A novel c.1037C > G (p.Ala346Gly) mutation in TP63 as cause of the ectrodactyly-ectodermal dysplasia and cleft lip/palate (EEC) syndrome

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Abstract

Ectrodactyly – ectodermal dysplasia and cleft lip/palate (EEC) syndrome (OMIM 604292) is a rare disorder determined by mutations in the TP63 gene. Most cases of EEC syndrome are associated to mutations in the DNA binding domain (DBD) region of the p63 protein. Here we report on a three-generation Brazilian family with three individuals (mother, son and grandfather) affected by EEC syndrome, determined by a novel mutation c.1037C > G (p.Ala346Gly). The disorder in this family exhibits a broad spectrum of phenotypes: two individuals were personally examined, one presenting the complete constellation of EEC syndrome manifestations and the other presenting an intermediate phenotype; the third affected, a deceased individual not examined personally and referred to by his daughter, exhibited only the split-hand/foot malformation (SHFM). Our findings contribute to elucidate the complex phenotype-genotype correlations in EEC syndrome and other related TP63-mutation syndromes. The possibility of the mutation c.1037C > G being related both to acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome and SHFM is also raised by the findings here reported.

Keywords: EEC syndrome, TP63-mutations, p63-associated disorders, SHFM.

Ectrodactyly-ectodermal dysplasia and cleft lip/palate (EEC) syndrome (OMIM 604292) is a rare autosomal-dominant disorder characterized by dry or eczematous skin, sparse hair on the scalp, eyebrows and eyelashes, nail dystrophy and hypodontia, in addition to ectrodactyly and orofacial cleft. Other clinical manifestations include syndactyly, absence or reduced number of sweat, sebaceous, and salivary glands, mammary gland/ nipple hypoplasia, nasolacrimal duct abnormalities, conductive or sensorineural hearing loss, facial dysmorphisms, chronic infections of the respiratory tract, genitourinary anomalies, and development delay (Clements et al., 2009; Celli et al., 1999; Shivaprakash et al., 2012). EEC is a complex pleiotropic condition of multiple congenital dysplasia and anomalies. The clinical diagnosis of the syndrome is hampered by its extremely variable expression (Rodini and Richieri-Costa, 1990; Celli et al., 1999; Rinne et al., 2006). The penetrance is practically complete and just a few cases of arguable lack of penetrance have been reported (Roelfsema and Cobben, 1996).

Most EEC cases, if not all, result from mutations in the TP63 gene, and more than 200 cases have already been reported in the literature (Clements et al., 2009). EEC is one out of five distinct ectodermal dysplasia (ED) syndromes caused by mutations in this gene, which include, besides EEC, acro-dermato-ungual-lacrimal-tooth syndrome (ADULT, OMIM 103285), ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC, OMIM 106260), limb-mammary syndrome (LMS, OMIM 603543), and Rapp-Hodgkin syndrome (RHS, OMIM 129400). Non-syndromic split hand/foot malformation (SHFM4, OMIM 605289) and, very rarely, nonsyndromic cleft lip may be caused by mutations in this same TP63 gene (Leoyklang et al., 2006; Rinne et al., 2006). All conditions related to TP63 mutations exhibit clinical features that largely overlap with EEC syndrome, complicating the differential diagnosis among them (Clements et al., 2009; Celik et al., 2011).

The protein p63, encoded by TP63 gene on 3q27, is a member of p53 family. Like the TP53 and TP73 genes, TP63 encodes a sequence-specific transcription factor that activates target genes involved in different cellular pathways. However, unlike the p53 protein, p63 protein is not involved in tumor suppression, but plays an important role as a transcription factor involved in limb, epithelial, and
craniofacial development (Rinne et al., 2007; van Bokhoven et al., 2011). p63 is expressed as at least six different isoforms, three of which include a transactivation domain (TAp63) and the other three, none (ΔNp63). Alternative splicing produces additional three C-terminus regions, α, β and γ isoforms. ΔNp63α is the major isoform expressed in the epidermis (Browne et al., 2011). The EEC syndrome is mainly caused by missense mutations in the DNA binding domain (DBD), a region with transactivation activity present in all isoforms (Celli et al., 1999; Rinne et al., 2007).

