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CASE REPORT

ULTRA-EARLY DIFFUSE LUNG DISEASE IN AN INFANT WITH PATHOGENIC VARIANT IN TELOMERASE REVERSE TRANSCRIPTASE (*TERT*) GENE

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ABSTRACT

The pathogenic variants in the telomerase reverse transcriptase (*TERT*) gene have been identified in adults with idiopathic pulmonary fibrosis, while their connection to childhood diffuse lung disease has not yet been described. Within this study, we present a case of a five-month-old, previously healthy infant, with early-onset respiratory failure. The clinical suspicion of diffuse lung disease triggered by cytomegalovirus (CMV) pneumonitis was based on clinical and radiological presentation. Multiorgan involvement was not confirmed. Considering the possible connection between CMV pneumonitis and early-onset respiratory failure, clinical exome sequencing was performed and a novel variant, classified as likely pathogenic in the *TERT* gene (c.280A>T, p.Lys94Ter) was detected. After segregation analysis yielded negative results, the *de novo* status of the variant was confirmed. Respiratory support, antiviral and anti-inflammatory therapy offered modest benefits, nevertheless, eighteen months after the initial presentation of disease, an unfavourable outcome occurred. In conclusion, severe viral pneumonia has the potential to induce extremely rare early-onset diffuse lung disease accompanied by chronic respiratory insufficiency. This is linked to pathogenic variants in the *TERT* gene. Our comprehensive presentation of the patient contributes to valuable insights into the intricate interplay of genetic factors, clinical presentations, and therapeutic outcomes in cases of early-onset respiratory failure.

Key words: cytomegalovirus (CMV), diffuse lung disease, early-onset respiratory failure, the *TERT* gene variants.

INTRODUCTION

Diffuse lung disease in children is characterized by a heterogeneous clinical presentation and radiological and prognostic features [1-3]. In adults, idiopathic pulmonary fibrosis (IPF) can be a consequence of telomere-related gene mutations, including telomerase reverse transcriptase (TERT) gene mutations which are associated with short telomere syndromes [4]. Telomeres are nucleoprotein structures with DNA repetitive sequences that protect chromosome ends and maintain chromosome stability, limit progressive shortening during cell replication, and prevent recombination at chromosome ends [5]. Telomere shortening leads to genomic instability, inducing DNA damage responses such as apoptosis and cell senescence [5].

IPF associated with pathogenic variants in the *TERT* gene is typically an age-dependent disease with clinical expression by the age of 50 years or later. The anticipation phenomenon refers to the earlier presentation of symptoms in younger generations [4].

In addition to previously described germline mutations in the *TERT* gene in children with malignant diseases, no association with childhood-onset pulmonary disease has been observed [6]. Here we present the case of a child with early onset respiratory failure associated with a pathogenic variant in the *TERT* gene.

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CASE PRESENTATION

The data of the patient were collected at the Department of Pulmonology at the Mother and Child Health Care Institute of Serbia "Dr. Vukan Cupic". This is a tertiary-level institution, recognized as the reference centre for rare diseases. This study was approved by the Ethics Committee of the Mother and Child Health Care Institute of Serbia "Dr. Vukan Cupic" in Belgrade, Serbia (Decision 8/106). Written informed consent was obtained for publication.

A full-term male infant was born to healthy non-consanguineous parents. His birth weight was 4020 g, and his APGAR score was 9. The patient met early developmental milestones. At the age of five months, the child was admitted to a regional hospital with fever, cough, tachypnoea, cyanosis, and increased breathing work. A chest radiography revealed diffusely decreased lung transparency with diffuse alveolar opacification. Therefore, a course of parenteral antibiotics and systemic corticosteroids as well as inhaled bronchodilators was administered. The respiratory viral PCR panel of the nasopharyngeal swab tested negative. A few days later, the patient was intubated due to clinical deterioration and transferred to our hospital. Upon admission, bilateral late inspiratory crackles were observed. A chest CT showed bilateral consolidation of the lung parenchyma with coarse intralobular thickening, minor ground-glass areas, and volume loss (Fig. 1A).

