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Overlap between EEC and AEC syndrome and immunodeficiency in a preterm infant with a TP63 variant

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Pathogenic variants in the transcription factor *TP63* gene cause a variety of clinical phenotypes, such as ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome and ankyloblepharon-ectodermal dysplasia-clefting (AEC) syndrome. Historically, *TP63*-related phenotypes have been divided into several syndromes based on both the clinical presentation and location of the pathogenic variant on the *TP63* gene. This division is complicated by significant overlap between syndromes.

Here we describe a patient with clinical characteristics of different *TP63*-associated syndromes (cleft lip and palate, split feet, ectropion, erosions of the skin and corneas), associated with a *de novo* heterozygous pathogenic variant c.1681 T>C, p.(Cys561Arg) in exon 13 of the *TP63* gene. Our patient also developed enlargement of the left-sided cardiac compartments and secondary mitral insufficiency, which is a novel finding, and immune deficiency, which has only rarely been reported. The clinical course was further complicated by prematurity and very low birth weight. We illustrate the overlapping features of EEC and AEC syndrome and multidisciplinary care needed to address the various clinical challenges.

1. Introduction

The *TP63* gene regulates the development of limbs, the orofacial region and structures that develop from the embryonal ectoderm such as skin, hair, teeth, nails and exocrine glands including sweat and sebaceous glands (Rinne et al., 2007). Pathogenic variants in the *TP63* gene can cause several phenotypically distinct syndromes. These include ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome, Rapp-Hodgkin syndrome, limb-mammary syndrome, ankyloblepharon-ectodermal dysplasia-clefting (AEC) syndrome and acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome (Brunner et al., 2002). Some *TP63* variants can cause non-syndromic malformations such as split hand-foot malformation and cleft lip. The distinction between syndromes can be difficult, and phenotypes may overlap especially between EEC and AEC syndromes and AEC and Rapp-Hodgkin syndrome (Chiu et al., 2011; Sutton et al., 2009).

Here we describe a very preterm infant with characteristics of both EEC and AEC syndromes, and additionally enlargement of the left cardiac compartments and immune deficiency.

2. Patient data

Our patient is a firstborn female infant of non-consanguineous Caucasian parents. Antenatal ultrasound screenings at 17, 28 and 31 gestational weeks, respectively, were unremarkable, non-invasive prenatal testing was negative for trisomies 13, 18 and 21. She was delivered by cesarean section at 31 + 6 weeks' gestational age due to maternal pre-eclampsia (birth weight 1450 g, −1.4 standard deviations (SD); birth length 41 cm, −0.5 SD; head circumference 28.7 cm, −0.5 SD). The mother received two doses of antenatal betamethasone for fetal lung maturation and magnesium sulphate for fetal neuroprotection prior to delivery. The infant had bilateral cleft lip and palate, split feet and abnormal skin with large, erythematous, scaly patches (Fig. 1a–b). She was started on nasal mask CPAP, started on intravenous nutrition via an

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umbilical venous catheter and cared for in an incubator to prevent water and heat loss.

Despite high incubator humidity (80%) and fluid intake of 220–250 ml/kg/day she developed dehydration and hypernatremia. Emollient creams and daily saline baths were administered. The skin lesions gradually worsened, and clinically showed absent or delayed epidermal development. The perianal area was persistently eroded, and several topical treatments were used with limited effect (Fig. 1c). Later potassium permanganate baths/soaks and medical honey were used to prevent infections. European Reference Network, ERN-Skin was consulted to guide the continued care of the infant. Erosive skin areas remain at 2 years of age especially in the head and neck region.

