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CASE REPORT

Germline variant of *CTC1* gene in a patient with pulmonary fibrosis and myelodysplastic syndrome

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ABSTRACT

Introduction: Telomeropathies are associated with a wide range of diseases and less common combinations of various pulmonary and extrapulmonary disorders.

Case presentation: In proband with high-risk myelodysplastic syndrome and interstitial pulmonary fibrosis, whole exome sequencing revealed a germline heterozygous variant of *CTC1* gene (c.1360delG). This "frameshift" variant results in a premature stop codon and is classified as likely pathogenic/pathogenic. So far, this gene variant has been described in a heterozygous state in adult patients with hematological diseases such as idiopathic aplastic anemia or paroxysmal nocturnal hemoglobinuria, but also in interstitial pulmonary fibrosis. Described *CTC1* gene variant affects telomere length and leads to telomeropathies.

Conclusions: In our case report, we describe a rare case of coincidence of pulmonary fibrosis and hematological malignancy caused by a germline gene mutation in *CTC1*. Lung diseases and hematologic malignancies associated with short telomeres do not respond well to standard treatment.

Key words: *CTC1* gene; interstitial pulmonary fibrosis; myelodysplastic syndrome.

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Contributions: MD, MiD, designed the study, analyzed and interpreted the data, and wrote the manuscript; ZV, SK, IB, SP, performed the genetic analysis; LČ, collected patient's data. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate: No ethical committee approval was required for this case report by the Department, because this article does not contain any studies with human participants or animals. Written informed consent was obtained from the patient who was enrolled in accordance with the Helsinki Declaration.

Introduction

Human telomeres consist of long TTAGGG sequence repeats that are bound and stabilized by two nucleoprotein complexes required for the protection and replication of chromosome ends [1]. A protein complex "shelterin" is comprised of six telomere-specific proteins (TRF1, TRF2, POT1, Rap1, TIN2 and TPP1), and a heterotrimeric "CST" complex is composed of the proteins Ctc1, Ten1 and Stn1 involved in telomere maintenance [2,3]. Genomic alterations in these proteins are causative of a number of disorders known as telomeropathies. Telomeropathies are closely associated with premature aging and a reduction of cell ability to cope with recurrent damage. Several pulmonary, hematological, or liver diseases are associated with telomeropathies. These diseases include pulmonary fibrosis as idiopathic pulmonary fibrosis (IPF) and myelodysplastic syndrome (MDS).

IPF manifests as bibasilar reticular abnormalities, bronchiectasis, honeycombing on high-resolution computed tomography, restrictive pulmonary function impairment, and decreased lung diffusion capacity for carbon monoxide. IPF is idiopathic interstitial pneumonia characterized by progressive fibrotic damage of lung parenchyma. MDS is a clonal hematopoietic tissue disorder manifesting as morphologic dysplasia in myeloid lineage and peripheral cytopenia [4].

Case report

A 69-year-old Caucasian male, a non-smoker, presented with a mild dry cough and mild shortness of breath after exercises. He was sent for suspected interstitial pulmonary fibrosis. The patient's family history was negative regarding pulmonary and malignant diseases. Until then he was only treated for hypertension. The patient was an old-age pensioner. He worked as an engineer (dust-free office work). Clinical assessment (including screening questionnaire, antibodies against specific antigens) was performed in order to exclude hypersensitivity pneumonitis or autoimmune disease. No exposure was found to cause exogenous allergic alveolitis. No causative antigens were found.

Physical examination revealed clubbing fingers and bilateral end-inspiratory crackles in the lower and middle lung areas. The posteroanterior chest-X ray showed bilateral reticular pulmonary infiltrates (Figure 1). High-resolution computed tomography (HRCT) of lungs identified reticular opacities, bronchiectasis, and honeycombing changes (Figures 2 and 3). Pulmonary function testing revealed no ventilation defect [forced vital capacity (FVC) = 3.54 L, 100% of the predicted value (p.v.); total lung capacity (TLC) = 5.13 L; 82%] and moderate decrease of diffuse lung capacity for carbon monoxide (DLco; 4.23 L; 53%p.v.). Arterial blood gas analysis was also normal. Bronchoscopy with bronchoalveolar lavage showed an increased number of neutrophilic granulocytes and lymphocytes (13%, 74%). Lymphocyte subtypes were investigated: CD3+CD4+ = 69.20%, CD3+CD8+ = 27.00%. The ratio CD4+/CD8+ was 2.5. The neutrophilic and lymphocytic alveolitis was caused by a previous lung infection in a patient immunocompromised by hematological disease. There was no yield of transbronchial lung biopsy. Due to the patient's condition and hematological disease, histological verification by transbronchial cryobiopsy was not indicated.

