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A novel mutation of *p63* in a Chinese family with inherited syndactyly and adactylism

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Abstract

p63 is a transcription factor homologous to p53 and p73; mutations in this gene have been identified in individuals with several types of developmental abnormalities, including EEC (ectrodactyly, ectodermal dysplasia, facial clefts) syndrome and split-hand/split-foot malformation (SHFM). Several mutations in the *p63* gene have previously been shown to be related to SHFM. In this study, we report on a Chinese family with intrafamilial clinical variability of SHFM that have a novel heterozygous mutation in all four affected individuals. The mutation is in exon 8 of *p63*, 1046G→A, which predicts an amino acid substitution G310E. SSCP analysis of the segregation pattern of the mutation strongly suggests a causal relationship to the SHFM phenotype in *p63*. This mutation has not been observed in other countries in the world.

Keywords: *p63*; SHFM; Missense mutation; SSCP; Chinese family

1. Introduction

Split-hand/split-foot malformation (SHFM, MIM18-3600) is a human limb malformation. It is an autosomal dominant disorder involving syndactyly, adactylism, median clefts of the hands and feet, and aplasia and/or hypoplasia of the phalanges, metacarpals and metatarsals [1]. At least five loci are responsible for this condition in humans: SHFM1 on 7q21.3–q22.1 [2,3], SHFM2 on Xq26 [4,5], SHFM3 on 10q24 [6,7], SHFM4 on 3q27 [8,9], and SHFM5 on 2q31 [10,11], and possible candidate genes include *DLX5*/*DLX6*, *FHL1*, *DAC*, *p63*, *HOXD13* [12–16].

The *p63* gene has previously been reported to be involved in SHFM4 [9]. This gene contains two promoters and is transcribed into different isoforms with dissimilar transcriptional activities. The two promoters are responsible for giving rise, by alternative splicing, to six isotypes with either full N-terminus containing transactivating domain (TAp63) or deleted N-terminus domain (ΔNp63). Alternative splicing at the 3′ end of the gene results in three different C-termini, alpha (α), beta (β), and gamma (γ). The largest p63 isotype, TA-p63α, has TA, DNA binding (DB), tetramerization

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(ISO), sterile alpha-motif (SAM), and transactivation inhibitory (TI) domains [17–19]. *p63* is expressed in the nuclei of basal cells of the skin, esophagus, mammary glands, tongue, cervix, urothelium, prostate, limb buds, branchial arches, and oral epithelium. The essential role of *p63* in epithelial development was demonstrated with the analysis of *p63*-null mice, which display perinatal lethality with dramatic defects in the limbs and skin, lack of ectodermal appendages such as teeth and mammary gland, and craniofacial malformations where the maxilla and mandibule are truncated and the secondary palate fails to close [20,21]. Various types of mutations related to the SHFM within the *p63* gene have been previously identified: splice-site mutation at the 3′ end of intron 4 (3′ss intron 4), missense mutations (R58C, K193E, K194E, R280C/H) and stop-mutations (Q634X, Q639X) [22–24]. Most of the mutations are point mutations clustered in the DNA binding domain (exons 5 and 7), except for one located in the TA domain (exon 3) and two located in the TI domain (exon 14). Some mutations of p63 with SHFM4 have been shown to cause alteration in the p63 protein activation and stability. Amino acids K193 and K194 are required for ubiquitin conjugation by E3 ubiquitin ligase (Itch) and naturally occurring mutations in these sites cause more stable p63 protein [25]. In addition, the Q634X and E639X are reported to disrupt the sumoylation site, and therefore increase the stability and transcriptional activity of the p63α isoform [17,26].

In this study, we report on one Chinese family with intrafamilial clinical variability of SHFM in which we have carried out mutation analysis for the genes of *DLX5*, *DLX6*, *DAC*, *p63 and HOXD13*. To determine whether mutations of these candidate genes are responsible for the affected individuals in the Chinese family, we amplified exons 3–8 and 14 of *p63*, and all exons of the other genes and sequenced the PCR products. We identified a novel mutation in the *p63 gene*.

2. Material and methods

2.1. Subjects

We examined a family in which five individuals (four living) in three generations were affected with SHFM (Fig. 1). Three of the affected individuals manifested limb abnormalities in X-rays (III-1 was not willing to take photograph), but a broad intrafamilial variability was noted. Peripheral blood samples were collected from the eight available individuals of this family (the proband's mother was not willing to test) and another 100 unrelated individuals were used as controls.

