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Conference Report: International Research Symposium on Ankyloblepharon-Ectodermal Defects-Cleft Lip and/or Palate (AEC) Syndrome

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Abstract

Ankyloblepharon-Ectodermal Defects-Cleft Lip/Palate (AEC) Syndrome (Hay-Wells syndrome, MIM #106220) is a rare autosomal dominant ectodermal dysplasia syndrome. It is due to mutations in the *p63* gene, known to be a regulatory gene with many downstream gene targets. TP63 is important in the differentiation and proliferation of the epidermis, as well as many other processes including limb and facial development. It is also known that mutations in *p63* lead to skin erosions. These erosions, especially on the scalp, are defining features of AEC syndrome and cause significant morbidity and mortality in these patients. It was this fact that led to the 2003 AEC Skin Erosion Workshop. That conference laid the groundwork for the International Research Symposium for AEC Syndrome held at Texas Children's Hospital in 2006. The conference brought together the largest cohort of individuals with AEC syndrome, along with a multitude of physicians and scientists. The overarching goals were to define the clinical and pathologic findings for improved diagnostic criteria, to obtain tissue samples for further study and to define future research directions. The symposium was successful in accomplishing these aims as detailed in this

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conference report. Following our report, we also present eleven manuscripts within this special section that outline the collective clinical, pathologic and mutational data from eighteen individuals enrolled in the concurrent Baylor College of Medicine IRB-approved protocol: Characterization of AEC syndrome. These collaborative findings will hopefully provide a stepping stone to future translational projects of *p63* and *p63*-related syndromes.

Keywords

ectodermal dysplasia; congenital ectodermal defect; skin; wound healing; p63; TP63; tumor protein p63; bone morphogenetic protein; BMP; fibroblast growth factor; FGF, ectodysplasin A receptor; EDAR; beta-catenin; NOTCH1; p53 apoptosis effector protein; Perp; activated protein kinase C; RACK1; stratifin; SFN; apobec-1-binding protein-1; ABBP1

CONFERENCE REPORT

Ankyloblepharon-Ectodermal Defect-Cleft Lip/Palate (AEC) Syndrome (Hay-Wells syndrome, MIM #106220) belongs to a large, heterogeneous group of ectodermal dysplasias (ED) that affect embryonic development of ectodermal tissues: hair, nails, teeth, sweat glands, and skin [McKusick, 1987]. The number and definition of distinct ED syndromes are ambiguous because of overlapping phenotypes and genotypes, and estimates of their overall incidence vary widely [Brunner et al., 2002].

Recent discoveries have linked AEC and several allelic disorders to mutations in the *p63* gene, a homologue of *p53* [McGrath et al., 2001]. These *p63* mutations give rise to a varied range of phenotypes in patients affected by AEC syndrome. The *p63* gene is an important regulatory gene and appears to control processes related to epidermal proliferation and differentiation [Koster et al., 2004].

The ground work for this conference was laid at the 2003 AEC Skin Erosion Workshop held in St. Louis, MO and sponsored by the National Foundation for Ectodermal Dysplasias (NFED), which provides support for ED families and advocates for clinical and basic science research. Participants at this workshop discussed directions for future research, formulated consensus recommendations for improved skin and wound care [Siegfried et al., 2005] and also recognized the need for more extensive clinical evaluation and tissue sampling to further the understanding of this disorder. Subsequently on November 8–10, 2006, the NFED convened the International Research Symposium on Ankyloblepharon-Ectodermal Defect-Cleft Lip/Palate (AEC) Syndrome in Houston, Texas. Participants included clinical professionals and biomedical researchers from North America and Europe and twenty-three affected individuals from thirteen families. Reaching beyond the scope of the first conference, participants worked together to examine and systematically document the clinical findings of these patients to develop a uniform, coordinated approach to diagnosis. The results of these subspecialty evaluations are detailed in the subsequent manuscripts within this special section [see Julapalli et al, Farrington et al, Cole et al, Sutton et al, Motil et al, Dishop et al, Lane et al, Bree, 2009 this issue]. Blood, hair and nail specimens were also obtained for mutational, microscopic and immunohistochemical evaluation, with these results also presented within this special section [see Rinne et al, Dishop et al, Koster et al, Beaudry et al, 2009 this issue].