The genetic results for primary immunodeficiency, cystic fibrosis, and metabolic disorders were negative. Flexible bronchoscopy revealed bronchomalacia. Bronchoalveolar fluid (BAL) analysis revealed significant lymphocytosis (12%) and neutrophilia (20%), while PCR was positive for cytomegalovirus (CMV). Therefore, parenteral ganciclovir was initiated. Immunophenotypic analysis of BAL showed <1% CD1+ cells with a normal CD4/CD8 ratio. The complete blood count and liver function test results were normal. The immunophenotype of lymphocytes in the peripheral blood showed a slightly decreased CD4 count and a CD4:CD8 ratio of 1.2.

One week later, the child developed life-threatening cardiac dysrhythmias requiring a pacemaker implantation. Echocardiographic findings were normal without pulmonary hypertension. A combination of respiratory insufficiency and cardiac arrhythmias arose clinical suspicion for central congenital hypoventilation syndrome. Genetic analysis for the *PHOX2B* gene mutations was negative.

Considering the possible connection between CMV pneumonitis and early-onset respiratory failure, clinical exome sequencing (CES) was performed using the TruSight One (TSO) panel (Illumina, San Diego, CA, USA). This panel includes all known disease-associated genes described in the OMIM database as of 2013 and is designed to cover all exons and flanking intronic regions of 4,813 genes (~62,000 exons). All genes in the TSO panel where pathogenic, likely pathogenic, or variants of uncertain significance (VUS) were detected were further analysed. Variant Interpreter (Illumina) software was used for systematic interpretation of detected variants, and the variants were classified according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) [7]. A novel heterozygous nonsense variant, c.280A>T, p.Lys94Ter (p.K94*), was detected in the *TERT* gene (NM_198253.3). This variant introduces a premature STOP codon in the second of the 16 exons in the gene (Fig. 2A), leading to protein truncation and degradation via nonsense-mediated mRNA decay. The variant was classified as "likely pathogenic" according to the ACMG classification recommendations based on the

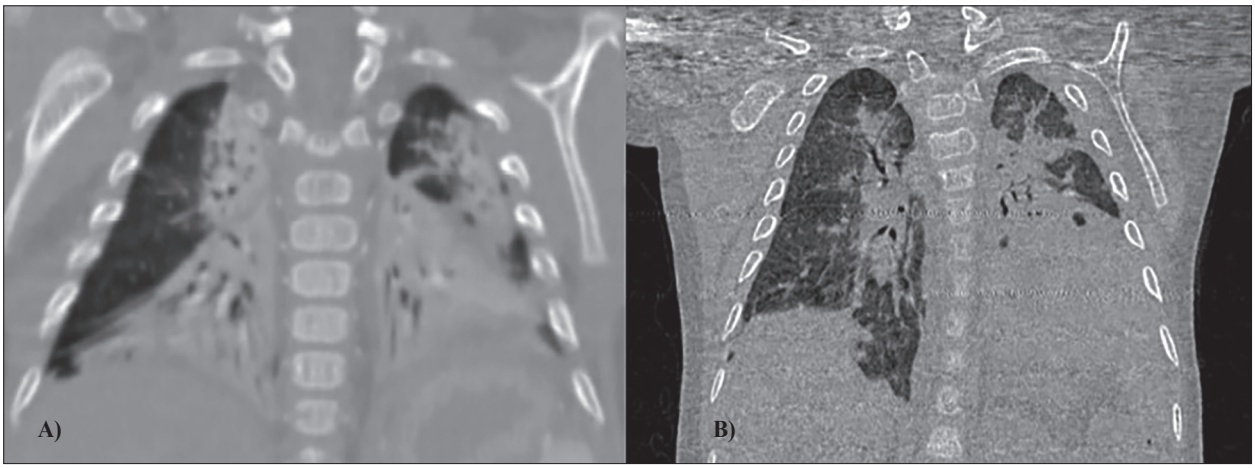

Figure 1. Chest CT scan. A) Initial finding – consolidations, intralobular thickening and mirror ground-glass opacifications. B) A follow up CT scan six months later – bilateral consolidations with fibroindurative lesion or plate-like atelectasis, and mild septal thickening.