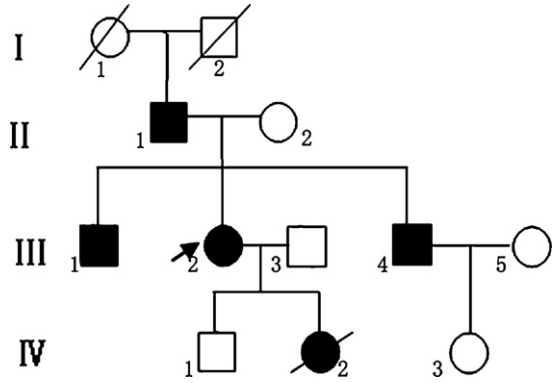

Fig. 1. The pedigree of Chinese family. The proband is indicated by the arrow. Symbols marked by a slash indicate that the subject is deceased. Males are indicated by squares, females are indicated by circles. Blackened symbols represent individuals in whom a digital abnormality was identified on clinical examination.

2.2. PCR amplification of the exons

Genomic DNA was isolated from peripheral blood from the family members and the 100 unrelated individuals using a standard method [27]. Approximately 20 ng of genomic DNA was used as template for PCR amplification. Oligonucleotide primers were designed for the exons 3–8 and exon 14 of *p63* using Primer Premier 5.0 and direct sequence analysis of the exons (including exon-intron boundaries) was performed. Moreover, one pair of primers was designed for each exon in the genes of *DLX5*, *DLX6*, *DAC* and *HOXD13*. The sequences of all the primers are shown in Table 1. Standard PCR protocols were performed as previously described [28]. Because the X-linked could be excluded by the analysis of the pedigree (Fig. 1), mutation analysis of *FHL1* was not performed.

2.3. Sequencing

The PCR products were excised from agrose gels, purified with TIANgel Midi columns (TIANGEN) and sequenced with a 3730 DNA Sequencer (ABI PRISM). Sequence analysis was performed using the DNASTAR program and BLAST program from NCBI (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).

2.4. Single-strand conformation polymorphism analysis

Segregation analysis of the G310E mutation by SSCP could not be performed because the exon 8 PCR fragment was more than 500 bp. For this analysis, another sense primer (5′-CTAATTCCTAGTGGGCAAGTC-3′) was designed, which produced an 187 bp fragment encompassing exon 8 that could be analyzed on polyacrylamide gels. DNA fragments were mixed with a twofold excess of 6X loading dye mix, denatured for 5 min in boiling water, immediately placed in ice water, and then loaded onto a 10% polyacrylamide gel (29:1

Table 1
Primers and PCR anneal temperature for the exons of DLX5, DLX6, DAC, P63, HOXD13

Exon	Forward primer (5′–3′)	Reverse primer (5′–3′)	T_m (°C)
DLX5			
1	aaggatttaaccaaggctgtgc	ctgtccatgtacctggctggt	53
2	ggcattgtctggtgacaagc	caaccagacgtgcagctca	52
3	ctttgttccccaactcagg	ctttgttccccaactcagg	48
DLX6			
1–2	gagctaaggtggctgcagag	ggcctctcacctgtgtcgt	52
3	ggtgaccgctcctctgtatt	aattcctcctgatgtgctcc	50
DAC			
1	gcaggaagggagggtggcga	gcgagcggacgcttacaggtcg	58
2	aacagttactccttcctggtctc	cattagcctctatgtaggaccag	42
3–4	agccttgttggttatccttacta	caggagggagtaatagtatgtaagg	50
5	attccccatttctccacctc	tctccaagacagtgactcaaagc	50
6	tacacccctcacagtgctcca	gcagtctcctccagctaaccct	52
7–8	tgaaggtggaacgaatgtgt	gtgccattgtaggcttcttc	50
9	cgtgcctagaaagggttggaag	gcatgtctcaacagccagcct	55
P63			
3	agccttgctgactttgaag	acatgactgaaaagacagg	54
4	gaggtggcacatagagctgagt	gacttttcccactaactggcact	55
5	tctccttcctttctccactggc	tgcccacagaatcttgaccttc	56
6	ccacgatgaggtcagagatg	ctgaaacagatgttctctcaagtc	57
7–8	ccgcaggcaagatgaaga	gattcaaccctaccccattt	54.5
14	cagggaatgataggatgctgt	ctagggagaaggctttgaaga	54
HOXD13			
1	gcgatgagctaacctgttgg	tgacggaggcagttgaaatc	55
2	accaaacagcatggcattt	aagataatcagtgctgggaatg	42