This unique research, educational, and patient-oriented symposium began with a day of structured clinical evaluations by multiple subspecialists (appendix II) in the areas of audiology, dentistry [see Farrington et al, 2009 this issue], dermatology [see Julapalli et al, 2009 this issue], genetics [see Sutton et al, 2009 this issue], growth/nutrition/

gastroenterology [see Motil et al, 2009 this issue], otorhinolaryngology [see Cole et al, 2009 this issue], ophthalmology, plastic surgery, and psychiatry [see Lane et al, 2009 this issue] to obtain and document clinical history and physical examination findings prospectively. The patients also donated tissue samples, including blood, hair and skin biopsies [see Rinne et al, Dishop et al, Koster et al, Beaudry et al, 2009 this issue]. On the second day, the patients participated in a Grand Rounds conference. The Baylor Dermatology Residents (appendix III) presented the case studies to the group for discussion, followed by participant lectures on AEC-related topics: medical history, nosology, genetics, and *p63*-associated developmental and molecular biological research. On the third day, the faculty participated in a round table discussion focused on defining better diagnostic markers, current care guidelines, and improved coordination between the clinical and research communities. The overarching goal was to establish a long-term direction for AEC research.

RESEARCH SYMPOSIUM PRESENTATIONS

The following are summations of the lectures and original research of the investigators presented during the research symposium (Appendix 1):

Overview of AEC Syndrome [Siegfried et al., 2005] - Alanna F. Bree, MD

Aklyoblepharon-ectodermal dysplasia-cleft lip and/or palate (AEC) syndrome, also known as “Hay-Wells syndrome”, is a rare, autosomal dominant disorder defined by ectodermal abnormalities of the skin, teeth, hair and nails, in combination with characteristic eyelid fusion and facial clefting. Denuded skin leading to life-threatening sequelae can occur in affected infants. Persistent scalp and skin erosions, complicated by infection, are common features of this syndrome. The clinical findings of AEC syndrome can overlap with those of other ectodermal dysplasia (ED) syndromes and are variable in presentation, which complicates the diagnosis and further characterization of these disorders. While the genetic basis for AEC syndrome has been identified and is due to mutations in *p63*, the utility of molecular testing in the clinical setting is limited. Skin biopsy might prove to be a useful diagnostic tool, but this has been inadequate due to the lack of existing information regarding histopathologic features of AEC syndrome. Previous reports of AEC skin histology with light microscopy have shown focally decreased sweat glands and miniaturized hair follicles. Prior electron microscopy findings include a decrease in basal and suprabasal keratins and disorganized stratum corneum keratin filaments. Until specific testing is more readily available, timely diagnosis relies on recognition of clinical features. The rarity of AEC syndrome also limits opportunities for further study and evaluation.

Skin Erosion and Wound Healing in Ankyloblepharon-Ectodermal Defect-Cleft and/or Palate (AEC) [Siegfried et al., 2005] - Elaine C. Siegfried, MD

The features of AEC syndrome described as a result of 12 patients presented at the 2003 Skin Erosions Workshop included: initial misdiagnosis of epidermolysis bullosa (25%), ankyloblepharon (100%), nasal-lacrimal duct atresia/obstruction (83%), cleft lip (83%), cleft palate (92%), dental abnormalities (100%), developmental delay (82%), death in infancy (17%), scalp/skin erosions (100%), syndactyly (42%), ear deformity (67%), recurrent otitis media (100%), hearing loss (64%), sparse hair (100%), heat intolerance (91%), hypospadias (50%), and neonatal tube feeding (58%). Nail abnormalities were seen in 100%. Affected nails were not characteristically “hyperconvex” as previously described, but were more often thin, soft, distorted or absent. Proximal nail folds were also characteristically absent, while pterygia and longitudinal hyper-ridging were common. The most dramatic ophthalmologic abnormality was blepharitis, which was disproportionately greater than conjunctivitis. Palpebral sweat glands were apparently absent and lacrimal apparatus

extrusia were common. There was misalignment, thinning and loss of eyelashes and eyebrows.

The scalp erosions were identified as a major source of morbidity for affected infants. Palms, soles and ear canals are similarly affected. Wound care recommendations included avoidance of aggressive measures, including wound debridement and grafting. Alternatively, gentle cleansing and bland emollients were recommended with careful monitoring and treatment of secondary infections. Tetracylcines, especially doxycyline, were recommended choices for their dual antimicrobial and matrix metalloproteninase activity.