We considered other interstitial lung diseases (ILDs) including hypersensitivity pneumonitis (HP), but we did not find any exposure in either work or home. The HRCT finding was discussed with the Multidisciplinary Team (MDT). Other ILDs were excluded, autoantibodies were negative, and no exposure leading to hypersensitivity pneumonitis (HP) was found (negative questionnaires, specific IgG were negative). The diagnosis was concluded as usual interstitial pneumonia on HRCT of the thorax with honeycombing at dorsal basal regions. The patient had been treated with corticoids before he was referred to us. Radiological progression of lung fibrosis occurred during treatment. We gradually discontinued corticoids. The duration of this treatment was 6 months. We considered antifibrotic treatment, but we did not administer it, as the patient did not meet the indication criteria for antifibrotic therapy. At the same time, the patient developed thrombocytopenia (77 x 10^9/L) and macrocytic anemia (hemoglobin 108 g/L; mean red cell

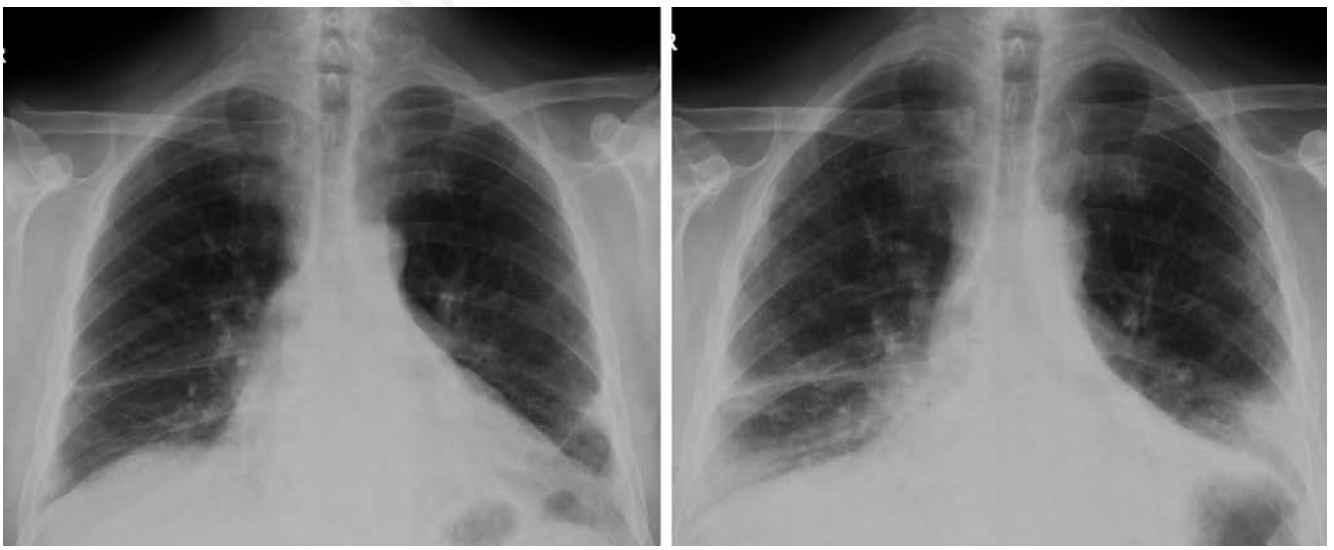

Figure 1. Chest X-ray before (left) and after COVID-19 infection (right). After COVID-19, new areas of bilateral decreased parenchyma transparency and consolidation are found.

volume 111 fL). Furthermore, high-risk MDS with blast excess (EB-2) was diagnosed (11.2% of myeloblasts in the bone marrow; deletion 5q in cytogenetics). Therapy with 5-azacytidine was initiated and the patient received 20 cycles of this treatment. Unfortunately, the patient was only temporarily stabilized by treatment and subsequently progressed into acute myeloid leukemia and died of severe COVID-19 pneumonia with respiratory failure.