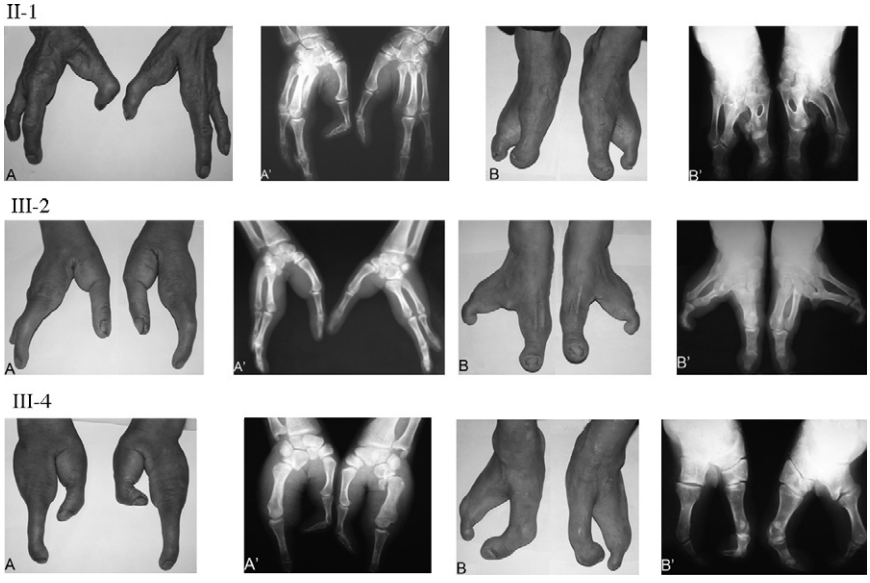

Fig. 2. Clinical feature of SHFM4 patients. The photographs and radiographs of hand and feet in II-1, III-2 and III-4, with the "lobster-claw" malformation. II-1 shows absence of the second metacarpus and phalanges and the third phalanges of hands, with hypoplasia of the third metacarpus (A), the median clefts and syndactyly in feet (B). III-2 shows absence of the second and third metacarpus and phalanges of the hands and 4/5 finger syndactyly (A), the median clefts and syndactyly in feet (B). III-4 shows severe absence of the phalanges of the second to fourth digits in both feet and hands (A and B).

acrylamide:*bis*-acrylamide ratio). Gels were run for 12 h at 10 V/cm at 4 °C, and fragments were visualized by silver staining.

3. Results

3.1. Clinical features of the family members

In this study, we examined eight individuals from a single Chinese family (six males and two females), in which three males and one female were affected with SHFM. Representative clinical observations from photographic and X-ray data are shown in Fig. 2. The phenotype of affected members is similar, and the III-4 is more severe than others. The abnormalities of these affected members present bilaterally in both the hands and feet, medial clefts from the metacarpus to finger/toe tip, producing a "lobster-claw" phenotype. Malformations of the hands and feet in the patients are due to primarily phalanx amalgamation with the absence of phalanx, metacarpus and metatarsals. The proband (III-2) showed an absence of the second and third metacarpus and phalanges of the hands, and soft tissue was completely missing between the fourth and fifth digits in both hands, resulting in 4/5 syndactyly of hands. Additional malformations included medial clefts, short phalanges and metatarsals, and missing third phalanges, with 1/2 and 4/5 toe syndactyly. On her left foot, the first and second phalanx/metatarsals, and peripheral soft tissue are completely fused, the fourth/fifth phalange and peripheral soft tissue are also fused. Her right foot is like the left foot except for the absence of fusion between the first and second metatarsals (Fig. 2 (III-2)). However, her father has no 4/5 finger syndactyly, the feet are also less severely affected (Fig. 2 (II-1)). Her brother presents with bilateral absence of three digits (the second to fourth) in the hands and feet, a more severe phenotype than in the other affected individuals (Fig. 2 (III-4)). The proband had a child who manifested the same phenotype as the mother and died after forty days. All the affected individuals of the family present somewhat waddling gait caused by the feet abnormalities, and have none of the significant abnormalities of the face, palate, skin, teeth, hair, or nails that characterize the EEC syndrome.

