synpolydactyly
Mutation
Homeodomain

ABSTRACT

Purpose: The 5′ *HoxD* genes and their paralogs in the *HoxD* cluster are crucial for normal vertebrate limb development. Mutations in *HOXD13* and *HOXD13* have been found to cause human limb malformation. Here we describe a two-generation Chinese family with a variant form of mild synpolydactyly.
Methods: Sequence analysis of *HOXD13* gene in a two-generation Chinese family with six individuals.
Results: Gene scan and linkage analysis suggested that *HOXD13* might be responsible for the disease of this family. An LOD around 1.8 was observed at three markers ($P=2E^{-3}$). We identified a novel c.893G>A (p.Arg298Gln) mutation in the HOXD13 homeodomain. And the mutation affected the transcriptional activation ability of HOXD13.
Conclusion: This finding expands the phenotypic spectrum associated with *HOXD13* mutations and advances our understanding of human limb development.

1. Introduction

The homeobox-containing (*Hox*) genes encode a set of highly conserved transcription factors that display important roles in controlling cell fates and regional identities along the primary body and limb axes [1,2]. There are 39 *Hox* genes arranged in four clusters (*HoxA–D*) in most vertebrate genomes including humans [3]. In general, the order of the genes within each cluster reflects their temporal and spatial expression patterns during development. Genes located at the 3′ end of each cluster are expressed early, in more anterior and proximal regions, whereas genes located at the 5′ end of each cluster are expressed later, in more posterior and distal regions [4,5]. Expression analysis, targeted mutagenesis and misexpression studies in the mouse and chicken have demonstrated that the 5′ *HoxD* genes and their paralogs in the *HoxA* cluster are critical for limb development [6].

To date, two such genes have been found among which mutations cause human limb malformation, *HOXD13* in synpolydactyly (SPD) and *HOXA13* in hand–foot–genital syndrome [7]. SPD is a rare, dominantly inherited limb malformation, which is characterized by syndactyly between the third and fourth fingers and between the fourth and fifth toes, with a partial or complete extra digit in the syndactylous web. Incomplete penetrance and variable expressivity both between and within affected families are common. Several kinds of mutations are observed in *HOXD13* (e.g., polyalanine expansions, truncations and missense mutations), which confer different properties to the mutant protein and result in a variable phenotype [8]. Mutations in *HOXD13* causing polyalanine expansion lead to typical SPD, whereas deletions and missense mutations are associated with atypical forms of SPD characterized by a distinctive foot phenotype [9–13].

To explore further the role of *HOXD13* in human limb development, we investigated a two-generation Chinese family with limb malformations. We identified a missense mutation that substitutes an arginine for a glutamine in position 31 (R31Q) in the homeodomain of HOXD13. This mutation caused a variant form of milder SPD phenotype among affected family members. We characterized the effects of this mutation and found that the c.893G>A (p.Arg298Gln) mutation impairs the capacity of HOXD13 to regulate transcription. It may act as a partial loss-of-function mutation and thereby affects human limb development.

* Correspondence to: J. Yang, Department of Hand Surgery, China–Japan Union Hospital of Ji Lin University, 126 Xiantai Street, Changchun 130033, China. Tel.: +86 431 85351802; fax: +86 431 85655031.
** Correspondence to: X. Ma, National Research Institute for Family Planning, Beijing 100081, PR China. Tel./fax: +86 10 6217 9059.
E-mail addresses: proyangjun@163.com (J. Yang), nicgr@263.net (X. Ma).
[1] These authors contribute equally to this work.

0009-8981/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.cca.2012.02.015

2. Materials and methods

2.1. Patients

A two-generation Chinese family in which six individuals were affected by different limb malformations was investigated (Fig. 1). Venous blood samples were collected from unaffected and affected individuals in this family with their informed consent and approval from the local ethics committee. Another 136 healthy individuals were used as controls. Genomic DNA was isolated from venous blood samples using standard methods.

2.2. Mutational analysis

We performed two-point linkage analysis and mutation identification. We genotyped four short tandem repeat (STR) markers around *HOXD13* (three upstream, D2S1379, D2S2981 and D2S2314; one downstream, D2S324). Physical positions of STR markers and the putative disease-causing mutation were obtained from the UCSC hg18 database (NCBI build 36). The genetic positions were interpolated from physical distances using Rutgers Map Interpolator (http://compgen.rutgers.edu/old/map-interpolator/) [14]. The parametric multiple locus logarithm of odds (LOD) score was calculated using Merlin [15] under an autosomal dominant inheritance model with complete penetrance. We assumed a disease allele frequency of 1/10,000. The LOD score for each marker is shown in Table 1.