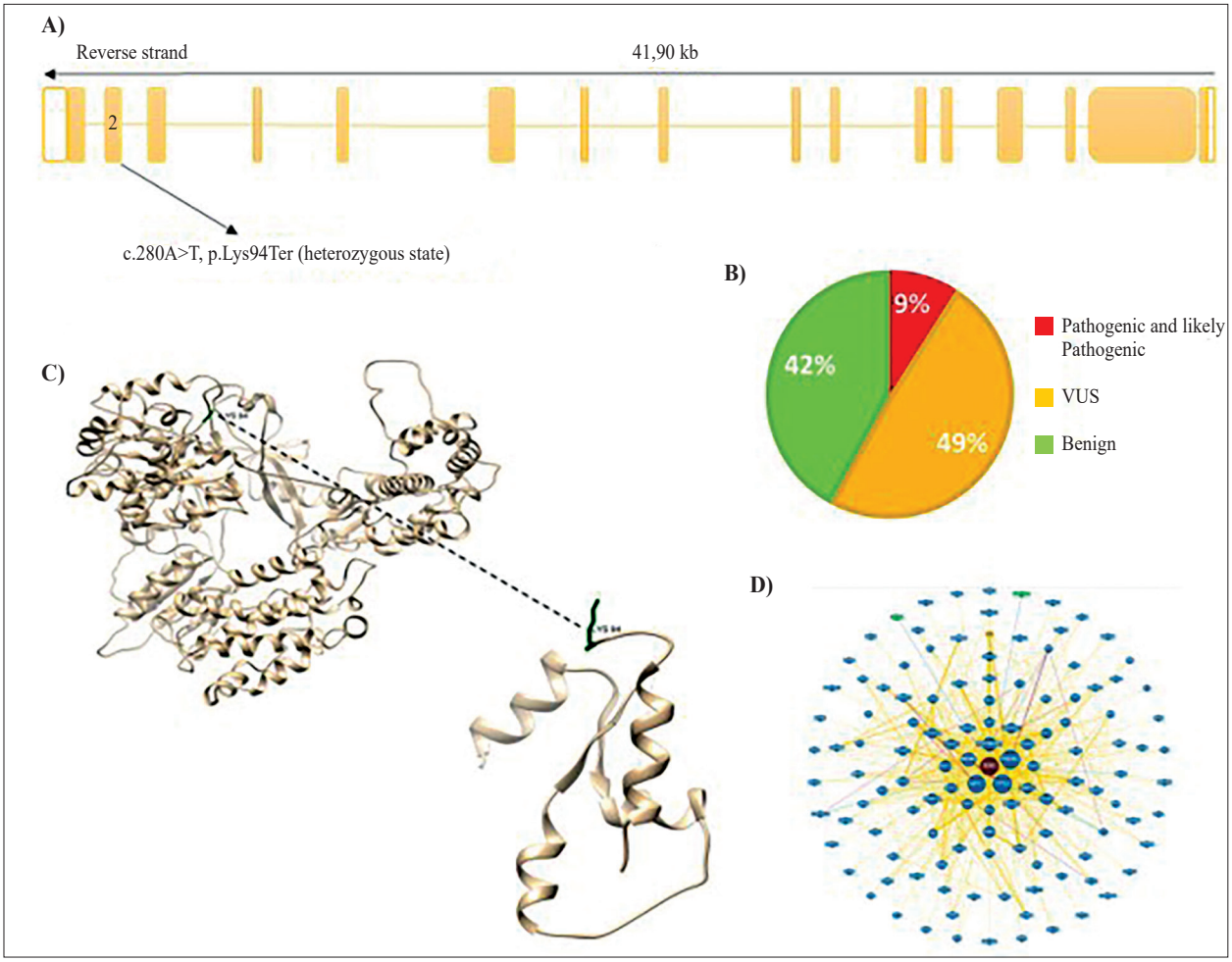

Figure 2. ***In silico*** **analysis of the impact of the detected variant in the TERT gene.** A) Schematic representation of sixteen exons in the *TERT* gene and marked detected loss of function variant in exon 2. B) Percentage ratio of pathogenic, likely pathogenic, VUS, and benign variants in the coding region of the *TERT* gene. C) Three-dimensional molecular models of *TERT* wild-type and mutated proteins. D) The protein-protein interaction network of *TERT* and interactors.

following criteria: PVS1 (Very Strong): Loss-of-function (LOF) variants in the *TERT* gene are a known mechanism of disease, with 76 reported pathogenic LOF variants; PM2 (Supporting): The variant has not been previously recorded in the GnomAD Exomes or GnomAD Genomes population databases, indicating it is rare. The results of the segregation analysis showed that neither parent was a carrier of the detected variant, indicating its *de novo* origin.