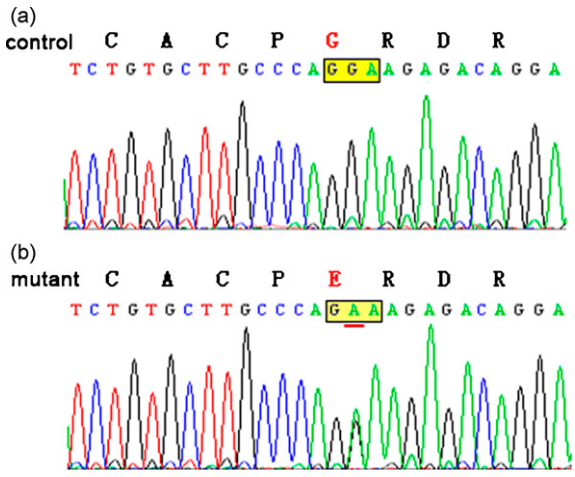

Fig. 3. A novel missense mutation (G310E) is located in the DNA binding domain of p63 gene was found in this family. (a and b) Sequencing of exon 8 from controls (upper fluorogram) and patients of a Chinese SHFM family. The G310E mutation, present in exon 8, compared with the nomal individual. The patient with the G310E mutation is heterozygous G/A at nucleotide 1046.

3.2. G310E point mutation detection

We used polymers chain reaction and direct sequencing analysis to analyze the *p63* genotypes from the four affected and four unaffected individuals of this family. Extensive sequencing analysis using Blast revealed a mutation in exon 8 of *p63*: G310E (1046 G→A) (Fig. 3) in the four patients, but not in the unaffected individuals in the family or in any of the 100 unrelated controls. This point mutation falls within the DNA binding domain of p63, whereas the adjacent mutations usually cause the EEC syndrome [9,22,29] (Fig. 4).

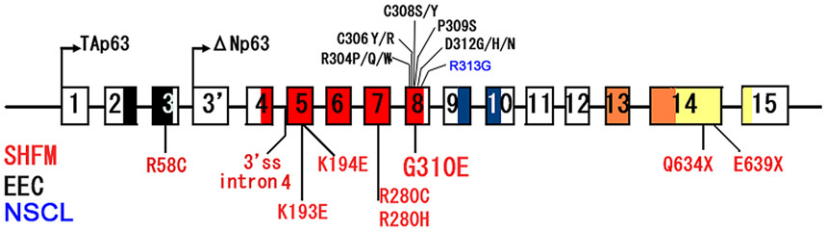

Fig. 4. Schematic diagram of human *p63* gene and mutations detected in cases of SHFM. Intron-exon structure and conserved protein domains are shown. *Arrows* indicate the different transcription initiation sites. The black, red, blue, oranger and yellow panes indicate the TA, DBD, ISO, SAM and TI domains of p63 protein, respectively. Mutations causing different diseases are illustrated in different font colours. Majority missense mutations are located in the highly conserved DNA binding domain of *p63*.

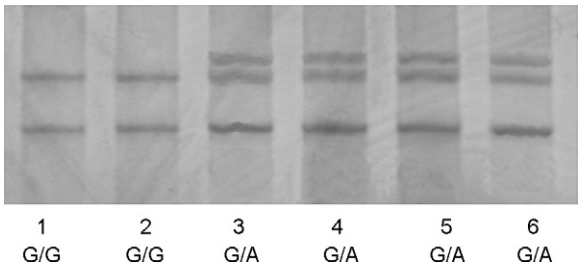

Fig. 5. Segregation of SSCP analysis using intronic primers flanking exon 8. Lane 1: unrelated control; lane 2: DNA from the unaffected individual of a Chinese SHFM family (IV-1 of pedigree), lanes 3, 4, 5, 6: DNA from the four affected individuals of the Chinese SHFM family. The G to A change is seen as a heterozygous mutation.

Mutations in the *DLX5*, *DLX6*, *DAC*, *and HOXD13* genes have also been suggested to be responsible for SHFM [12,14,15]. In order to eliminate the possibility that mutations of these genes cause the clinical symptoms in the patients of this family, we performed similar analyses on these genes as for *p63*. Our analysis indicated that none of the individuals in the family had mutations in these genes, indicating that the clinical symptoms described here are likely to be due to the identified mutation in *p63*.

