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# Tissue-specific telomere shortening and degenerative changes in a patient with *TINF2* mutation and dyskeratosis congenita

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## Abstract

Dyskeratosis congenita is a disease of impaired tissue maintenance downstream of telomere dysfunction. Characteristically, patients present with the clinical triad of nail dystrophy, oral leukoplakia, and skin pigmentation defects, but the disease involves degenerative changes in multiple organs. Mutations in telomere-binding proteins such as TINF2 (TRF1-interacting nuclear factor 2) or in telomerase, the enzyme that counteracts age related telomere shortening, are causative in dyskeratosis congenita. We present a patient who presented with severe hypoxemia at age 13. The patient had a history of myelodysplastic syndrome treated with bone marrow transplant at the age of 5. At age 18 she was hospitalized for an acute pneumonia progressing to respiratory failure, developed renal failure and ultimately, she and her family opted to withdraw support as she was not a candidate for a lung transplant. Sequencing of the patient's TINF2 locus revealed a heterozygous mutation (c.844C > T, Arg282Cys) which has previously been reported in a subset of dyskeratosis congenita patients. Tissue sections from multiple organs showed degenerative changes including disorganized bone remodeling, diffuse alveolar damage and small vessel proliferation in the lung, and hyperkeratosis with hyperpigmentation of the skin.



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Author contributions

M.J. and C.K. conceived the study. C.M.R and M.J. and C.K. analyzed the data and wrote the paper. S.A. supported the study and the telomere experiments. R.A. reviewed the manuscript and Bone Marrow Transplant specifics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

Patient and family consented to participating in research studies and autopsy. A chart review was also conducted and approved by Stanford University's research compliance office (IRB # 30096).

Autopsy samples revealed a bimodal distribution of telomere length, with telomeres from donor hematopoietic tissues being an age-appropriate length and those from patient tissues showing pathogenic shortening, with the shortest telomeres in lung, liver, and kidney. We report for the first time a survey of degenerative changes and telomere lengths in multiple organs in a patient with dyskeratosis congenita.

**Keywords**

Dyskeratosis congenita; Telomere length; Hypoxemia; Pediatric bone marrow failure

## 1. Introduction

Dyskeratosis congenita (DC) is a disease of short telomeres and is characterized by the clinical triad of nail dystrophy, oral leukoplakia, and lacy reticular skin pigmentation [1]. However, the list of minor features associated with this disease is long and involves many organs [2] though there is considerable variability across organ systems in timing and severity of presentation. Clinical manifestations may range from alopecia or early grey hair to developmental delay, low birth weight, liver and pulmonary fibrosis. Germline inheritance may also show disease anticipation, with relatives suffering from only cytopenias or pulmonary fibrosis while later generations are more affected by the inheritance of shorter telomeres [3]. Patients may present with only one or two of these non-specific clinical features making the ultimate diagnosis of DC based on clinical symptoms a major challenge.

Telomere shortening is broadly associated with aging, increased cancer risk, and cellular senescence [4]. Telomeres are repetitive DNA sequences at the end of chromosomes that are subject to shortening as a consequence of cell division, termed the “end replication problem” [5]. Telomerase, a ribonucleoprotein complex, specifically counteracts telomere shortening by adding telomeric repeats to chromosome ends, while the shelterin complex (a complex of telomere-associated proteins) coats the telomere and provides a docking site for telomerase [6,7].

Diseases of telomere dysfunction, such as DC, aplastic anemia, and idiopathic pulmonary fibrosis [8,9] are caused by mutations in genes critical to the telomerase/shelterin pathway. Germline mutations in the genes important for the telomerase holoenzyme, such as *TERT*, *TERC, DKC1*, *NHP1*, *NOP10*, *NHP2*, *TCAB1, NAF1, and PARN* as well as the shelterin components *TINF2 and TPP1* have been linked to patients with DC and other syndromes of short telomeres [9–12]. DNA processing is also critical to telomere maintenance as genes such as *CTC1* and *RTEL1* are also mutated in patients with DC [13–15].