Pulse doses of methylprednisolone were initiated, with prednisone between doses and hydroxychloroquine and azithromycin, showing modest clinical benefits. Unfortunately, failure to wean off MV in a further course led to a tracheotomy, and MV was continued at home. An open lung biopsy was not performed. A control chest CT scan six months later revealed progression of the lung disease (Fig. 1B). Head CT, performed before steroid therapy commenced, showed supratentorial parenchymal volume loss with compensatory enlargement of CSF spaces. Eighteen months after the initial presentation, a new severe bilateral pneumonia led to multi-organ failure and death. The parents did not provide consent for autopsy.

DISCUSSION

The proposed mechanism of pulmonary involvement emphasises the importance of triggers such as smoking, stress, obesity, and inflammation [8]. CMV infection could be the trigger, as it has been described as a presenting feature in some *TERT*-variant associated disorders, such as dyskeratosis congenita (DC) [6]. The propensity of patients with confirmed pathogenic variants in the *TERT* gene to develop pneumonitis has been previously demonstrated [9]. Additional evidence suggests an impaired T cell immunologic response to CMV in lung transplant recipients with short telomere syndromes [10]. Although the relationship between viral infection and pulmonary fibrosis in adults

is not fully understood, direct damage caused by a virus and immune-mediated injury are proposed mechanisms [11]. However, in this case, the reason for the early onset of the disease remains unclear since ventilation-induced lung injury or CMV-associated acute respiratory distress syndrome could have similar consequences.

The typical clinical course in adults diagnosed with *TERT* mutation-associated conditions is insidious, with typical onset after the fourth decade of life [4,12]. Although the phenomenon of genetic anticipation based on the progressive shortening of telomeres is a characteristic of *TERT* gene mutations, no childhood presentation has currently been reported [4,13].

According to the literature and databases, each of the *TERT* mutations is referred to as "private mutations", and a total of 2625 clinically known and classified variants within the coding region of the *TERT* gene, of which only 9% are classified as pathogenic/likely pathogenic (Fig. 2B). Within the coding region of the *TERT* gene, loss of function (LOF) variants are very rare and have been detected only in the heterozygous state, thus indicating that the *TERT* gene is almost completely intolerant to LOF variants [14]. The detected heterozygous variant, c.280A>T (p.Lys94Ter) in the patient, was an LOF variant. The results of *in silico* modelling of wild-type and mutated TERT demonstrated that amino acid changes and consequential downstream introduction of the STOP codon led to protein truncation and consequent removal of protein-binding sites (Fig. 2C). Since TERT interacts with 122 different interactors (Fig. 2D) in cells, its function is further disrupted due to the absence of accurate amino acids for protein-protein interactions. Zaug et al. described families suffering from pulmonary fibrosis due to *TERT* mutations with a highly variable degree of telomerase functional impairment. The results of their study showed that the degree of functional impairment of telomerase was highly variable and many *TERT* mutations were shown to retain high—near normal—telomerase enzyme activity [15]. A limitation of this study was the absence of telomere length measurements or telomerase enzyme activity, which could have provided insights into the association between the detected germline variants, telomere length, and unusual early onset diseases.

In conclusion, the identification of a pathogenic variant in the *TERT* gene underlines the importance of genetic testing in paediatric patients presenting with respiratory failure, especially when confronted with atypical clinical features. The association between CMV infection and *TERT* mutations sheds light on potential disease mechanisms involving impaired telomerase function and immune response dysregulation and may lead to extremely rare early onset lung disease with chronic respiratory insufficiency and an unfavourable final outcome.

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