3.3. Results of SSCP

SSCP analysis of the coding region and flanking intronic sequences of exon 8 in *P63* was performed. The denatured PCR products from individuals in the family and controls analyzed on polyacrylamide gels (Fig. 5), showed the differential segregation in both affected and unaffected individuals in the family. Similarly, the different DNA bands segregation was also observed in both the control and the affected individuals with the SHFM phenotype. No additional sequence alteration was found in unaffected individuals of the family, and the segregation pattern of the mutation strongly suggests a causal relationship to the SHFM phenotype in *p63*.

4. Discussion

4.1. The analysis of genotype–phenotype correlation for SHFM

A number of studies have reported that mutations in several different genes are responsible for SHFM. Human *DLX5* and *DLX6* (distal-less homeobox) genes located on chromosome 7q22 have been identified as possible candidate genes for the autosomal dominant form of the SHFM1, which is a heterogeneous limb disorder characterized by missing central digits and claw-like distal extremities [30]. *DLX5* expression in the apical ectodermal ridge of *Dlx5/6* null mice can fully rescue *Dlx/Dll* function in limb outgrowth. Recently, it has been proposed that the *DLX5/DLX6* homeobox genes could play essential roles in the pathogenesis of SHFM1 [12]. Synpolydactyly (SPD; MIM 186000), a characteristic of this syndrome, is a rare, dominantly inherited limb malformation that typically consists of 3/4 syndactyly in the hands and 4/5 syndactyly in the feet, with digit duplication in the syndactylous web [15]. SPD is the first human malformation syndrome shown to be caused by mutations in one of HOX genes, *HOXD13* (homeobox D13), which maps to chromosome 2q31. The mutation in question is specific and highly unusual, an imperfect trinucleotide repeat sequence in exon 1 of the gene, which in normal individuals encodes a 15-residue polyalanine tract, is expanded, resulting in a tract of 22–29 residues [31,32]. Recently, 0.5 Mb tandem genomic duplications at 10q24 were identified in SHFM3 families as well as duplications in 10q24 that comprise dactylin (*DAC*) [33]. *DAC* is the best candidate gene for SHFM3 because of its role in the Dactylaplasia mice phenotype [14]. Mutations underlying SHFM4 have been found in the *p63* gene, which encodes a homologue of the tumour-suppressor p53 [34,35].

In this study, we analyzed the *DLX5/DLX6*, *DAC*, *p63* and *HOXD13* sequences of four affected and four unaffected individuals of a single Chinese family. Through NCBI Blast and DNASTAR program, extensive sequencing analysis revealed no mutations in the *DLX5/DLX6*, *DAC* and *HOXD13* genes (data not shown), however, a novel missense mutation in the DNA binding domain and at position 310 of the p63 protein was revealed (Fig. 4). The mutation results in an animo acid substitution of glycine to glutamic acid (Fig. 3), and was not found in the four unaffected individuals in the family. In order to distinguish this mutation from a rare polymorphism, we sequenced 100 unaffected individuals from the general population and were unable to find this substitution. Therefore, we concluded that G310E of *p63* gene is a novel mutation, but not a polymorphism.

Genotype–phenotype analysis of human syndromes, knockout experiments in mice, and genetic data in zebrafish unambiguously pointed to *p63* as a master regulator of ectoderm and, more specifically, of multilayered epithelia formation [36]. Genetic experiments in mice confirmed the specificities of the *p63* gene function and are in agreement with the phenotypes observed in humans. The forelimbs of Tp63 knockout mice are severely malformed, lacking the radius and the complete autopod, while the hindlimbs do not develop at all, and

mice lacking *p63* die soon after birth with severe defects in limb, craniofacial and skin development [13,37].

4.2. Expounding the pathogenicity of p63 mutation

p63, along with p53 and p73, belongs to the p53 transcription factor family [38]. The p63 DNA binding domain shares approximately 60% and 85% amino acid identity with *p53* and *p73*, respectively [35]. The glycine at position 310 is strictly conserved in all three homologues and six isoforms of p63, suggesting that this amino acid could confer functional specificity to p63 protein. The majority of missense mutations causing EEC syndrome or SHFM are located in the DNA binding domain of the p63 protein, affecting all six isoforms. It is conceivable that these mutations may regulate the function of p53 family proteins to disrupt normal cell proliferation and development [39]. According to the protein model, it can be deduced that the p63 DNA binding domain assumes a loop-sheet-helix motif and two large loops that make up the DNA binding surface of the protein [40,41]. The prediction that p63 mutant proteins in EEC syndrome disrupts the DNA binding capacity was supported by data that showed the inability of mutated *p63*γ to promote expression of a reporter gene, and the inability of Δ*Np63*α to compete with *p53* for binding to the specific site [42].