Because of suspected inherited predisposition leading to IPF and MDS, genetic testing focused on whole exome sequencing (WES) was performed. The WES variants evaluation process was aimed at the analysis of single nucleotide variants (SNV) and short indels (indel) within virtual genes panel associated with myeloid malignancies and predispositions to both myeloid and pulmonary disorders (Supplementary Table 1). A germline heterozygous variant c.1360delG (NM_025099.5; g.8235132) encoding the p.Glu454Serfs*9 substitution in 8 exons of the *CTC1* gene was identified. The coverage of c.1360delG was 73 and variant allele frequency (VAF) was 47.95% in a patient. The sequencing result is shown in Figure 4. Deleting guanine at position 1360 creates a new reading frame, resulting in a premature stop codon at position 9. Variant c.1360delG is classified as pathogenic/likely pathogenic, according to the consensus guidelines by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. The presence of variant c.1360delG was verified by Sanger sequencing. Shen *et al*. [5] reported a 31-years-old man with heterozygous c.1360delG variant in *CTC1* which was diagnosed with adult-onset severe idiopathic aplastic anemia (AA) and development of secondary paroxysmal nocturnal hemoglobinuria. In addition, a c.1049delC variant (VAF: 21.21%) in the *RUNX1* gene was identified and classified as a variant with uncertain significance (VUS).

Discussion

Telomeropathies are very heterogeneous diseases. Therefore, patients with the same mutation can present different manifestations. This report describes a rare case of coincidence of pulmonary fibrosis and hematological malignancy caused by a germline gene variant in *CTC1* (CST telomere maintenance complex component [1]. There is no similar case of the coincidence of these two diseases described in the medical literature. We did not find any articles describing the coincidence of the two diseases (aplastic anemia and pulmonary fibrosis).

The *CTC1* gene (OMIM #613129), located on chromosome 17p13.1, encodes a 1,217 amino acids nuclear protein Ctc1 which is a component of the conserved telomere maintenance CST complex along with the Stn1 and Ten1 proteins [3,6,7]. *CTC1* variants alter the function of the CST complex, which may result in the shortening of telomeres and DNA damage responses [8,9]. In that case, telomeres are recognized as damaged DNA that can result in cell-cycle arrest, cell apoptosis, or senescence. The CST complex was initially proposed to play a role in telomere length homeostasis by reducing access of telomerase to telomeres in order to prevent excessive telomere lengthening [10]. Subsequently, the CST complex was shown to promote telomere replication [6]. Therefore, the Ctc1 protein is involved in the maintenance of telomeres. Telomeropathies are very heterogeneous diseases depending on the gene mutated and the specific variants, their penetrance, and the existence of anticipation effects. Therefore, patients with the same variant can present different manifestations. Some patients present severe symptoms early, such as those of dyskeratosis congenita (DC) or the related Hoyeraal-Hreidarsson syndrome, Resvesz syndrome, and Coats plus syndrome. Moreover, diseases associated with telomeropathies may occur at a younger age than usual in sporadic forms: AA median age 20-30 years at diagnosis [11], idiopathic pulmonary fibrosis (IPF) median age 40-60 years at diagnosis [12,13]. A study by Arias-Salgado *et al*. describes genetic analyses of aplastic anemia and idiopathic pulmonary fibrosis patients with short telomeres caused by *CTC1* gene mutation but not as a coin-