Laboratory tests for DC include genetic testing and flow cytometry FISH (flow-FISH) for telomeres in peripheral blood. Telomere length is short in most, but not all patients with DC, and shortness of telomeres is associated with disease severity when compared to age matched controls [16,17]. Patients with *TINF2* mutations have particularly short telomeres, even compared to other DC individuals [18]. Given the accessibility of peripheral blood testing, telomere length analysis is performed most commonly using flow-cytometry fluorescence in situ hybridization (flow-FISH) with a telomere PNA probe on peripheral

blood leukocytes and it is unknown how DC affects telomere lengths in tissues other than blood. Since DC patients show clinical signs and symptoms in multiple organ systems, correlating telomere length with specific tissue pathology will help to better understand the pathophysiology of telomere disease.

## 2. Methods

### 2.1. Telomere restriction fragment analysis

Tissue blocks were flash frozen at the time of autopsy and stored at −80 degrees Celsius. Then, tissue blocks were ground over liquid nitrogen with a mortar and pestle, digested in cell lysis buffer, followed by DNA extraction [19]. To measure telomere lengths, DNA was digested with Proteinase K at 6 ug/mL overnight. DNA was extracted by the phenol chloroform method and digested overnight with HinfI and RsaI before electrophoresis and southern blotting with end labeled $(CCCTAA)_4$ oligonucleotide probe. Digested DNA was run on an ethidium bromide gel to assess DNA quality. A phosphor screen was exposed to the hybridized membrane overnight and the image was obtained. Image J was used to obtain median telomere lengths for each lane. Two technical replicates were averaged to obtain mean telomere lengths for each tissue.

### 2.2. Topo cloning and sequencing of TINF2 alleles

DNA was extracted from tissues by overnight digestion with Proteinase K at 6 ug/mL. DNA was extracted by the phenol chloroform method and the *TINF2* locus was amplified from kidney using primers Exon6-For: GGCTCCGGGCATAAGAAAC, Exon 6-Rev: TGAGGTGAGAGCAAGCAAAG [18]. Amplicons were ligated into TOPO blunt plasmids (ThermoFisher K280020) and transformed into competent bacteria. Clones were selected and sequenced by Sanger sequencing.

## 3. Results

### 3.1. Clinical presentation and family history

A 13-year-old female presented with severe hypoxemia (baseline oxygen saturation 80%) on room air. Clinical history revealed an allogeneic bone marrow transplant (BMT) for myelodysplastic syndrome (MDS) at the age of 5 in 1999 (Fig. 1A). Her conditioning regimen consisted of fractionated total body irradiation (FTBI) to a total of 1320 cGy and high-dose cyclophosphamide. She also received cyclosporine for GVHD prophylaxis. There was no clinical evidence of pulmonary disease or any other organ system involvement during evaluation for BMT. Although her BMT was successful, she did have a brief period of renal insufficiency 7 months after BMT. She subsequently had a renal biopsy that showed thrombotic microangiopathy with associated chronic systemic hypertension treated with Enalapril. She was referred to pulmonary clinic during a routine visit to her nephrologist 9 years after her transplant. At that clinic visit, she was noted to be hypoxic ($SaO_2$ 80% in room air), but without symptoms of acute respiratory distress. Physical examination and interview revealed a thin, dark-skinned young woman of Filipino ancestry who was active in school activities and excelling academically. She had clear breath sounds and was mildly

tachypneic at baseline. Her exam was notable for dystrophic, ragged nail-beds and abnormal patches of skin pigmentation.