In this family, the missense mutation G310E only causes SHFM and has not been reported elsewhere. However, several other mutations of nearby amino acids are also associated with the EEC syndrome (R304, C306, C308, P309, D312) with the exception of one nearby mutation R313G that causes the non-syndromic cleft lip/palate (NSCL) in Vietnamese families [9,22,29] (Fig. 4). The arginine at position 304, directly contacts a phosphate in the backbone of the DNA. Amino acid substitutions at R304 result in loss of this contact and can also impose steric hindrance on DNA binding, thus impeding activity of all isoforms of p63 [9]. A C306R mutation is predicted to abolish the DNA binding activity of p63 through structural deformation of the protein [42], and D312 plays an important role in the positioning of the p63 DNA binding surface by forming salt bridges with R304 and R311 [9]. The G310 is located among these amino acids, and the mutation results in conversion of a glycine to glutamic acid. Glycine is a non-polar amino acid, while glutamic acid is a polar, negativly charged amino acid. The substitute of polar amino acids has been suggested that these substitutions create extensive structural rearrangements affecting the DNA binding activity of p63 [9]. Herein, we speculated that the substitution of glutamic acid for glycine is likely to have a similar effect on the DNA binding capacity of p63.

5. Conclusion

A novel p63 missense mutation was found in a Chinese family with inherited syndactyly and adactylism. This mutation was not found either in the unaffected members in the family or the 100 controls, suggesting that it is not a polymorphism. Furthermore, the difference of SSCP in the exon 8 of normal and mutant in p63, strongly suggest that the p63 G310E mutation is associated with the Chinese SHFM family. The phenotype of these affected members suggests that the *p63* isotypes may be crucial upstream regulators of signaling pathways regulating epidermal soft tissue and ectodermal appendage development, and the p63 mutant proteins may also contribute to the molecular mechanisms underlying SHFM and other human ectodermal malformation. The molecular mechanism of SHFM underlying the novel mutation of *p63* is remained to be determined further.

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References

[1] X.J. de Mollerat, D.B. Everman, C.T. Morgan, K.B. Clarkson, R.C. Rogers, R.S. Colby, et al., P63 mutations are not a major cause of non-syndromic split hand/foot malformation, J. Med. Genet. 40 (2003) 55–61.

[2] S.W. Scherer, P. Poorkaj, T. Allen, J. Kim, D. Geshuri, M. Nunes, et al., M. A Patton. Fine mapping of the autosomal dominant split hand/split foot locus on chromosome 7, band q21. 3-q22. 1, Am. J. Hum. Genet. 55 (1994) 12–20.

[3] S.W. Scherer, P. Poorkaj, H. Massa, S. Soder, T. Allen, M. Nunes, et al., Physical mapping of the split hand/split foot locus on chromosome 7 and implication in syndromic ectrodactyly, Hum. Mol. Genet. 8 (1994) 1345–1354.