Here we report a novel heterozygous missense mutation in TP63 (c.1037C > G) that results in p.Ala346Gly, causing EEC in a Brazilian family (Figure 1).

The proband, a woman born in the Northern region of Brazil, presents the complete manifestation of the EEC syndrome (Figure 2), bilateral split hand and split foot malformation (SHFM), cleft lip and palate, dracryocystitis and hypodontia (Figure 2A, C and E). In addition, she had hypothyroidism.

Her affected son also had bilateral SHFM. In the lower limbs, the splitting was accompanied by malformation of the leg bones, probably tibial and/or fibular hemimelia (lower limb extremities and distal region of the leg bones were amputated and no X-rays were available). He also presented dracryocystitis, hypodontia, thin and hypopigmented hair, blepharitis, and blepharophimosis, but no labiopalatine cleft (Figure 2B and D).

The deceased father of the proband was reported to have only SHFM, without cleft lip/palate or other features associated with the syndrome.

Blood samples were obtained with informed consent from the proband and her children. Genomic DNA was extracted from peripheral blood leukocytes using standard techniques. Initially, the DNA was amplified by PCR using primers that flank the entire coding sequence of the TP63 gene. The amplified product was sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing Kit method in an ABI 3730 DNA Analyzer equipment (Applied Biosystems, Foster City, CA, USA). Sequencing of both strands was performed at least twice for each sample.

The results were analyzed by the Bioedit program (Ibis Biosciences, Carlsbad, CA, USA). Prediction of the effect of the mutation was performed with the Polyphen-2, SIFT and MutationTaster2 online programs. The protein alignment analysis was done using the Universal Protein Resource (Uniprot) catalog available online. The mutation was also searched in the databases of the 1000 Genomes and the NHLBI Exome Sequencing Project. The numbering of nucleotides and amino acids used in this report is in accordance with systematic nomenclature approved by the Human Genome Variation Society (HGVS).

Molecular analysis of the TP63 gene identified a novel heterozygous missense change c.1037C > G in exon 8, present in the proband and in her affected son. It was absent in her unaffected daughter. This mutation has never been described before and is not present in the 1000 Genomes and NHLBI exome sequencing project databases. The mutation c.1037C > G in TP63 that results in a p.Ala346Gly amino acid substitution, was predicted to be probably damaging by PolyPhen-2 (score of 0.997) and SIFT (score of 0), and to be disease causing by MutationTaster2. The protein sequence alignment showed that alanine at position 346 lies in the DBD region and is highly conserved across different species (Uniprot). The TP63 mutation here described has been submitted to the ClinVar Database (SCV000189629). More than 30 different mutations have been described in the literature, and only two of them are located outside the DBD region (Celli et al., 1999; Rinne et al., 2006; Clements et al., 2009).

Mutations in the DBD region were reported to disrupt the DNA-binding ability of p63, which results in reduced transactivation activity (Celli et al., 1999). p63 protein plays an important role in the development, proliferation, and maintenance of stratified epithelia, such as the epidermis (Koster et al., 2004). Mice lacking Tp63 display defects in epithelial lineage development and fail to develop stratified epithelia and epithelial appendages (Mills et al., 1999; Yang et al., 1999), which explains the skin, glands, teeth and hair defects in the EEC syndrome.

Irf6, one of p63 target genes, is needed for skin differentiation and palate development, including palate closure (Moretti et al., 2010). Mutations in Irf6 were associated

Figure 1 - Pedigree of family with ectrodactyly-ectodermal dysplasia and cleft lip/palate (EEC) syndrome.
with cleft lip/palate and other developmental disorders in mice (Richardson et al., 2006). A feedback regulatory loop between Irf6 and p63 was suggested (Guerrini et al., 2011), and, therefore, mutations in TP63 could explain orofacial cleft.