A detailed family history did not reveal any rheumatologic or pulmonary diseases, cancers, or bone marrow failure syndromes. A review of systems was positive for short stature, delayed puberty, primary ovarian failure, and hypothyroidism. Baseline pulmonary function testing showed a restrictive ventilatory and significant diffusion defect with forced vital capacity (FVC) = 1.56 L; 65% predicted, forced expiratory volume (FEV1) = 1.43 L; 55% predicted, and corrected diffusion capacity of the lung for carbon monoxide (DLCO) = 4.3 ml/min/mmHg; 18% predicted. Inspiratory and expiratory views of the chest by CT angiography revealed mildly dilated pulmonary arteries but no focal arteriovenous malformation or parenchymal disease. No fibrosis was observed at this time. Given the severe hypoxemia, she was evaluated for intra and extrapulmonary shunting. Contrast cardiac echocardiogram revealed no intracardiac shunting, but was diagnostic for intrapulmonary shunting with bubbles returning to left atrium 4 beats after detection in right atrium. Because no focal arteriovenous (AVMs) were identified there was no obvious target site for intervention. Hepatopulmonary syndrome was a primary consideration in the differential diagnosis, but she had a magnetic resonance arteriogram of the abdomen that did not reveal evidence of portal vein dilation, splenomegaly, or demonstration of extrapulmonary arteriovenous malformation. She also had repeated abdominal ultrasounds to screen for interval changes and no interval evidence for portal-systemic shunting was detected. Her serum liver enzyme measurements (AST and ALT) were also normal, thus she did not have classic signs or features of hepatopulmonary disease previously reported in patients with telomere disorders [20]. Thus, given her nail dystrophy and cutaneous findings, she was referred for skin biopsy as well as genetic testing for dyskeratosis congenita.
A buccal sample was sent for targeted exome sequencing and identified a heterozygous mutation in *TINF2* at Arg282 (c.844C > T, Arg282Cys). We confirmed the mutation by sequencing this region of exon 6 (Fig. 1B). This mutation had been previously reported to be associated with clinical disease in a subset of patients with severe dyskeratosis [18]. Prior to her formal diagnosis of dyskeratosis congenita, she was treated for less than one year with oral immunosuppression (prednisone and cyclosporine) for possible chronic graft vs. host disease on the basis of her skin biopsy.

### 3.2. Treatment Outcomes:

Over the course of 3–4 years, the patient had progressive worsening of baseline hypoxemia with saturations at baseline to mid 70′s. There was no evidence that she was improving on an immunosuppressive regimen, thus immunosuppression was discontinued and she was managed supportively. She was hospitalized for the first time at the age of 18 for an acute pneumonia and respiratory failure. Repeat chest CT at this time showed interval progression to mild fibrosis in the upper lobes bilaterally and diffusely dilated pulmonary vasculature similar to the findings previously noted. She was hospitalized over 1 month in the intensive care unit and developed renal failure requiring dialysis and ultimately, she and her family opted to withdraw support.

### 3.3. Pathological and histological findings:

Upon autopsy, the patient was found to have an overall short stature, estimated to be the size of an 11 year old, with ectoderm findings including prominent left central incisor, dystrophic fingernail, and mottled skin hyperpigmentation with regions of lichenification (Note: image from clinic visit, age 14y) (Fig. 2A). The chest wall showed a mild pectus carinatum. The breasts were not developed (Tanner I) and external genitalia were those of a normal female with sparse pubic hair (Tanner II).

Examination of the bone marrow aspirate smears pre-transplant demonstrate frequent erythroid precursors with both nuclear and cytoplasmic bridging, a feature found in both high turnover states and myelodysplasia (Fig. 2B). The bone marrow trephine biopsy pre-transplant showed features of morphologic dysplasia with overall decreased cellularity for age that was further reduced at a second biopsy taken one month later (per report).

Biopsy results of the skin lesions at age 14y from the palms and soles showed hyperpigmented patches with regions of lichenification. Histology of these areas demonstrated hyperkeratosis with hyperpigmentation of the basal keratinocytes and slightly increased number of melanophages in the upper dermis (Fig. 2C). The skin findings at autopsy also showed melanin incontinence, a non-specific finding that can be seen in dyskeratosis congenita [21].

The kidney H&E stains showed glomeruli with extensive mesangiolysis, capillary loop double contours, and blood-less glomeruli. Kidneys also showed segmental and global glomerulosclerosis. There was extensive renal tubular atrophy and fibrosis. The blood vessels in the kidneys demonstrate intimal sclerosis and arterial hyalinosis (Fig. 2D–F). These findings are consistent with her acute renal failure at end of life in the context of chronic systemic hypertension.

