Two Novel TP63 Mutations Associated
With the Ankyloblepharon, Ectodermal Defects,
and Cleft Lip and Palate Syndrome

A Skin Fragility Phenotype

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Background: Ankyloblepharon, ectodermal defects, and cleft lip and palate (AEC) syndrome is a rare autosomal dominant disorder caused by mutations in the sterile α motif region of TP63, a homologue of the tumor suppressor TP53. Recent structure-function studies have identified complexities in the genotype-phenotype correlation of the p63 syndromes.

Observations: We report 2 sporadic cases of AEC syndrome in infants. Both patients demonstrated skin erosions with prominent scalp involvement. Histologic studies demonstrated mild basal layer vacuolization and rare dyskeratotic keratinocytes, with evidence of both acantholysis and cytolysis at the blister edge. Immunohistochemistry using anti-p63 monoclonal antibody demonstrated basal epidermal nuclear staining in both healthy control and patient tissue samples. Ultrastructural studies showed focal disruption of anchoring fibrils near the blister edge of one patient and normal desmosomes, hemidesmosomes, and basement membrane zone in the nonblistered skin of the other patient. The DNA analysis of each patient revealed 2 novel missense mutations in the TP63 gene that resulted in L514S and R555P amino acid substitutions within the sterile α motif region of the p63 protein.

Conclusions: We report 2 novel TP63 mutations resulting in AEC syndrome. The R555P mutation is the most carboxy-terminal of all the reported AEC missense mutations of p63. The presence of skin fragility, manifested as erosive skin lesions in body areas in addition to the scalp, is postulated to be an important diagnostic feature of AEC syndrome.

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Hay and Wells described the syndrome of ankyloblepharon, ectodermal defects, and clefting of the lip and palate (AEC) in a series of 7 patients from 4 affected families. The trait is transmitted in an autosomal dominant fashion with variable expressivity, although many sporadic cases have been described. In addition to the classic triad of congenital anomalies, affected individuals may demonstrate varying degrees of alopecia, onychodystrophy, hypohidrosis, hypodontia, maxillary hypoplasia, alveolar synechiae, syndactyly, auricular deformities, and supernumerary nipples. Recalcitrant scalp dermatitis was proposed to be a distinguishing feature in AEC syndrome.

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Mutations in TP63, a homologue of the tumor suppressor TP53, are responsible for AEC syndrome as well as ectodactyly, ectodermal defects, and cleft lip and palate (EEC) syndrome; limb-mammary syndrome (LMS) (an EEC-like syndrome with ectrodactyly, mammary gland and nipple hypoplasia, and clefting of only the palate with no other hair or skin defects); split-hand and split-foot malformation (SHFM) (or isolated ectrodactyly); acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome (an LMS-like syndrome with ectrodactyly and mammary hypoplasia plus excessive freckling but without facial clefting); and Rapp-Hodgkin ectodermal dysplasia syndrome (RHS) (an AEC-like syndrome with characteristic midfacial hypoplasia). Structure-function analysis has revealed a complex genotype-phenotype correlation within the p63 syndromes; p63, like p53, contains a transactivation domain, DNA-binding domain, and oligomerization domain in addition to a unique carboxy-terminal sterile α motif (SAM). Generally, AEC syndrome and RHS are associated with mutations in the SAM region of p63 that are thought to disrupt...
protein-protein interactions, whereas p63 mutations associated with EEC syndrome occur in the DNA-binding domain of the protein and are expected to affect transcriptional activity of the protein.\textsuperscript{7,10} Although more difficult to characterize because of the fewer reported cases, LMS has been associated with 2 frameshift mutations within or immediately downstream of the SAM domain,\textsuperscript{7} and ADULT syndrome mutations map to the amino-terminal transactivation or DNA-binding domains.\textsuperscript{9,13} A variety of isolated SHFM mutations have been reported, including missense and splice site mutations within the amino-terminal DNA-binding domains and a nonsense mutation in the carboxy-terminal transcription inhibitory domain.\textsuperscript{8}

We report 2 sporadic cases of AEC syndrome for which genetic analysis has revealed 2 novel mutations in \textit{TP63}. Both of our patients had extensive skin erosions, including the scalp. A review of the literature suggests that patients with AEC syndrome often demonstrate widespread skin erosions at birth, most prominently on the scalp, suggesting that the scalp dermatitis in AEC syndrome may occur as part of a more generalized skin fragility phenotype.