Allelic variants of p63 syndromes and patient presentations [Rinne et al., 2007] - Virginia Sybert, MD

The allelic disorders caused by p63 mutations, including AEC syndrome, EEC (ectrodactyly-ectodermal dysplasia-clefting) syndrome, ADULT (acro-dermato-ungual-lacrimal-tooth) syndrome, Rapp–Hodgkin syndrome, limb-mammary syndrome and split hand/foot malformation, have phenotypic overlap but distinguishing clinical features can be helpful for diagnosis.

Getting Under The Skin of *p63* [Rinne et al., 2006] - Hans vanBokhoven, PhD

Ectodermal dysplasias are a group of inherited developmental disorders characterized by ectodermal malformations and hypoplasias, which can be accompanied with craniofacial and limb abnormalities. Seven different clinical entities of this group have been linked to mutations in the p63 gene, of which four present with skin problems combined with other defects in ectodermally derived structures such as teeth, nail, hair and lacrimal ducts: EEC (ectrodactyly-ectodermal dysplasia-clefting syndrome; MIM 129900), AEC (ankyloblepharon-ectodermal dysplasia-clefting; Hay Wells; MIM 106260) syndrome, ADULT (acro-dermato-ungual-lacrimal-tooth) syndrome (MIM 103285) and, most recently, Rapp–Hodgkin syndrome (MIM 129400). Skin and hair defects are most pronounced for AEC syndrome. Causative *p63* mutations in these syndromes have been identified in well over 100 different families. These results have been presented as a clinical study based on 227 patients carrying a *p63* mutation. This analysis has established strong genotype-phenotype associations. The differential pattern of mutations is most prominent for EEC syndrome and AEC syndrome, which are mostly caused by missense mutations in the DNA-binding domain and SAM domain of the gene, respectively. Most other mutations identified to date are also syndrome-specific, such as the R298Q/G mutations responsible for ADULT syndrome. These patients illustrate the minor differences between AEC and Rapp-Hodgkin syndrome, which suggests that these syndromes are variants of a single clinical entity.

The domain-specificity of *p63* mutations indicates that there are different molecular mechanisms behind the various *p63*-associated syndromes. Our knowledge about these mechanisms is only superficial. The aim of current research is to increase insights into the role of p63 in normal and abnormal epidermal development and the molecular mechanisms of the various pathogenic *p63* mutations. To this end, primary keratinocyte lines have been established from skin biopsies of patients with a known *p63* mutation, such as EEC Y163C, R279H, R304W; AEC T537P, and a stop-mutation in the very beginning of the ΔNp63 isoform (Q11X) from a Rapp-Hodgkin syndrome patient. These cells are used in cultures and in vitro skin reconstitution experiments to identify abnormalities in molecular processes involving *p63* and their effects on cell growth and skin development.

AEC syndrome, Rapp-Hodgkin Syndrome and the Tail of *p63* [Sahin et al., 2004] - Dr. Suzanne Clements, MRCP (UK)

The molecular basis of AEC syndrome has been shown mainly to involve heterozygous missense mutations in the sterile-alpha-motif (SAM) domain within the tail region of *p63*. Atypical mutations include an intronic splice site mutation and a single amino acid insertion, but these also occur within the SAM domain. This region of *p63* is thought to be important in protein-protein interactions, for example in binding *p63* to the RNA splicing protein, apopbec-1 binding protein (ABBP1). Adjacent to the SAM domain is a transactivation inhibition domain (TID), which may have a repressive role in balancing the effects of different isoforms of *p63*. A small number of mutations within the SAM domain have also been reported in Rapp–Hodgkin syndrome. Mutations in the SAM or TID domains have also been reported in EEC syndrome, limb-mammary syndrome and split-hand split-foot malformation syndrome. Of note, several of the mutations described in AEC and Rapp-Hodgkin syndrome are very similar and sometimes identical, thus highlighting the considerable clinical and molecular overlap between these two ectodermal dysplasia syndromes.