Lung histopathology revealed diffuse alveolar damage mostly in the organizing phase likely secondary to the history of respiratory syncytial virus (RSV) and subsequent pneumonia. There was only mild interstitial fibrosis most prominent at the lung apices (Fig. 2G). The bronchioles contained focal squamous metaplasia. The superior upper lobes had bands of old dense fibrosis, but no areas progressed to “honeycomb” stage of remodeling. No areas of lung were seen with an intermediate stage of healing, only old fibrosis and diffuse alveolar damage that is estimated to be a few weeks old. Although definitive etiology for chronic hypoxia was not identified, there were numerous dilated thin walled interstitial pulmonary vessels that may have contributed to the physiologically-defined intrapulmonary shunts (Fig. 2G). Cultures, molecular tests, and stains for infectious agents were negative.

Liver histopathology revealed mild macrosteatosis, patchy hepatocyte necrosis, and mild expansion of the sinusoidal spaces (Fig. 2H). Trichrome and reticulin stains showed mild to moderate centrilobular fibrosis and mild expansion of sinusoidal spaces (Fig. 2I, J). An iron stain of the liver showed mild to moderate iron deposition, although this was in the context of the patient receiving multiple blood transfusions (Fig. 2K). Notably, these findings are distinct from those found in hepatopulmonary syndrome [20].

Other histopathological findings at autopsy demonstrated systemic changes in the ovaries and small intestine. The ovaries were small and streak like and microscopic sections showed primordial follicles without folliculogenesis (Fig. 2L). Microscopic sections of the small intestine showed loss of the intervening crypt progenitor cells and an overall increase in Paneth cells. A summary of the pathological findings for tissues obtained during clinical care and at autopsy are summarized in Table 1.

### 3.4. Telomere length analysis

Patients with *TINF2* mutations have short telomeres in peripheral blood lymphocytes compared to age-matched controls [18,22]. Because of accessibility, telomere length is usually measured in peripheral blood lymphocytes. Dyskeratosis Congenita is a multisystemic disease, affecting many organs, suggesting that telomere length may be reduced in other tissues [23]. However, it is not known if shorter telomeres in other organs are responsible for the variable clinical symptoms. Autopsy studies of normal tissues show that the shortest telomeres are found in the blood, followed by the lung and liver, and are roughly associated with more proliferative tissues [24,25]. Longer telomeres are associated with tissues that have lower proliferative rates, such as skeletal muscle, large intestine, and cardiac muscles [24]. In DC patients, the model would predict that tissues with increased proliferation would have shorter telomeres than unaffected tissues with less proliferation. Therefore, to preserve DNA required for telomere length analysis, tissues obtained at autopsy from 16 different organs were flash-frozen with liquid nitrogen. Telomere lengths were analyzed using a modified southern blot approach [19]. The Southern Blot method is more robust when assessing telomere length across different sample types when compared to other methods [26]. The shortened telomere signal is not secondary to degraded DNA since DNA integrity is monitored prior to the Southern Blot step by assessment of fragmented DNA in a separate ethidium bromide stained gel prior to restriction enzyme digestion [26]. Degraded DNA from pancreas, small bowel, and vertebrae were removed from the analysis (data not shown).

In our patient, hematopoietic organs, such as the lymph node and spleen, demonstrated longer telomeres since the cells are derived from her sibling BMT donor (Fig. 3). All tissues we examined had shorter telomeres than the transplant-derived hematopoietic cells. Comparison of the average telomere lengths in patient tissues by densitometry shows that telomeres were the shortest in lung (even despite infiltrates of transplant-derived cells), liver and kidney tissue, and longest in the myocardium. Although normative data was not available for all the tissues we measured, a study of telomere lengths in surgically resected tissue provides a reference for age-dependent telomere lengths in a subset of tissues [27]. Comparing the telomere lengths in this patient to this data shows shorter than predicted telomere lengths in the patient's non-hematopoietic comparison tissues. Skin telomere length in the patient was 7.3 kb compared to a predicted 7.9 kb and muscle telomere length in the patient was 6.9 kb compared to a predicted 9.1 kb. For hematopoietic tissues including spleen and lymph nodes patient telomere lengths were 8.5 and 10.5 kb respectively compared to a predicted leukocyte telomere length of 7.7 kb. The longer telomere length in these tissues likely reflect the high proportion of donor-derived cells. These data are in accordance with previous studies that have found rates of telomere shortening with age to be

highest in hepatic tissue and blood lineages and lowest in myocardial and brain tissues, with measurable shortening also recorded in skin and lung [24,25].

