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p63 gene analysis in Mexican patients with syndromic and non-syndromic ectrodactyly

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Abstract

Ectrodactyly is a congenital limb malformation that involves a central reduction defect of the hands and/or feet which is frequently associated with other phenotypic abnormalities. The condition appears to be genetically heterogeneous and recently it has been demonstrated that mutations in the *p63* gene, a homologue of the tumor suppressor gene *p53*, are the cause of at least four autosomal dominant genetic syndromes which feature ectrodactyly: ectrodactyly, ectodermal dysplasia, and facial clefting (EEC), split hand/split foot malformation (SHFM), limb-mammary syndrome (LMS), and acro-dermato-ungual-lacrimal-tooth syndrome (ADULT). In this study, genetic analysis of the p63 gene in a group of 13 patients with ectrodactyly (syndromic and isolated) was performed. Four patients with syndromic ectrodactyly had *p63* heterozygous point mutations that affect the DNA binding domain of the protein. One of these subjects exhibited the typical features of EEC syndrome as well as ankyloblepharon being, to our knowledge, the first case combining these traits. This finding supports the view of a clinical overlap in this group of autosomal dominant syndromes caused by p63 mutations and demonstrates that there are exceptions in the previously established *p63* genotype–phenotype correlation.

Keywords: Ectrodactyly; *p63* gene; EEC syndrome; Limb malformation

Introduction

Ectrodactyly or split hand–split foot malformation (SHFM) is a congenital defect of the distal limbs occurring in approximately 1 in 18,000 newborns [7]. It is characterized by varying degrees of central longitudinal fissuring and central ray deficiency frequently associated to syndactyly, and aplasia and/or hypoplasia of the phalanges, metacarpals, and metatarsals. Ectrodactyly is associated with other physical anomalies in up to 40% of the cases [5,7]. Syndromatic ectrodactyly includes autosomal dominant entities like EEC syndrome which is defined by ectrodactyly, ectodermal dysplasia and cleft lip with or without cleft palate [14], limb-mammary-syndrome (LMS) which associates ectrodactyly with mammary gland defects as aplasia or hypoplasia of the mammary gland and nipple [18], and ADULT syndrome which displays acro-dermato-ungual-lacrimal-tooth defects [13]. These syndromes [1,2,4,6,9,16,19], as well as isolated SHFM [8,19] have been shown to result from mutations in the *p63* gene, a homologue of the archetypal tumor suppressor p53. In addition, mutations in *p63* have been found in patients with AEC syndrome, characterized by ankyloblepharon (congenital fusion of the eyelids), ectodermal dysplasia, orofacial clefting, but minimal to absent limb involvement [11].

To date, approximately 40 different *p63* gene mutations have been described in individuals with these five different entities (reviewed in [20]) and interestingly, the distribution of mutations over the various p63 protein domains and the structural and functional implications of these mutations have revealed a striking genotype–phenotype correlation as the pattern of mutations is distinct for each of these syndromes [3,11,19].

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This report presents the results of *p63* gene molecular analysis in a group of 13 Mexican patients with syndromatic and isolated ectrodactyly.

Subjects and methods

Thirteen Mexican patients with syndromatic or isolated ectrodactyly were included in the study. Three of them had a diagnosis of EEC syndrome, two had ectrodactyly and ectodermal dysplasia but no clefting (EE phenotype); the remaining eight subjects had isolated ectrodactyly. Patients #9 and #10 were mother and daughter. The detailed clinical description of each patient is shown in Table 1. After approval of the Institutional Review Board and given patient's informed consent, DNA was extracted from peripheral blood leukocytes in all subjects according to standard procedures. PCR amplification of the 16 exons of the *p63* gene was achieved using 16 pairs of primers previously described [19]. Each 25 μl PCR amplification reaction contained 1X buffer, 100–200 ng of genomic DNA, 0.2 mM of each dNTP, 1U Taq polymerase, 1 mM of forward and reverse primers, and MgCl2 between 1 and 3 mM. Temperatures program was identical as reported elsewhere [19]. PCR products were analyzed in 1.5% agarose gels from which the bands with the amplified templates were excised and the DNA subsequently purified with the help of the Qiaex II (Qiagen) kit. Direct sequencing was performed with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) adding ~10 ng of template DNA in each reaction and using a temperature program which included 25 cycles of denaturation at 97 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for 4 min. All samples were analyzed in an ABI Prism 310 (Applied Biosystems) Genetic Analyzer. Sequence variations were confirmed in each case using a newly amplified fragment.