A key target of future research will be to identify abnormal signaling pathways in *p63*-associated ED syndromes by assessing both gene and protein expression in affected patients' skin. Assessing the downstream consequences of *p63* mutations on known *p63*-responsive pathways (such as Notch, BMP, FGF, EDAR and beta-catenin), will give new insight into how *p63* mutations disrupt normal ectodermal development. Keratinocytes will be isolated from the skin biopsy samples and grown using standard cell culture methods. This will provide further RNA for molecular analysis, as well as material for in vitro cell biological studies of cell migration, adhesion, differentiation, proliferation, and other processes. The keratinocytes will also be used to establish organotypic skin cultures in which wound healing and other physiological parameters can be assessed.

p63 and Molecular Mechanisms of Ectodermal Dysplasias [Yang et al., 2006] - Frank McKeon, PhD

Murine embryos lacking the p63 gene display striking defects in all stratified epithelia, including breast, prostate, urothelia, and epidermis. These epithelial abnormalities have been attributed to the key roles of isoforms TAp63 and ΔNp63 in epithelial lineage commitment and differentiation, respectively. One hypothesis holds that p63 is necessary for the regenerative proliferation of epithelial progenitor cells.

ΔNp63 Knockdown Mice: A Mouse Model for AEC Syndrome [Koster et al., 2004; Koster et al., 2005; Koster et al., 2007] - Dennis R. Roop, PhD and Maranke I. Koster PhD

AEC syndrome is characterized by skin fragility and is caused by dominant mutations in *p63*, a transcription factor expressed as six isoforms. It has been proposed that *p63* is essential for the maintenance of epidermal stem cells. In addition, *p63* is essential for morphogenesis and maintenance of the epidermis by regulating proliferation and differentiation of epidermal keratinocytes. More specifically, TAp63Δ functions during early stages of epidermal morphogenesis, while ΔNp63Δ functions during late stages of epidermal morphogenesis and in postnatal skin. Since AEC patients display postnatal skin fragility, mutant ΔNp63Δ proteins are most likely responsible for skin erosions in AEC patients. To determine the role of ΔNp63Δ in the epidermis, a mouse model was generated that allows the selective downregulation of ΔNp63 in the epidermis. Interestingly, downregulating ΔNp63 caused severe epidermal defects, including aberrant keratinocyte differentiation and impaired basement membrane formation, culminating in the development of skin erosions. This skin phenotype is indistinguishable from that of patients with AEC, in which expression of mutant ΔNp63Δ proteins in essence creates a functional knockdown of

ΔNp63Δ. This suggested that ΔNp63Δ induces genes required for normal differentiation of keratinocytes, as well as genes that are critical for establishing and/or maintaining the basement membrane. Microarray analyses identified Fras1 and IΔB kinase-alpha (IKKΔ) as putative ΔNp63Δ target genes. Fras1 is a keratinocyte-produced extracellular matrix protein, which is required for maintaining the integrity of the basement membrane; whereas IKKΔ is required for terminal differentiation of keratinocytes. It has been demonstrated that ΔNp63Δ directly induces transcription of Fras1 and IKKΔ, further suggesting that skin fragility in AEC patients is caused, in part, by a failure to properly express these ΔNp63Δ target genes. Taken together, the inability of keratinocytes that express mutant ΔNp63Δ to produce a stable basement membrane and to commit to terminal differentiation may contribute to skin fragility in AEC patients by failing to provide structural stability to the epidermis.

Toward an Understanding of the Molecular Basis of AEC Syndrome [Nguyen et al., 2006] - Caterina Missero, PhD

Complete abrogation of the *p63* gene function in mouse models revealed an absolute requirement for *p63* for the proper development of ectodermally-derived tissues. In spite of these findings, *p63* molecular function in skin development remains largely obscure. In freshly isolated epidermal cells, *p63* is responsible for maintaining cell adhesion, for favoring the expression of early differentiation markers, while inhibiting the expression of late differentiation markers. We found that *p63*'s effect on epidermal differentiation occurs, at least in part, through selective modulation of *Notch1*-dependent transcription and function (Nguyen et al., Genes & Devel.2006). Importantly, *p63* is also crucial to determine the identity of epidermal cells. These data indicate that: a) mutations in the SAM domain affect only selective functions of the *p63* gene; b) *p63* is involved in the rather unique role of maintaining epidermal cells identity. Thus, looking at the global regulation of gene expression upon induction and downregulation by wild-type and mutant *p63*, we aim at deciphering the molecular function of this essential gene in normal and in AEC skin.