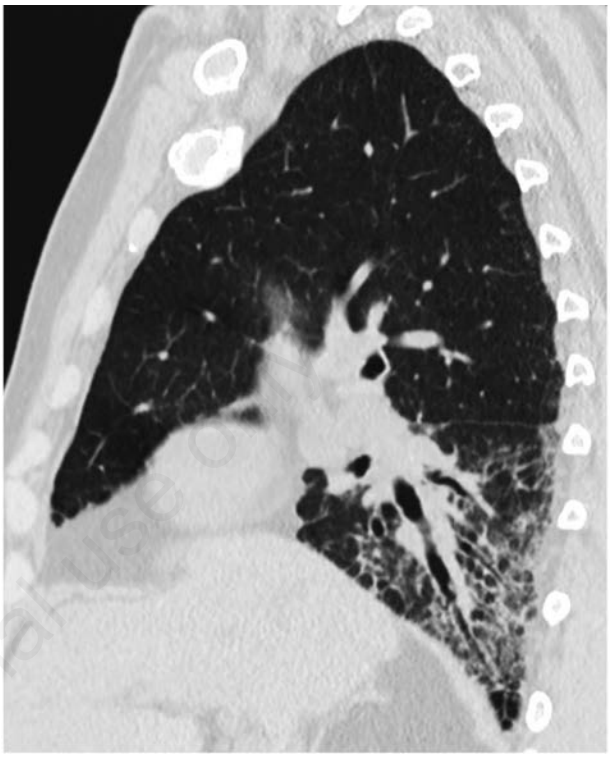

Figure 2. Our patient high-resolution computed tomography (HRCT) at diagnosis. Typical UIP HRCT pattern shown on sagittal view. Honeycombing, reticulation and peripheral bronchiectasis with basal and subpleural predominance are present.

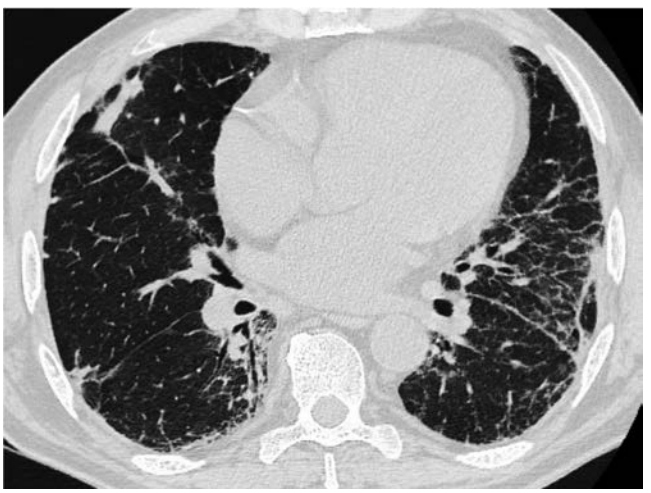

Figure 3. Our patient high-resolution computed tomography (HRCT) at diagnosis. Axial HRCT view through both lungs under tracheal bifurcation. Peripheral bronchiectasis on the right side in the paravertebral location and bilateral reticulation with subpleural predominance are present.

cidence of both diseases, but each disease separately. However, we did not find any articles describing the coincidence of the two diseases (aplastic anemia and pulmonary fibrosis) [13].

There is an increasing number of inherited disorders in which excessive telomere shortening underlies the molecular defect, with dyskeratosis congenita (DC) being the archetypal short telomere syndrome. Excessive telomere shortening can affect almost any organ system, so the clinical manifestations are protean, including developmental delay, cerebellar hypoplasia, exudative retinopathy, AA, acute myeloid leukemia, IPF, idiopathic hepatic cirrhosis, head and neck cancer and dental abnormalities, and may be multisystemic [14]. Unfortunately, there are no "age-specific" telomere length standards, because their length is reflected in an incredible number of individual factors (age is only one of them) [15]. For this reason, it is still difficult to use telomere length for routine diagnosis. Diseases and conditions associated with pathogenic variants of *CTC1* include cerebroretinal microangiopathy with calcifications and cysts (CRMCC) and DC. The majority of *CTC1* pathogenic variants found in either CRMCC or DC are compound heterozygotes of a missense variant and a truncation variant, with a few exceptions of compound heterozygotes for two missense variants [16,17]. Blood cells from *CTC1* mutated patients were shown to exhibit shortened telomere lengths or telomere lengths at the lower range of normal [8,18]. However, one study reported no significant differences between the leukocyte telomere lengths of *CTC1* mutant patients and those of controls, raising the possibility that the disease mechanism of *CTC1* mutations may involve non-telomeric functions [19].

Short telomere syndromes associated with pulmonary diseases, particularly fibrosis, respond poorly to standard treatments, such as corticosteroids and bronchodilators. Androgen derivatives could be a potential therapeutic option able to re-elongate previously shortened telomeres. However clinical trials are needed to develop pharmacological agents aimed at correcting disease-causing genetic defects and determine if androgen therapy is effective for telomere-related interstitial lung diseases [20,21]. Lung transplantation may be required in end-stage disease. Higher rates of hematological, renal and infectious complications were seen, requiring reduced immunosuppressive regimens in all cases [14]. The prognosis of high-risk MDS is also poor. It is even worse in cases with a congenital predisposition. In our patient, there was only a temporary stabilization of the disease during 5-azacytidine therapy.