Results

In all cases, the PCR amplified products were of the expected size excluding gross rearrangements at the *p63* locus. After sequencing the entire coding sequence and the intron/exon junctions of the *p63* gene, four heterozygous mutations were identified in our group of Mexican patients with syndromatic or isolated ectrodactyly. In patient 1, which had a diagnosis of EEC syndrome, a CGC to CAC mutation in exon 7 predicted an arginine to hystidine change at position 279 of the p63 protein (R279H) which is located in the DNA binding domain of p63 (Fig. 1); it is important to emphasize that this patient had in addition ankyloblepharon (AEEC phenotype), which is an unusual finding in EEC. In patients 9 and 10 (a mother with EE phenotype and her daughter with EEC syndrome), a change from CGG to TGG at codon 204 in *p63* exon 5 predicted a shift from arginine to tryptophan (R204W), also situated in the DNA binding domain of the protein (Fig. 2); the third mutation was identified in patient 11 and was identical to that described in patient 1 (R279H); this patient exhibited a phenotype characterized by ectrodactyly associated to ectodermal dysplasia without facial clefting (EE phenotype). In addition, a previously undescribed intronic *p63* polymorphism was identified: in intron five, at position −4 of the acceptor splice site of exon 6, a deletion of an adenine was observed (IVS5-4A-del). This variant was observed in DNA from both control subjects and patients.

Discussion

Epithelial–mesenchymal interactions are essential for many aspects of vertebrate limb development. During the early stages of development, the limb bud mesenchyme induces the overlying distal tip ectoderm to

Table 1
Clinical features of patients included in the study

Patient #	Gender	Limb defects	Additional features	Diagnosis
1	Female	Ectrodactyly/syndactyly hands and feet	Cleft lip and palate/ectodermal dysplasia/ankyloblepharon/blepharitis	EEC
2	Male	Ectrodactyly in hands	Cleft lip/ectodermal dysplasia	EEC
3	Male	Ectrodactyly/brachydactyly in hands	...	SHM
4	Female	Ectrodactyly/syndactyly in hands	...	SHM
5	Male	Ectrodactyly in hands	Hypospadias	SHM
6	Male	Ectrodactyly in hands	Facial dysmorphism	SHM
7	Male	Ectrodactyly in hands and feet	...	SHFM
8	Female	Ectrodactyly in hands/syndactyly in feet	...	SHM
9*	Female	Ectrodactyly in feet/hypoplastic left hand second finger/brachydactyly in hands and feet	Ectodermal dysplasia	EE
10*	Female	Ectrodactyly in hands and feet	Cleft lip and palate/ectodermal dysplasia	EEC
11	Female	Ectrodactyly in hands and feet	Ectodermal dysplasia/lacrimal duct obstruction	EE
12	Female	Ectrodactyly in feet/hypoplastic thumbs	...	SFM
13	Female	Ectrodactyly in hands and feet	...	SHFM

EEC = ectrodactyly, ectodermal dysplasia, and facial clefting; EE = ectrodactyly and ectodermal dysplasia; SHFM = split hand–split foot malformation; SHM = split hand malformation; SFM = split foot malformation.

* Patients #9 and #10 were mother and daughter.