[4] M. Ahmad, H. Abbas, S. Haque, G. Flatz, X-chromosomally inherited split hand/split foot anomaly in a Pakistani kindred, Hum. Genet. 75 (1987) 169–173.
[5] M.F. ul Haque, S. Uhlhaas, M. Knapp, H. Schuler, W. Friedl, M. Ahmad, P. Propping, Mapping of the gene for X-chromosomal split-hand/split-foot anomaly to Xq26-q26.1, Hum. Genet. 91 (1993) 17–19.
[6] F. Gurrieri, P. Prinos, D. Tackels, M.W. Kilpatrick, J. Allanson, M. Genuardi, et al., A split hand-split foot (SHFM3) gene is located at 10q24-25, Am. J. Med. Genet. 62 (1996) 427–436.
[7] A. Raas-Rothschild, S. Manouvrier, M. Gonzales, J.P. Farriaux, S. Lyonnet, A. Munnich, Refined mapping of a gene for split hand–split foot malformation (SHFM3) on chromosome 10q25, J. Med. Genet. 33 (1996) 996–1001.
[8] M.A. Delrue, D. Lacombe, Association of ectrodactyly and distal phocomelia, Genet Couns. 13 (2002) 319–325.
[9] J. Celli, P. Duijf, B.C. Hamel, M. Bamshad, B. Kramer, A.P. Smits, Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome, Cell 99 (1999) 143–153.
[10] R.G. Boles, B.R. Pober, L.H. Gibson, C.R. Willis, J. McGrath, D.J. Roberts, T.L. Yang-Feng, Deletion of chromosome 2q24-q31 causes characteristic digital anomalies: case report and review, Am. J. Med. Genet. 55 (1995) 155–160.
[11] F.R. Goodman, F. Majewski, A.L. Collins, P.J. Scambler, A 117-kb microdeletion removing HOXD9-HOXD13 and EVX2 causes synpolydactyly, Am. J. Hum. Genet. 70 (2002) 547–555.
[12] R.F. Robledo, L. Rajan, X. Li, T. Lufkin, The Dlx5 and Dlx6 homeobox genes are essential for craniofacial, axial, and appendicular skeletal development, Genes. Dev. 16 (2002) 1089–1101.
[13] P.H. Duijf, H. van Bokhoven, H.G. Brunner, Pathogenesis of split-hand/split-foot malformation, Hum. Mol. Genet. 1 (2003) 51–60.
[14] A. Sidow, M.S. Bulotsky, A.W. Kerrebrock, B.W. Birren, D. Altshuler, R. Jaenisch, K.R. Johnson, E.S. Lander, A novel member of the F-box/WD40 gene family, encoding dactylin, is disrupted in the mouse dactylaplasia mutant, Nat. Genet. 23 (1999) 104–107.
[15] G. Frances, G.U. Maria-Luisa, H. Christine, R. William, W. Robin, S. Peter, Deletions in *HOXD13* segregate with an identical, novel foot malformation in two unrelated families, Am. J. Hum. Genet. 63 (1998) 992–1000.
[16] M. Faiyaz-Ul-Haque, S.H.E. Zaidi, L.M. King, S. Haque, M. Patel, M. Ahmad, T. Siddique, W. Ahmad, L.C. Tsui, D.H. Cohn, Fine mapping of the X-linked split-hand/split-foot malformation (SHFM2) locus to a 5.1-Mb region on Xq26.3 and analysis of candidate genes, Clin. Genet. 67 (2005) 93–97.
[17] Y.P. Huang, G. Wu, Z. Guo, T. Fomenkov, H.L. Park, B. Trink, D. Sidransky, A. Fomenkov, E.A. Ratovitski, Altered sumoylation of p63α contributes to the split-hand/foot malformation phenotype, Cell Cycle 3 (2004) 1587–1596.
[18] H.G. Brunner, C.J. Ben Hamel, H. van Bokhoven, P63 Gene Mutations and Human Developmental Syndromes, Am. J. Med. Genet. 112 (2002) 284–290.
[19] V. Wessagowit, J.E. Mellerio, A.C. Pembroke, J.A. McGrath, Heterozygous germ-line missense mutation in the p63 gene underlying EEC syndrome, Clin. Exp. Dermatol. 25 (2000) 441–443.
[20] A. Yang, R. Schweitzer, D. Sun, M. Kaghad, N. Walker, R.T. Bronson, et al., p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development, Nature 398 (1999) 714–718.
[21] G. Blandino, M. Dobbelstein, p73 and p63: why do we still need them? Cell Cycle 2 (2004) 886–894.
[22] P. Ianakiev, M.W. Kilpatrick, I. Toudjarska, D. Basel, P. Beighton, P. Tsipouras, Split-hand/split-foot malformation is caused by mutations in the p63 gene on 3q27, Am. J. Hum. Genet. 67 (2000) 59–66.