Conclusions

Telomeropathies are associated with a wide range of diseases and combinations of various pulmonary and extrapulmonary disorders. Lung diseases associated with short telomeres, such as pulmonary fibrosis, and also hematologic malignancies do not respond well to standard treatment. With our case, we want to draw attention to the fact that even apparently different diseases can have the same genetic basis in one patient.

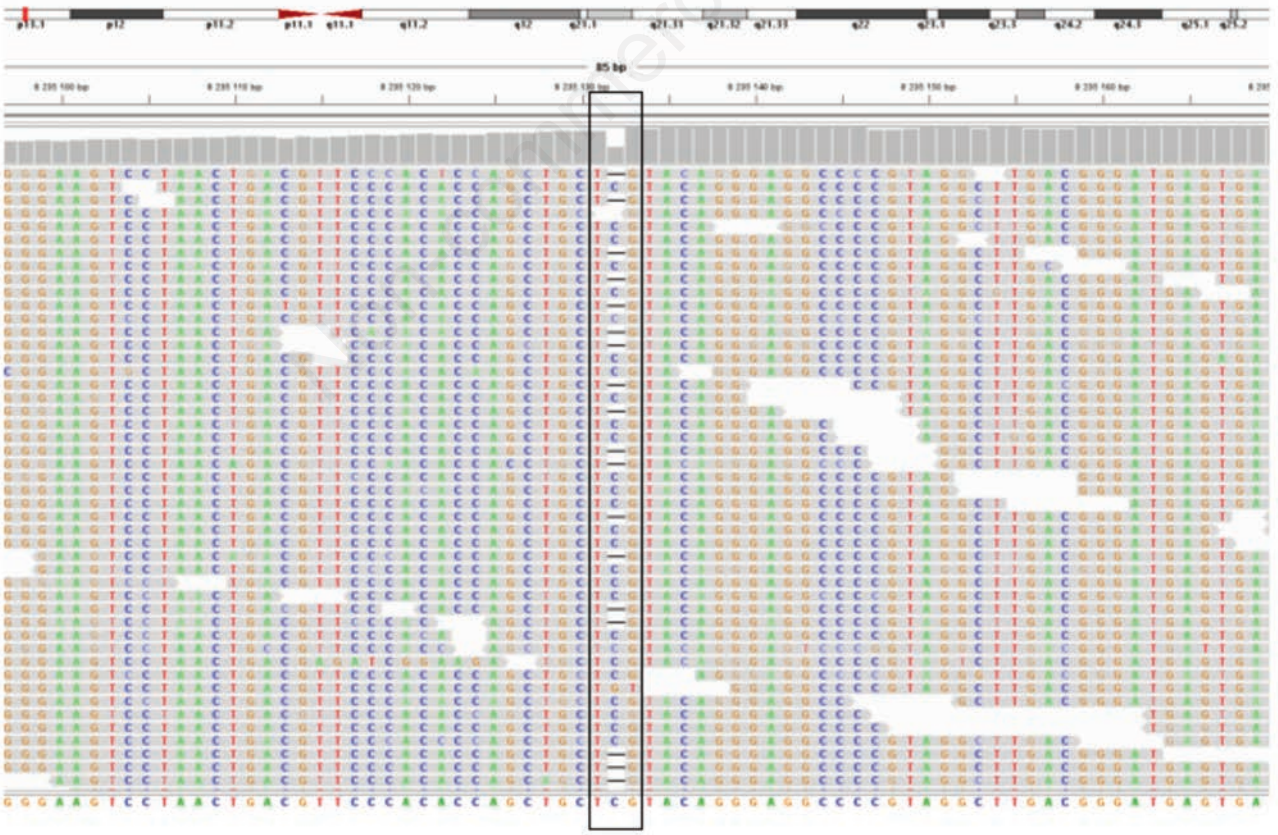

Figure 4. Visualization of c.1360delG variant (NM_025099.5; g.8235132) detected by whole exome sequencing using Integrative Genomics Viewer. Nucleotide deletion is marked by a black frame.