Perp Is A *p63*-Regulated Gene Essential For Ectodermal Derivative Integrity [Ihrie et al., 2005] - Laura D. Attardi, PhD

Perp (p53 apoptosis Effector Related to PMP-22) is a tetraspan membrane protein originally isolated because of its involvement in *p53*-dependent apoptosis. *Perp* also plays a novel and essential role in cell-cell adhesion in stratified epithelia. *Perp*-deficient mice exhibit postnatal lethality at a frequency of greater than 95% within the first week after birth. *In situ* hybridization and immunohistochemical analysis revealed that *Perp* is strongly expressed in a variety of stratified epithelia during development, including the tongue, palate, and skin, in both mice and humans. *Perp* null newborn mice exhibit blistering in the oral epithelia and skin reminiscent of that seen in certain human diseases associated with defects in desmosomal cell-cell adhesion complexes, such as pemphigus vulgaris and ectodermal dysplasia/skin fragility syndrome, suggesting an important function for *Perp* in cell-cell adhesion. *Perp* localizes to desmosomes in the skin and oral mucosa, and desmosome organization and structure is severely compromised in epithelia from *Perp* knockout mice. Together, these findings illustrate the critical role that *Perp* plays in desmosomal adhesion in stratified epithelia.

Perp expression in stratified epithelia has additionally been shown to be dependent on the transcription factor *p63*, which is known to be required for the establishment and maintenance of skin, oral mucosa, and other stratified epithelia. The importance of *p63* is underscored by the consequences of mutation or loss of this protein. As *Perp* is a *p63* target gene and a critical desmosomal protein, it is hypothesized that loss of *Perp* in adult tissues might affect the function of various ectoderm-derived tissues. Consistent with this notion, the small cohort of *Perp-/-* mice that survived to adulthood displayed a substantially reduced

lifespan and exhibited several abnormalities in ectoderm-derived tissues. Symptoms include keratoderma on the soles of the feet, abnormally thickened skin, malformed nails, greasy and disorganized hair, and a high predisposition to severe bacterial infection.

Tools And Methods To Identify And Characterize Novel Proteins Using The Yeast, Saccharomyces Cerevisiae [Ranish et al., 2004] - Vivian A. Lombillo, MD, PhD

There is an armamentarium of yeast tools that could potentially be useful to investigate genes and proteins implicated in the ectodermal dysplasias. The yeast, *Saccharomyces cerevisiae*, is a useful model system to study human skin diseases. *S. cerevisiae* is a single cell eukaryote with approximately 6,000 genes. Over 50% of these yeast genes have a similar counterpart in humans, in particular those involved in cell division and DNA repair. There are numerous genetic, biochemical and molecular tools that yeast biologists utilize to probe mechanisms of human disease.

p63 Degradation Pathway And Ectodermal Dysplasia and P63 Protein Interactions in AEC Syndrome [Huang et al., 2005; Fomenkov et al., 2003] - Edward Ratovitski, PhD and Alexy Fromenkov, PhD

Histological staining of skin samples from patients with Hay-Wells and Rapp-Hodgkins syndromes demonstrate aberrant *p63* localization associated with the suprabasal layers of epidermis. This process may be associated with the deregulation of the *p63* degradation pathway. That in turn may contribute to increased proliferative, anti-apoptotic potential and prevent normal differentiation programming in these cells. It has been shown that *p63* function is regulated by a specific proteasome-dependent degradation mechanism through association with a receptor for activated protein kinase C (*RACK1*) and stratifin (*SFN*).

Environmental stimuli (e.g. ultraviolet, chemical agents, etc.) that induce DNA damage or differentiated factors like Ca++ activate a cascade of specific protein kinases that can enhance p63 protein modifications leading to selective degradation of *p63*. Indeed drug or radiation induced genotoxic stress in squamous cell carcinomas lead to protein stabilization of well known tumor suppressor *p53*, but activated degradation of ΔNp63alpha. Moreover homozygous mice with mutation in the *SFN* gene produce a strikingly similar phenotype to *p63* null mice with many ectodermal dysplasia features. Therefore, identification of *p63*-specific protein kinases and *p63* modification sites that mark this protein to degradation could lead to improvement in current therapeutic approaches.