[23] H. van Bokhoven, B.C. Hamel, M. Bamshad, E. Sangiorgi, F. Gurrieri, P.H. Duijf, et al., p63 Gene mutations in eec syndrome, limb-mammary syndrome, and isolated split hand–split foot malformation suggest a genotype–phenotype correlation, Am. J. Hum. Genet. 69 (2001) 481–492.
[24] J.C. Zenteno, V. Berdo′n-Zapata, S. Kofman-Alfaro, O.M. Mutchinick, Isolated Ectrodactyly Caused by a Heterozygous Missense Mutation in the Transactivation Domain of TP63 American, J. Med. Genet. 134 (2005) 74–76.
[25] M. Rossi, S.M. De, A. Pollice, R. Santoro, M.G. La, L. Guerrini, V. Calabro, Itch/AIP4 Associates with and Promotes p63 Protein Degradation, Cell Cycle 5 (2006) 1816–1822.
[26] P. Ghioni, Y.D. Alessandra, G. Mansueto, E. Jaffray, R.T. Hay, M.G. La, L. Guerrini, The protein stability and transcriptional activity of p63alpha are regulated by SUMO-1 conjugation, Cell Cycle 4 (2005) 183–190.
[27] L. Rudbeck, J. Dissing, Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR, Biotechniques 25 (1998) 588–592.
[28] O. Yamada, T. Matsumoto, M. Nakashima, S. Hagari, T. Kamahora, H. Ueyama, Y. Kishi, H. Uemura, T. Kurimura, A new method for extracting DNA or RNA for polymerase chain reaction, J. Virol. Methods 27 (1990) 203–209.
[29] M.A. Crackower, S.W. Scherer, J.M. Rommens, C.C. Hui, P. Poorkaj, S. Soder, J.M. Cobben, L. Hudgins, J.P. Evans, L.C. Tsui, Characterization of the split hand/split foot malformation locus SHFM1 at 7q21.3-q22.1 and analysis of a candidate gene for its expression during limb development, Hum. Mol. Genet. 5 (1996) 571–579.
[30] P. Leoyklang, P. Siriwan, V. Shotelersuk, A mutation of the p63 gene in non-syndromic cleft lip, J. Med. Genet. 43 (2006) 28–32.
[31] E.K. David, W.H. Ferrier Peter, Holland ancient orign of the *Hox* gene cluster, Nat. Rev. Genet. 2 (2001) 33–38.
[32] K. Marie, F. Nadine, H. Yann, D. Denis, Serial deletion and duplications suggest a mechanism for the collinearity of *Hoxd* genes in limbs, Nature 420 (2002) 145–150.
[33] X.J. de Mollerat, F. Gurrieri, C.T. Morgan, E. Sangiorgi, D.B. Everman, P. Gaspari, et al., A genomic rearrangement resulting in a tandem duplication is associated with split hand–split foot malformation 3 (SHFM3) at 10q24, Hum. Mol. Genet. 12 (2003) 1959–1971.
[34] V. Calabro, G. Mansueto, T. Parisi, M. Vivo, R.A. Calogero, G. La Mantia, et al., The human MDM2 oncoprotein increases the transcriptional activity and the protein level of the p53 homolog p63, J. Biol. Chem. 277 (2002) 2674–2681.
[35] A. Yang, M. Kaghad, Y. Wang, E. Gillett, M.D. Fleming, V. Dotsch, N.C. Andrews, et al., p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death inducing, and dominant-negative activities, Mol. Cell 2 (1998) 305–316.
[36] T. Barbara, M. Roberto, Mechanisms of transcriptional repression of cell-cycle G2/M promoters by p63, Nucl. Acid Res. 34 (2006) 928–938.
[37] A.A. Mills, B. Zheng, X.J. Wang, H. Vogel, D.R. Roop, A. Bradley, p63 is a homologue required for limb and epidermal morphogenesis, Nature 398 (1999) 708–713.
[38] M.I. Morasso, N. Radoja, Dlx genes, p63, and ectodermal dysplasias, Birth Defects Res. 75 (2005) 163–171.

[39] N.P. Pavletich, K.A. Chambers, C.O. Pabo, The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots, Genes Dev. 7 (1993) 2556–2564.
[40] Y. Cho, S. Gorina, P.D. Jeffrey, N.P. Pavletich, Crystal structure of a *p53* tumor suppressor–DNA complex: understanding tumorigenic mutations, Science 265 (1994) 346–355.
[41] D.W. Matthewand, A.P. Jennifer, p63: molecular complexity in development and cancer, Carcinogenesis 25 (2004) 857–864.
[42] H.Q. Ying, D.L. Chang, H.W. Zheng, F. McKeon, Z.X. Xiao, DNA-binding and transactivation activities are essential for tap63 protein degradation, Mol. Cell Biol. 25 (2005) 6154–6164.