The patient showed partial ectropion and absent lacrimal puncta. Regular eye lubricants and courses of topical chloramphenicol or dexpanthenol-retinol were used. At ten weeks of age worsening corneal erosions developed and sodium hyaluronate-chondroitin sulphate,

Fig. 1. a-e. Photographs illustrating clinical findings in a very premature infant with a rare *TP63* variant. a) Cleft lip and palate, widespread ectodermal dysplasia and split feet; b) split feet; c) severe skin erosion in the perianal area; d) corneal scarring in the right eye; e) descemetocele in the left eye.

paternally derived topical serum drops and soft corneal lenses were applied, with limited clinical effect. Partial tarsorraphy was first carried out at 5 months of age to avoid further corneal erosions. Topical cyclosporin, levofloxacin and dexamethasone were added to the care regimen. At 17 months of age, corneal erosion and scarring continued to enlarge, and a left-sided descemetocele developed (Fig. 1d–e), which was treated with amniotic membrane patching. At 2 years of age descemetocele was noted also in the right eye and treated similarly.

Neonatal screening showed absent T cell excision circles (TREC), and a marked T-cell lymphopenia was found. B lymphocytes and NK cells were normal. Intravenous immunoglobulin infusions and trimethoprim/sulfamethoxazole prophylaxis were administered to avoid *Pneumocystis jirovecii* infection. Vaccines containing live attenuated viruses were withheld. The patient developed four septic staphylococcal infections during the first four months of age.

During *Staphylococcus aureus* sepsis at three months of age, echocardiography excluded endocarditis but revealed left-sided atrial and ventricular enlargement of unknown origin and secondary mitral insufficiency. This finding was not present at birth. The cardiac findings have persisted, but overall cardiac function has remained stable.

3. Genetic testing

A clinical geneticist was consulted, and based on the clinical findings, sequencing of the *TP63* gene was carried out. Sequence analysis identified a *de novo* heterozygous missense variant c.1681 T>C, p.(Cys561Arg) in exon 13 (NM_003722.4). This variant is absent in the reference population database gnomAD and in the clinical variation database ClinVar, but it is described once in the medical literature in a patient who had features of both EEC and AEC phenotypes (Chiu et al., 2011). It causes the change of a highly conserved amino acid, and all utilized in silico tools (Mutation Taster, Align GVGD, Sift, Polyphen 2, CADD) predicted the alteration to be deleterious. According to the ACMG, the variant was classified as likely pathogenic (PS2, PM1, PM2, PP3), and was submitted to the LOVD database (variant #0000908392). The gene locus encodes the sterile alpha motif (SAM) domain which is involved in protein binding.

4. Discussion

The presented patient is a very preterm infant with findings suggestive of EEC/AEC syndrome. None of the severe malformations were noticed despite repeated prenatal ultrasound screenings. She was diagnosed with cardiac abnormalities and immune deficiency, which have only rarely been reported in association with *TP63* variants.

Previously, most of the reported pathogenic variants of *TP63* associated with EEC syndrome have been in the exons encoding the DNA-binding domain of the protein, with only few reports of variants in other domains (Rinne et al., 2007). Our patient had a variant in exon 13 encoding the SAM domain, which is usually clinically associated with AEC syndrome. Celik and colleagues reported a patient with a variant in the DNA-binding domain of *TP63*, but clinical characteristics overlapping both EEC and AEC syndromes (Celik et al., 2011). Chiu and colleagues reported a patient with features of both AEC and EEC syndromes (ankyloblepharon and split hands and feet), with the same variant as our patient (Chiu et al., 2011, Supplementary Table 1). Ankyloblepharon, the hallmark of AEC syndrome, is present only in less than half of patients diagnosed with AEC syndrome. In addition, Brueggeman and Bartsch reported a case of a mother and child with the same variants but different phenotypes (Brueggemann and Bartsch, 2016), which demonstrates that the genotype-phenotype correlations of the *TP63* variants are not particularly strong. Some authors have suggested that some of the *TP63*-associated syndromes should be combined rather than considered as separate (Otsuki et al., 2020; Prontera et al., 2011).

The dermatological features of our patient were quite severe, and resembled the ectodermal dysplasia usually associated with AEC syndrome (Maillard et al., 2019). Two previously reported cases of EEC phenotype with a variant in sequences encoding the SAM domain of the *TP63* did not reportedly have severe ectodermal dysplasia, in contrast to AEC patients whose variants usually affect the SAM domain (Celli et al., 1999; Rinne et al., 2006).