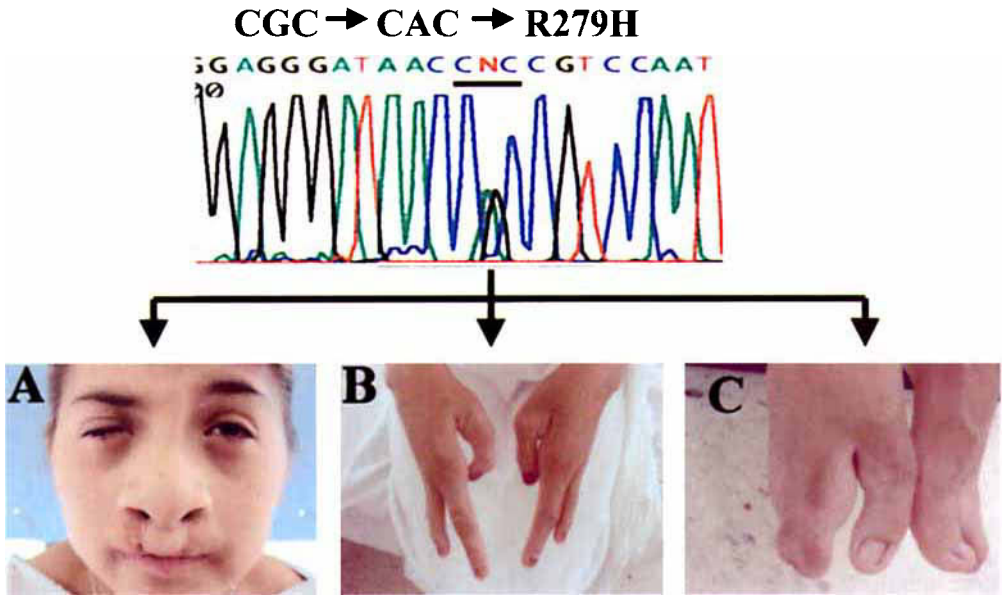

Fig. 1. Upper panel: p63 exon 7 nucleotide sequencing in patient 1 demonstrates a heterozygous G to A substitution that converts an arginine residue (CGC) to a hystidine (CAC), designated R279H. Lower panel: phenotypic features of patient 1. (A) Repaired bilateral cleft lip and right chronic blepharitis. (B) Bilateral ectrodactyly of the hands and nail dysplasia. (C) Bilateral ectrodactyly of the feet.

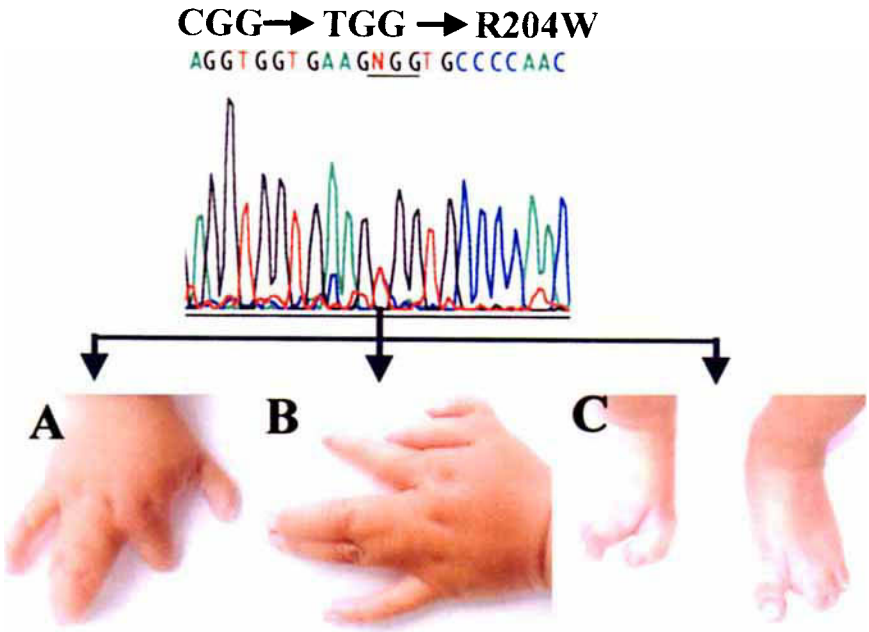

Fig. 2. Upper panel: p63 exon 5 nucleotide sequencing in patient 10 demonstrates a heterozygous C to T substitution that converts an arginine residue (CGG) to tryptophan (TGG), designated R204W. Lower panel: limb features of patient 10. (A) Right hand showing ectrodactyly and thumb hypoplasia. (B) Left hand showing polydactyly, syndactyly between the third and fourth digits, and hypoplastic thumb. (C) Bilateral ectrodactyly of the feet.

thicken and form a morphologically distinct structure known as the apical ectodermal ridge (AER). Subsequent interactions between the AER and the underlying mesenchyme are responsible for proliferation, patterning and outgrowth of the developing limb. The AER is required for proliferation of the mesenchyme along the proximodistal axis of the limb and the maintenance of the antero-posterior patterning center in the posterior mesenchyme, the zone of polarizing activity. Similarly, the mesenchyme is required for the maintenance of the AER (reviewed in [16,17]. Therefore, proper signaling along the antero-posterior axis between the AER and

the underlying mesoderm is crucial for normal formation of the distal limb. Recent evidence indicates that a number of factors direct ridge–mesenchyme interactions, including members of the fibroblast growth factor family (FGFs) and sonic hedgehog (SHH) [10].