Two exons and split sites of *HOXD13* were amplified by polymerase chain reaction (PCR) using two pairs of gene-specific primers (Supplementary Table 1). Amplified fragments were sequenced using an ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Site-directed mutagenesis and plasmid construction

Human *HOXD13* open reading frame (ORF) cDNA was obtained from GeneCopoeia (Rockville, MD, USA). Site-directed mutagenesis was performed with appropriate primers to generate *HOXD13* carrying the c.893G>A (p.Arg298Gln) or the c.940A>C (p.Ile314Leu) mutation using the QuickChange Lightning Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA). Presence of the desired base changes was verified by DNA sequencing.

The ORFs of wild-type and mutant *HOXD13* were amplified by PCR and cloned into a HindIII- and EcoRI-digested pcDNA3.1(+) vector (Invitrogen, Carlsbad, CA, USA) to create expression plasmid pcDNA3.1-HOXD13. The human *EPHA7* promoter of 779 bp (from −708 to +71), which contains a HOXD13-binding site, was obtained by PCR from human genomic DNA and insert into the NheI and HindIII sites of a pGL3-basic vector (Promega, Madison, WI, USA) to generate a pGL3-EPHA7 reporter construct. A complementary 97 bp oligonucleotide containing the *HOXD9* promoter-derived HCR region as described previously [11], was annealed and ligated to pGL3 basic vector (Promega) at the NheI and HindIII sites to generate pGL3-HCR reporter constructs. All the clones were confirmed by sequencing. The PCR primers used for mutagenesis and plasmid construction are shown in Supplementary Table 2.

2.4. Luciferase assays

293T cells were cultured in Iscove's modified Dulbecco's medium with 10% fetal bovine serum, 100 mg/mL penicillin and 100 mg/mL streptomycin. Cells were seeded in 24-well tissue culture plates 24 h prior to transfection, at about 60% confluence. Transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

HOXD13 expression constructs (wild type and mutants) or a pcDNA3.1 empty vector was cotransfected together with pGL3-EPHA7 or pGL3-HCR reporter constructs respectively. *Renilla* luciferase control plasmid pREP7-RLuc was also cotransfected in each well for normalization. At 30 h after transfection, cells were washed, lysed and assayed for firefly and *Renilla* luciferase expression using the Dual Luciferase Reporter Assay System (Promega). The values represent the means of three independent experiments performed in triplicate and the bars in figures denote the S.D. Student's *t* test was used to test for statistical significance of differences between means of unpaired samples.

3. Results

3.1. Clinical report

We investigated a Han Chinese family with distinctive syndactyly phenotypes (Fig. 1). There were 6 affected individuals in the 2 generations of family. Digital photographs of 5 of the affected individuals and

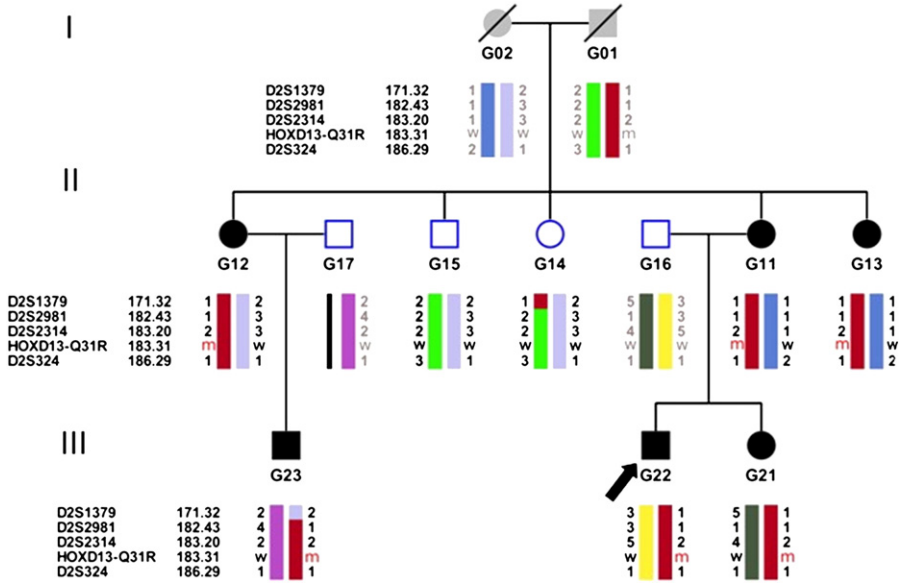

Fig. 1. Family pedigree of a two generation Chinese SPD-2 family. Squares are males and circles are females. Blackened symbols indicated affected individuals, open symbols are unaffected individuals. Gray shaded symbols indicate unknown affection status. Decreased first-generation parents and non-directly related family members were not genotyped. Marker names and their genetics positions are shown on the left side. Inferred genotypes are shown in gray. The disease haplotype (shown in red) carries the mutation HOXD13-Q31R co-segregates with the affection status.