The skin condition of our patient caused her eyelids to remain protracted from birth, which caused severe challenges in maintaining corneal patency. Lacrimal duct dysfunction has been reported in association with *TP63* variants, as was the case in our patient, and limbal stem cell deficiency (di Iorio et al., 2012). Despite very frequent treatment, she developed severe corneal erosions and needed serial invasive surgical procedures.

An interesting feature of our patient was the $CD8^+$ T-cell lymphopenia diagnosed during the first weeks of life after she failed her TREC screening test. Wenger and colleagues described a set of monozygotic twins born prematurely, who similarly both failed TREC screening and were subsequently diagnosed with T-cell lymphopenia (Wenger et al., 2018). The detected *TP63* variant in these patients was however in a different domain compared to our patient. Another *TP63* variant in a patient with congenital T-cell lymphopenia and EEC syndrome was reported by Giampietro and colleagues (Giampietro et al., 2013). Interestingly, they proposed that the observed T-cell lymphopenia could be a result of delayed or inhibited maturation of thymic epithelial cells induced by the *TP63* variant.

Our patient developed enlargement of the left cardiac compartments with secondary mitral insufficiency. The etiology of this finding remains unknown, and at the time of diagnosis it was hypothesized that this could have been secondary to sepsis. This is however unlikely, as the cardiac findings persisted at 16 months of age. Some authors have described cardiac anomalies in association with *TP63* variants. Valenzise and colleagues described a patient with ADULT phenotype, p. Arg298Gln variant and associated arrhythmogenic right ventricular cardiomyopathy (Valenzise et al., 2008). Sharma and colleagues described a patient with EEC phenotype, p.Arg280Cys variant and tetralogy of Fallot (Sharma et al., 2015). A potential molecular link between cardiac development and *TP63* variants has been established in murine models (Paris et al., 2012; Rouleau et al., 2011). These prior observations and the findings of our patient suggest there might be a causal link between *TP63* variants and abnormal cardiac development.

Our patient demonstrates an unusual genotype-phenotype association, with a variant in the SAM domain of the *TP63* gene normally associated with AEC syndrome but displaying clinical features suggestive of EEC syndrome. Our findings and those reported by others highlight the possibility of genotype-phenotype overlapping. Despite the difficulty of distinguishing *TP63*-associated syndromes, the focus of care needs to be on alleviating the burden of disease manifestations. In our patient, skin care, hydration and ophthalmological care have been the most important factors of the clinical course, which was furthermore complicated by T-cell lymphopenia, frequent septic infections, left-sided atrial and ventricular enlargement with mitral insufficiency, prematurity and associated additional respiratory, nutritional and fluid balance issues. The care of such a diversity of clinical challenges requires a multidisciplinary approach.

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Disclosure of conflict of interest

The authors report no conflicts of interest relevant to this report.

CRediT authorship contribution statement

Kjell Helenius: Conceptualization, of the clinical report, Investigation, and interpretation of current evidence, Writing – original draft, corresponding author. **Liisa Ojala:** Conceptualization, of the clinical report, Investigation, and interpretation of current evidence, clinical data acquisition (ophthalmology), Writing – review & editing, manuscript reviewing and editing. **Leena Kainulainen:** Conceptualization, of the clinical report, Investigation, and interpretation of current evidence, clinical data acquisition (immunology), Writing – review & editing, manuscript reviewing and editing. **Sirkku Peltonen:** Conceptualization, of the clinical report, Investigation, and interpretation of current evidence, clinical data acquisition (dermatology), Writing – review & editing, manuscript reviewing and editing. **Marja Hietala:** Conceptualization, of the clinical report, Investigation, and interpretation of current evidence; clinical data acquisition (clinical genetics), Writing – review & editing, manuscript reviewing and editing. **Pia Pohjola:** Genetical, Formal analysis, and data synthetization, Investigation, and interpretation of current evidence, Writing – review & editing, manuscript reviewing and editing. **Vilhelmiina Parikka:** Conceptualization, of the clinical report, Investigation, and interpretation of current evidence, Writing – review & editing, manuscript reviewing and editing, Supervision, of the study group.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2023.104735.

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