p63, a gene homolog to the cell cycle regulator *p53*, is an essential transcription factor also involved in the mesenchymal–ectodermal signaling of the AER. The *p63* gene is expressed in the progenitor cells of many epithelial tissues, particularly in the limb bud AER, branchial arches, and the epidermal appendages [21,22]. Mice knockout for *p63* display a severe phenotype with absence of most of the tissues that are derived from these progenitor cells and newborn mice usually die due to dehydration shortly after birth [12,22]. These mutant animals exhibit a severe phenotype characterized by limb truncations, craniofacial abnormalities, lack of the epidermal components and defects in structures dependant on epidermal–mesenchymal interactions such as hair follicles, teeth primordia, and mammary glands [12,22].

Ectrodactyly is a distal limb malformation that can occur isolatedly or as part of complex genetic malformation syndromes [15]. Recently, it has been demonstrated that *p63* is a major locus for syndromatic (EEC, LMS, and ADULT syndromes) or isolated (SHFM) autosomal dominant ectrodactyly [3]. In addition, AEC syndrome which is characterized by ankyloblepharon, ectodermal dysplasia, orofacial clefting and minimal to absent limb defects has been associated to mutations in the SAM domain of p63 [11].

Our study confirms that the mutation R279H is the most common p63 disease-causing mutation as it accounts for 13% (11 out 84, including our results) of all pathogenic p63 mutations described so far. In fact, approximately 18% of p63 mutations (15 out 84) affect amino acid number 279, located within the DNA binding domain of the protein. However, combined mutational data indicate extensive genotype–phenotype correlation with each of the above mentioned syndromes having a distinct pattern and type of *p63* mutations.

It is interesting to note that in the 3 population in which *p63* mutations have been described to date (Caucasic, Asiatic, and Hispanic, in this study), a *p63* mutational homogeneity is evident. These observations indicate that there are p63 nucleotides highly mutable as those in positions 727, 728, 797, 952, 953, 955, and 1028, which accounts for approximately 47% of all *p63* mutations. This remarkable mutational pattern may be explained by the fact that all these nucleotides are located at CpG sites [19], which are mutational hotspots. Another possibility is that this mutational profile is caused by a specific pathogenetic gain of function-mechanism, as was previously suggested [19].

An interesting finding in this study was the antecedent of ankyloblepharon in patient 1, which exhibited the typical features of EEC syndrome (ectrodactyly, ectodermal dysplasia, and facial clefts). Ankyloblepharon is not considered part of EEC syndrome but is a cardinal feature in AEC syndrome which associates ankyloblepharon, severe ectodermal dysplasia, and facial clefting with minimal or absent limb anomalies. To our knowledge this is the first case in which a mutation in *p63* is associated with an EEC syndrome phenotype in addition to ankyloblepharon. This finding supports the view of a clinical overlap in this group of autosomal dominant syndromes caused by *p63* mutations and is in opposition to the strict genotype–phenotype correlation previously observed in *p63*-related human syndromes [19].

Cases 9 and 10 (mother and daughter) carried a R204W mutation that affects the DNA-binding domain of p63. However, the phenotypic expression of this alteration was significantly different in both patients as the mother exhibited incomplete ectrodactyly in feet, minimal hand involvement, ectodermal dysplasia and no facial clefts, while the daughter showed a severe phenotype characterized by bilateral cleft lip and palate, ectodermal dysplasia and complete ectrodactyly in hands and feet (Fig. 2). This striking intrafamilial phenotypic difference associated with a particular *p63* mutation could be attributed to the involvement of additional genetic factors (modifier genes), as previously suggested [20]. Finally, given that *p63* is essential for early limb development, it would be interesting to investigate the potential role of this gene in the etiology of other autosomal dominant limb malformations and thus identify new members of the expanding *p63* mutation family of human malformation syndromes.

In conclusion, our results are in agreement with previous reports in which *p63* mutations are frequently associated to EEC or EEC-like disorders but are uncommon in isolated SHFM. *p63* mutations in Mexican patients with syndromic ectrodactyly correspond to those found in other populations reflecting a mutational homogeneity in this gene. In addition, we identify a p63 mutation which is associated with an EEC syndrome phenotype as well as ankyloblepharon, which to our knowledge is a non-previously described association. This case illustrates that there are exceptions in the uniform genotype–phenotype correlation observed in the group of *p63* related disorders.

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