Several proteins have been identified that interact with the p63 carboxyl terminus and its sterile alpha-motif, including the apobec-1-binding protein-1 (*ABBP1*). AEC-associated *p63* mutations completely abolished the physical interaction between ABBP1 and p63. Physical association of *p63* and *ABBP1* led to a specific shift of fibroblast growth factor receptor (FGFR) 2 alternative splicing from the BEK-isoform (mesenchymal differentiation) toward the K-SAM isoform essential for epithelial differentiation. Thus, a p63-ABBP1 complex differentially regulates FGFR2 expression by supporting alternative splicing of the K-SAM isoform of FGFR2. It has therefore been hypothesized that the inability of mutated *p63* to support this splicing likely leads to the inhibition of epithelial differentiation and, in turn, accounts for the AEC phenotype. Similarly, the AEC-derived mutated ^{p63}. failed to interact with other members of RNA splicing machinery (e.g. SCAF4) supporting the critical role for *p63* in RNA processing. The search will continue for RNA inhibitors that prevent RNA splicing of FGFR2-BEK in favor of FGFR2-K-SAM.

COLLABORATIVE ROUNDTABLE DISCUSSION

The following is a brief summation of the roundtable discussion (Appendix IV):

The Friday afternoon roundtable discussion was an opportunity for the clinical and basic science perspectives to come together for a common goal: improving the lives of individuals affected by AEC syndrome.

Clinical information from patients collectively known to the group, especially during their neonatal course, was shared. Eye findings, clefting and scalp erosions were defining features seen with variable expression among patients. It was posited that the ankyloblepharon filiforme adnatum may occur due to a failure of apoptosis that typically occurs in the cells adhering the eyelids. The skin erosions are somewhat mystifying and one of the most challenging aspects of the disease. They are typically present in the neonatal period, but can also develop during the first year of life. Additionally, hair and nails changes, pigmentary anomolies, skin fragility, and atrophic or cribriform scarring were also seen in the patients.

Some affected individuals were also reported to have neutropenia of undetermined etiology and many have recurrent infections involving the ears and compromised skin. Other affected infants have developed bacteremia and succumbed to sepsis. This may be related to an impaired skin barrier, but may also have other causes. Immunologic work-up of a few patients have revealed no definable abnormalities. One child has been diagnosed with autoimmune lymphoproliferative syndrome (ALPS). More study is needed in this area.

Many of the children have nutritional issues. A majority have suffered failure to thrive and have been prescribed nutritional supplementation during infancy and childhood, which is seemingly out of proportion to other children who have isolated cleft lip and/or palate. The reason for this is not clear. Oral and esophageal changes, as seen in animal models, and potential gastrointestinal involvement may have a relationship with altered digestion and absorption. None of the affected children have been evaluated with endoscopy, and we do not know if similar mucosal changes occur in humans. Further study regarding the esophageal and gastrointestinal changes in animal models is needed to explore this issue. Mucosal biopsies from affected patients when undergoing other surgeries, especially cleft repairs, might be useful. Many patients have required G-tubes, and this might be anticipated and given early consideration for those patients who are failing to thrive.

Skin biopsies, hair and blood samples were donated by many affected participants through a Baylor College of Medicine IRB-approved protocol. These specimens were evaluated for histopathologic changes [see Dishop et al, 2009 this issue] and the DNA sequenced to determine the location of mutations [see Rinne et al, 2009 this issue]. The tissues were also evaluated for *p63* and other associated genes through immunohistochemistry and in-situ hybridization [see Koster et al and Beaudry et al, 2009 this issue]. This information was entered, along with the clinical findings, into a dataset which was analyzed to make correlations (Appendix V). While interesting associations were found, it was difficult to make specific genotype phenotype correlations due to the limited sample size, as well as undefined epigenetic factors [see Bree, 2009 this issue].

The tissues have been archived and will be used for further studies, while also providing a resource for researchers interested in studying *p63* and/or *p63*-related syndromes. Protocols have been developed for requesting these tissues for scientific study, in addition to protocols for enrolling additional subjects and obtaining future tissues. This information will be available on the NFED website (www.nfed.org), as well as disseminated at national meetings.

Discussion of future research directions included: culturing of keratinocytes, although technically difficult due to their growth disadvantage, for evaluation of *p63*; microarray analysis to assess other associated genes affected by *p63* mutations; and the possible development of a line of holoclones that could be used for future translational study.

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APPENDIX I

Research Presentation Participants

(professional appointments listed at time of conference)

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NFED Director of Research

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Appendix II

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(professional appointments listed at time of conference)

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APPENDIX IV

Roundtable Discussion Participants

Alanna F. Bree, MD

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APPENDIX V

Database entry and analysis

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APPENDIX VI

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Astra Tech

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Sigma-Tau

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