The apical ectodermal ridge (AER) on limb-bud, which is responsible for limb development, is maintained by self-renewing stem cells. It was speculated that p63 has an essential role in preserving self-renewal capacity of progenitor epithelial cells (Yang et al., 1999). Moreover, there is evidence that p63 is able to induce the transcription of Dlx5 and Dlx6 promoters in the AER (Lo Iacono et al., 2008), and the DLX5 gene was already associated with SHFM (type I) in humans (Shamseldin et al., 2012). Double knockout mice for Dlx5 and Dlx6 present SHFM (Merlo et al., 2002). Restelli et al. (2014) proposed a model for a regulatory loop during limb development. According to their model: p63 activates Dlx5/Dlx6 transcription; Dlx5/Dlx6 positively controls Fgf8; p63 also activates Fgf8 transcription; Pin1 (peptidyl-prolyl cis/trans isomerase NIMA-interacting-1) induces p63 destabilization mediated by proteasome degradation; on the other hand, Fgf8 acts on the AER cells and promotes p63 stability, apparently because it protects p63 from Pin-1 interaction. The activation of this loop and maintenance of p63 levels allows stratification and specialization of ectoderm cells in the AER. These findings, taken together, can explain the clinical features of isolated forms of SHFM and SHFM in EEC syndrome.

In defects associated to TP63 mutations, genotype-phenotype-correlations are hampered by extremely variable expressivity both among and within families (Rinne et al., 2007; Di Lorio et al., 2012). The situation is further
complicated by the fact that affected individuals by (apparently) different diseases associated with the TP63 gene may be carriers of identical mutations: such is the case of the mutation p.R243W associated with EEC (Celli et al., 1999) and ADULT (Avitan-Hersh et al., 2010) syndromes, and the case of the mutation p.R319H, that can cause the EEC syndrome (van Bokhoven et al., 2001) as well as non-syndromic SHFM (Ianakiev et al., 2000).

This variable expressivity of EEC is evident in the present study: besides the proband’s son not having cleft lip/palate, features present in his mother, his leg malformation is much more severe when compared to the one presented by his mother. Since the proband’s son does not have cleft lip/palate, his phenotype is similar to that of ADULT syndrome, which may not include clefting, breast hypoplasia or freckling (Berk et al., 2012). The significant clinical overlap between the two phenotypes demonstrates that the distinctions between EEC and ADULT syndromes are somewhat artificial, as already pointed out by Reisler et al. (2006). The father of the proband allegedly presented only SHFM malformation affecting his four limbs, without other manifestations of EEC. This could indicate that the mutation c.1037C>G might also be associated with non-syndromic SHFM, in spite of a certain degree of uncertainty due to the fact that this individual is already deceased and could not be personally examined by one of us.

As already stressed, this is the first report of the mutation c.1037C>G (p.Ala346Gly). Clements et al. (2009) found a mutation at the same amino acid position, a change from alanine to asparagine (p.Ala346Asp) (in their report the mutation was formerly described as p.Ala307Asp), which caused the EEC syndrome in two patients of the same family, mother and son. They both displayed hypodontia and bilateral SHFM. The ectodermal anomalies were similar in mother and son. Some differences between the patients from his and our report were observed in the clefting pattern, since it was bilateral in the case of p.Ala346Asp mutation and unilateral in ours. Another important difference between these two reports concerns the large spectrum of clinical variability observed within families: while the patients studied by Clements et al. (2009) differed only in relation to the presence of lacrimal ducts dysplasia, our patients differed in relation to the presence of cleft lip/palate and to the variability spectrum of limb malformation.

The present findings contribute to elucidate the complex genotype-phenotype correlations in EEC syndrome and other related TP63-mutation syndromes. The possibility of the mutation c.1037C>G being related to both ADULT syndrome and SHFM is also raised by the findings here reported.

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