\section*{REPORT OF CASES}

\subsection*{CASE 1}

A 3270-g female infant born at 40 6/7 weeks’ gestation presented with right-sided cleft lip and cleft palate, ankyloblepharon of the right eyelid, hypertelorism, hyponychia, syndactyly of the third through fifth toes on the right foot and the fourth and fifth toes on the left, and skin erosions manifested as widespread areas of denudation on the extremities and scalp (Figure 1). Additionally, the patient demonstrated small, low-set ears and auditory canals, widely spaced hypoplastic nipples, hyponychia, hypoplastic labia majora, bilateral atresia of the lacrimal glands and ducts, alopecia with nearly absent eyebrows and eyelashes, and sparse white, coarse, and wiry scalp hair. There was no family history of consanguinity, and both parents were unaffected. A 1-year-old sister was reported to have had 20-nail dystrophy with thickened nail beds and ridging shortly after birth, which spontaneously resolved.

Skin bacterial, fungal, and herpesvirus cultures were negative at birth. On day 2 of life, her white blood cell count decreased to 1200/µL, reaching a nadir of 900/µL on day 3. Cultures of skin erosions at this time revealed methicillin-resistant \textit{Staphylococcus aureus} and \textit{Escherichia coli}. Blood cultures were negative. The patient received vancomycin hydrochloride and gentamicin sulfate therapy, and a 4-day course of filgrastim was administered on days 3 to 6, resulting in recovery of her white blood cell count to normal range by day 5. On day 12, the patient developed widespread, superficial white pustules across the scalp, trunk, extremities, and oropharynx. Potassium hydroxide examination of a pustule scraping was positive for budding yeast forms, and superficial swabs of scalp erosions grew \textit{S} aureus, \textit{Enterococcus} species, and \textit{Pseudomonas} species, for which she received broad-spectrum systemic antibiotics and antifungal therapy. Her skin erosions were treated with bacitracin ointment under petrolatum gauze. Despite aggressive treatment with topical and systemic antibiotics, she continues to have local erosions and secondary infections of the scalp. Subsequent immunologic studies, including total immunoglobulin and lymphocyte subset analysis, have not documented any quantitative defects in cellular or humoral immunity. She also has significant developmental delay.

\subsection*{CASE 2}

A 2840-g male infant born at 38 weeks’ gestation presented with ankyloblepharon, cleft palate, erosive scalp dermatitis, hyponychia, hypoplastic nipples, webbed penis, and scattered superficial skin erosions on the planar surfaces of the feet, dorsal hands, and trunk (Figure 2). Both parents were unaffected and denied a history of consanguinity. On day 2 of life, the patient developed neutropenia with a white blood cell count nadir of 2300/µL and an absolute neutrophil count of 415/µL. Blood cultures were negative, and the patient’s white blood cell count recovered by day 6. The patient had repeated apneic episodes, and subsequent echocar-
diography revealed a bidirectional patent foramen ovale. Superficial swabs of skin erosions from the scalp on day 4 revealed *Candida albicans*. The patient’s scalp erosions resolved transiently with topical nystatin cream with petrolatum gauze. The scalp erosions subsequently recurred and persisted despite aggressive topical therapy with petrolatum gauze, silicone-based dressings, and topical antibiotics where needed. A trial of becaplermin (recombinant platelet-derived growth factor) gel for the scalp erosions was discontinued after 3 weeks owing to worsening of the affected areas.

Skin biopsies were performed. Samples from both patients were obtained from perilesional skin after mechanical rubbing and submitted for both routine and ultrastructural histologic analysis. Skin samples from the left arm of patient 1 on day 1 of life demonstrated intraepidermal blister formation with areas of both cytolyis and acantholysis, with focal dyskeratotic keratinocytes (Figure 3A). In skin samples from the thigh of patient 2 obtained on day 2 of life, mild papillary dermal edema was observed in association with rare dyskeratotic cells and epidermal basal layer vacuolization (Figure 3B). Both biopsy specimens demonstrated normal adnexal structures.

Immunohistochemical analysis using anti-p63 monoclonal antibody (4A4; BD Pharmingen, San Diego, Calif) demonstrated basal epidermal nuclear localization of p63 in healthy control and patient samples (Figure 4). Ultrastructural studies of skin biopsy samples from patient 1 demonstrated focal disruption of anchoring fibrils (Figure 5A). Immunoelectron microscopy showed focal absence of type VII collagen with normal distribution of type IV collagen and laminin (data not shown). Electron microscopy of skin samples from patient 2 revealed intact desmosomes and hemidesmosomes, including anchoring fibrils and basal laminae (Figure 5B).