References

1. Broccoli D, Smogorzewska A, Chong L, de Lange T. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. Nat Genet 1997;17:231-5.
2. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 2005;19:2100-10.
3. Wellinger RJ. The CST complex and telomere maintenance: the exception becomes the rule. Mol Cell 2009;36:168-9.
4. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia 2022;36:1703-19.
5. Shen W, Kerr CM, Przychozen B, Mahfouz RZ, LaFramboise T, Nagata Y, et al. Impact of germline CTC1 alterations on telomere length in acquired bone marrow failure. Br J Haematol 2019;185:935-9.
6. Rice C, Skordalakes E. Structure and function of the telomeric CST complex. Comput Struct Biotechnol J 2016;14:161-7.
7. Sargolzaeiaval F, Zhang J, Schleit J, Lessel D, Kubisch C, Precioso DR, et al. CTC1 mutations in a Brazilian family with progeroid features and recurrent bone fractures. Mol Genet Genomic Med 2018;6:1148-56.
8. Anderson BH, Kasher PR, Mayer J, Szynkiewicz M, Jenkinson EM, Bhaskar SS, et al. Mutations in CTC1, encoding conserved telomere maintenance component 1, cause Coats plus. Nat Genet 2012;44:338-42.
9. Huang C, Jia P, Chastain M, Shiva O, Chai W. The human CTC1/STN1/TEN1 complex regulates telomere maintenance in ALT cancer cells. Exp Cell Res 2017;355:95-104.
10. Chen LY, Redon S, Lingner J. The human CST complex is a terminator of telomerase activity. Nature 2012;488:540-4.
11. Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. Blood 2014;124:2775-83.
12. Armanios M. Telomerase and idiopathic pulmonary fibrosis. Mutat Res 2012;730:52-8.
13. Arias-Salgado EG, Galvez E, Planas-Cerezales L, Pintado-Berninches L, Vallespin E, Martinez P, et al. Genetic analyses of aplastic anemia and idiopathic pulmonary fibrosis patients with short telomeres, possible implication of DNA-repair genes. Orphanet J Rare Dis 2019;14:82.
14. Barbaro PM, Ziegler DS, Reddel RR. The wide-ranging clinical implications of the short telomere syndromes. Intern Med J 2016;46:393-403.
15. Rizvi S, Raza ST, Mahdi F. Telomere length variations in aging and age-related diseases. Curr Aging Sci 2014;7:161-7.
16. Keller RB, Gagne KE, Usmani GN, Asdourian GK, Williams DA, Hofmann I, et al. CTC1 Mutations in a patient with dyskeratosis congenita. Pediatr Blood Cancer 2012;59:311-4.
17. Lin H, Gong L, Zhan S, Wang Y, Liu A. Novel biallelic missense mutations in CTC1 gene identified in a Chinese family with Coats plus syndrome. J Neurol Sci 2017;382:142-5.
18. Walne AJ, Bhagat T, Kirwan M, Gitiaux C, Desguerre I, Leonard N, et al. Mutations in the telomere capping complex in bone marrow failure and related syndromes. Haematologica 2013;98:334-8.
19. Polvi A, Linnankivi T, Kivelä T, Herva R, Keating JP, Mäkitie O, et al. Mutations in CTC1, encoding the CTS telomere maintenance complex component 1, cause cerebroretinal microangiopathy with calcifications and cysts. Am J Hum Genet 2012;90:540-9.
20. Vieri M, Kirschner M, Tometten M, Abels A, Rolles B, Isfort S, et al. Comparable effects of the androgen derivatives danazol, oxymetholone and nandrolone on telomerase activity in human primary hematopoietic cells from patients with dyskeratosis congenita. Int J Mol Sci 2020;21:7196.
21. Chambers DC, Lutzky VP, Apte SH, Godbolt D, Feenstra J, Mackintosh J. Successful treatment of telomeropathy-related interstitial lung disease with immunosuppression and danazol. Respirol Case Rep 2020;8:e00607.

Online supplementary material:

Methods - Whole exome sequencing (WES)

Table 1. Virtual genes panel associated with myeloid malignancies and predispositions to myeloid and pulmonary disorders.

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