The DNA analysis of peripheral blood (GeneDx, Gaithersburg, Md) from patient 1 revealed a T-to-C point mutation in exon 13 of the *TP63* gene, which resulted in an L514S amino acid substitution in the SAM of the p63 protein (numbered according to GenBank accession No. AF075430). Genetic analysis of buccal swab DNA from patient 2 demonstrated a G-to-C mutation in exon 14 of *TP63*, predicting an R555P amino acid substitution in the carboxy-terminus of the SAM region of p63.

**COMMENT**

The features of ectodermal dysplasia in conjunction with cleft abnormalities occur in the p63 syndromes AEC syndrome, EEC syndrome, RHS, and LMS. In addition, fa-
clefting and ectodermal dysplasia are also seen in the non-p63 disorder cleft lip and palate ectodermal dysplasia (CLPED1) syndrome. Recent genetic and molecular biological studies have shed light on the syndromic association of limb, craniofacial, and epithelial anomalies related to p63. Early studies showed that p63, a homolog of the tumor suppressor p53 and the related protein p73, is a transcription factor expressed in the basal or progenitor layers of many epithelial tissues. Mice that lack p63 demonstrate severe developmental malformations, including craniofacial abnormalities; limb truncations; anodontia; alopecia; complete absence of mammary, lacrimal, and salivary glands; and stratified squamous epithelia.

The complexity of genotype-phenotype correlation in the p63 syndromes is in part attributed to the complex biochemical structure of p63. At least 6 isoforms of p63 exist because of the presence of 2 transcriptional start sites and 3 alternative splice sites within the TP63 gene. Proteins generated from the 5' proximal transcriptional start site contain an amino-terminal p53-like transactivation domain and are termed TAp63, whereas proteins that lack this domain are termed ΔNp63. Three alternatively spliced variants with different carboxy-termini can be generated from each of these transcripts (labeled with the suffix α, β, or γ), with only the α isoforms containing the SAM region. Different isoforms of p63 vary in their expression patterns, transcriptional activity, and targets. The TAp63 isoforms are the first to be expressed during development and are required for initiation of epithelial stratification, whereas expression of ΔNp63 isoforms allows for maturation of embryonic epidermis. The ΔNp63α, the predominant isoform in the mature epidermis, has been shown to function as a transcriptional repressor, which may regulate the proliferative capacity of basal keratinocytes.

Also complicating the genotype-phenotype correlation in the p63 syndromes is the observation that the same mutation for both SHFM and classic EEC syndrome has been reported, suggesting that the phenotypic expression of these mutations may be highly variable. Additionally, 1 patient with skeletal reduction defects classic for EEC syndrome together with ectodermal defects, including bilateral mammary gland aplasia, had a single nucleotide insertion leading to a premature stop codon in the SAM region of p63, a region associated with AEC syndrome, RHS, and LMS, the latter of which may best classify this patient. Recent work has identified a transcriptional inhibitory domain downstream of the SAM domain that inhibits the transcriptional activity of p63, possibly explaining the skeletal phenotype of the carboxy-terminal p63 deletion.

The difficulty of clinical diagnosis even with the advent of DNA mutation analysis underscores the importance of reporting complete clinical descriptions of patients for whom genetic data are available to make more accurate genotype-phenotype correlations. In addition to the distinguishing feature of ankyloblepharon, AEC syndrome may be differentiated from EEC syndrome by the common occurrence of erosive scalp lesions that are often colonized with mixed flora and may necessitate early topical and systemic antibiotic therapy. Although all 7 of the original patients described by Hay and Wells were noted to have varying degrees of alopecia, only 2 were described with other forms of scalp involvement: one with “recurrent bacterial infections” and the other with “cradle cap eczema with folliculitis.” Interestingly, these 2 patients were the only children evaluated in the series, suggesting that the scalp lesions may improve with age and/or resolve with alopecia. In contrast, EEC syndrome often displays ectodactyly and genitourinary abnormalities, including renal agenesis. Clefting in EEC syndrome usually includes cleft lip (with or without cleft palate), whereas isolated cleft palate (as noted in patient 2) can occur in AEC syndrome, RHS, or LMS.

The clinical distinctions between RHS and AEC syndrome are no longer considered significant. In Syndromes of the Head and Neck, only the feature of ankyloblepharon was proposed to distinguish the 2 clinical diagnoses. However, 2 unrelated patients, one with RHS (no ankyloblepharon) and the other with AEC syndrome (with ankyloblepharon), have recently been associated with the identical p63 mutation (I510T). Both patients had erosive scalp lesions. At least 4 other patients previously classified as having RHS, including 1 with solitary ankyloblepharon, have been linked to mutations within or near the SAM domain associated with AEC syndrome. Interestingly, some of these were

Figure 3. Hematoxylin-eosin-stained skin sections. A, Upper arm of patient 1 showing intraepidermal blister formation with cytolysis and focal dyskeratotic keratinocytes. B, Thigh of patient 2 showing mild papillary dermal edema, basal layer vacuolization, and rare dyskeratotic keratinocytes (original magnification ×40).
frameshift mutations, resulting in an elongated (rather than truncated) protein. One patient with RHS\textsuperscript{27} had a mutation at R279 in the DNA-binding domain of p63, a region that has been associated with EEC mutations. However, this patient did not have clefting, ankyloblepharon, or erosive scalp dermatitis and had several EEC-like skeletal variations, which argues for reclassification of this patient as having the EEC syndrome. Thus, a review of the recent literature suggests that scalp involvement, rather than ankyloblepharon, seems to be the most distinguishing feature of the group of AEC syndrome and RHS relative to the other p63-associated clefting and ectodermal dysplasia syndromes and appears to correlate with missense mutations in the SAM domain.

Although none of the original 7 patients described clinically by Hay and Wells\textsuperscript{1} were noted to have skin involvement outside the scalp, 6 of the 8 patients with AEC syndrome genetically characterized by McGrath et al\textsuperscript{5} were observed to have widespread areas of skin fragility with cutaneous erosions. A review of the original series of patients for each syndrome in which a TP63 genetic mutation was first assigned shows that in comparison, skin or scalp fragility was observed in 0 of 14 patients with the EEC syndrome,\textsuperscript{28,29} 0 of 7 with ADULT syndrome,\textsuperscript{30} and 0 of 2 with LMS.\textsuperscript{7} (Although the original LMS kindred had 21 patients,\textsuperscript{31} TP63 mutations were not detected in subsequent genetic testing of these individuals.\textsuperscript{7}) Of note, the absence of skin or scalp involvement is typically a diagnostic feature to differentiate LMS from the other ectodermal dysplasia and facial clefting syndromes.

Both of our patients presented with erosive scalp lesions in addition to the classic clinical triad of ankyloblepharon, ectodermal defects, and clefting, indicating the diagnosis of AEC syndrome. Patient 1 also had significant 4- to 5-toe syndactyly. Both patients developed neutropenia during the neonatal period, which may have occurred as a result of incipient bacterial or candidal septicemia in these patients, as skin cultures showed growth of pathogens, but repeated blood cultures were negative. As immunologic evaluations were unrevealing in both patients, recurrent infections on the scalp are presumed to occur on the basis of impaired skin integrity and not impaired immunity.

The skin biopsy histologic findings of basal layer vacuolization and dyskeratotic cells are of interest because p63, like p53, contains a proline-rich proapoptotic domain, and apoptosis has previously been described in skin biopsy specimens from a patient with RHS.\textsuperscript{10} However, no specific increase in terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate–biotin nick end labeling (TUNEL) staining was observed in skin samples from multiple patients with AEC syndrome,\textsuperscript{5} although dyskeratotic cells were not a prominent histologic feature within these skin sections.

The presence of acantholytic cells in the area of blistering suggests desmosomal disruption, although desmosomes appeared normal on electron microscopy. The pres-
tions may include genetic testing of the parents to elucidate the structure-function relationship of p63 and identify its downstream targets will clarify the genotype-phenotype correlation highlights the difficulty of a purely genetic diagnosis with novel p63 mutations, especially in cases that may demonstrate variable expressivity. We propose that the scalp dermatitis classically associated with AEC syndrome represents a more generalized skin fragility phenotype and may be a diagnostic feature of AEC syndrome. Future investigations may include genetic testing of the parents to explore the possibility of nonpenetrance or gonadal mosaicism. In particular, genetic testing of the parents of patient 1 and her sibling with the history of 20-nail dystrophy would be of interest in regard to the possibility of variable expressivity. It is hoped that further molecular studies to elucidate the structure-function relationship of p63 and identify its downstream targets will clarify the genotype-phenotype correlation of the p63 syndromes to allow for better genetic testing and counseling of affected patients and their families.

Additionally, CLPED1, previously known as Zlotogora-Ogur syndrome or Margarita Island ectodermal dysplasia, was shown to be caused by mutations in the PVRL1 gene. This gene encodes the immunoglobulin-related transmembrane protein nectin 1, which localizes to adherens junctions and appears to mediate entry of α-herpesviruses into human cells. Patients with CLPED1 demonstrate varying degrees of alopecia, hypodontia, onychodysplasia, syndactyly, and clefting, with some cases of developmental delay. Given the phenotypic overlap of CLPED1 with the p63 syndromes, it is possible that p63 may also regulate the expression of PVRL1 or other genes involved in cell adhesion signaling pathways.

Genetic analysis of patient 1 demonstrated a missense mutation of the TP63 gene, resulting in a novel L514S amino acid substitution in the SAM region of p63. Two different amino acid substitutions at this site (L514F and L514V) have previously been reported in AEC syndrome. This mutation is predicted to lie within the first α-helical domain of the SAM region and disrupt proper packing of the α-helices based on models of SAM domains in p73 and the ephrin tyrosine kinases for which crystal structures have been solved. The L514F mutation has been shown to interrupt the binding of p63 to an RNA-processing protein, ABBP1, which leads to aberrant splicing of the keratinocyte growth factor receptor and inhibition of epithelial differentiation. Patient 2 demonstrated an R555P amino acid substitution in p63, which is predicted to lie in the fifth α-helical domain at the carboxy-terminus of the SAM region. This is the most carboxy-terminal location of any of the AEC syndrome and RHS missense mutations previously reported.

Previously, it was reported that p63 demonstrates aberrant nuclear staining in the epidermis from patients with AEC syndrome, with expression in the more superficial layers of the epidermis. However, immunohistochemical analysis using the same p63 antibody (which recognizes all isoforms of p63) demonstrated similar patterns of nuclear localization of p63 in both control and patient tissue samples, indicating that aberrant p63 expression likely does not contribute to the pathophysiologic findings in these 2 cases.

We present 2 cases with the clinical diagnosis of AEC syndrome confirmed by genetic testing. The complex genotype-phenotype correlation highlights the difficulty of a purely genetic diagnosis with novel p63 mutations, especially in cases that may demonstrate variable expressivity. We propose that the scalp dermatitis classically associated with AEC syndrome represents a more generalized skin fragility phenotype and may be a diagnostic feature of AEC syndrome. Future investigations may include genetic testing of the parents to explore the possibility of nonpenetrance or gonadal mosaicism. In particular, genetic testing of the parents of patient 1 and her sibling with the history of 20-nail dystrophy would be of interest in regard to the possibility of variable expressivity. It is hoped that further molecular studies to elucidate the structure-function relationship of p63 and identify its downstream targets will clarify the genotype-phenotype correlation of the p63 syndromes to allow for better genetic testing and counseling of affected patients and their families.

ence of cytolysis in the histology of blistered skin from patient 1, as well as the focal disruption of anchoring fibrils on electron microscopy, indicates structural disruption of the hemidesmosome and may be secondary to mechanical rubbing before biopsy. The finding of acantholysis is of some interest because the skin fragility seen in patients with AEC syndrome is reminiscent of the phenotype observed in the ectodermal dysplasia and skin fragility syndrome due to mutations in plakophilin 1, an intercellular component of desmosomes. Additionally, desmosomal diseases such as pemphigus tend to be more severe on the scalp, which has been proposed to be a result of the regional expression of desmosomal proteins. Recently, the desmosomal protein Perp, a homologue of the tight junction protein claudin, was shown to be a p63-regulated gene, with Perp-deficient mice demonstrating significant suprabasilar acantholytic blistering of the skin and mucous membranes. These findings suggest that p63 may be an important upstream regulator of desmosomal cell adhesion, which may contribute to the skin fragility observed in patients with AEC syndrome.

Figure 5. Electron microscopy of skin. A, Normal intracellular hemidesmosomal structure with focal disruption of anchoring fibrils in patient 1 (original magnification x23 800). B, Electron microscopy of tissue from patient 2 demonstrates normal hemidesmosomes and basement membrane zone (original magnification x40 800). Stars indicate hemidesmosomes; arrowheads, anchoring fibrils; and long arrows, basal lamina.
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