## 4. Discussion

We analyzed telomere length and pathology in 16 tissues from 10 organs and report the first systematic collection of diverse organs for telomere length analysis from a pediatric patient with dyskeratosis congenita due to the Arg282Cys mutation in *TINF2* (encoding a shelterin complex protein) that has previously been associated with telomere shortening. At the time of initial presentation at the age of five, the patient had isolated bone marrow failure and she was only diagnosed with DC years later when she developed associated skin and buccal mucosal lesions. In the interim, she developed significant hypoxemia from intrapulmonary shunting not classically known to be associated with disorders of telomere shortening at that time. This observation prompted a collaborative effort to identify DC patients across the country and indeed, we identified and described a small group of patients with hypoxemia and symptoms consistent with pulmonary AVMs, though our patient (Patient #7) was the youngest member reported in this report of 13 patients [28].

To eliminate diagnostic delay, we advocate evaluating for DC along with a full panel of inherited genetic conditions including Fanconi anemia and Diamond-Blackfan anemia in pediatric patients and young adults who present with myelodysplastic syndromes and bone marrow failure [29]. An estimated 2 to 5 percent of bone marrow failure patients have DC, and many more likely go unidentified [30].

Although DC patients may be identified by characteristic clinical findings, overt clinical presentation in other organ systems may not occur until years later and early identification would modify therapeutic approach and management, including reduced conditioning regimen for bone marrow transplant that would have immediate implications for the patient as well as long-term considerations for diagnostic monitoring [31]. Initial evaluation should include screening for telomere lengths most commonly by flow-FISH, and NGS panel testing on skin fibroblasts for genetic mutations. Patients with DC or those who will go on to develop DC in their lifetimes typically have telomeres below the first percentile of age matched controls, and telomere length analysis by flow-FISH has greater than 90% sensitivity and specificity for dyskeratosis congenita [32]. Because circulating lymphocytes are most accessible, telomere length is typically measured from this tissue, thus pre-BMT evaluation is required.

For the patient presented here, telomere lengths were shortened compared to transplanted tissue in all organs examined, underscoring the multisystem nature of the disease. Commonly DC patients initially present with cytopenias and bone marrow failure; however, this genetic disease affects virtually every major organ system, though the severity of clinical manifestation differs across organs and changes over time. DC patients are at a higher risk for pulmonary fibrosis and while pulmonary complications are a recognized complication associated with poor outcomes [23], the sub-populations or cell types affected within the lung are unknown. In our analysis, we found organs with a brisk lymphocytic infiltrate, such as the colon and lung, also show two populations of telomere lengths, likely

corresponding to 1) the transplant-derived lymphocytes with longer telomeres and 2) the patient's underlying tissue with shorter telomeres. The potential contributions of infiltrating donor-derived immune populations in an organ-specific context is an important area for future investigation. In addition, only recently have the varied physiologic manifestations in the lung (e.g. AVMs) been recognized. More consistent and systematic screens for lung function over time are required to understand the trajectory of affected patients. A high priority will be to study affected organs at the single cell level to identify which cell types are most susceptible. In the human lung, it is not clear if type 2 alveolar epithelial cells are susceptible to defects in telomere maintenance, as is predicted by genetic mouse models inducing pulmonary fibrosis [33] in an alveolar epithelial progenitor cell population [34] and whether fibrosis is directly related to shortened telomeres. In patients with DC there is a higher incidence of liver cirrhosis and portal hypertension. Endocrine dysfunction is also common and may lead to pubertal delay and poor bone health, among other problems [1,2]. Individuals with DC are also at higher risk for certain cancers, and management now includes screening for cancer subtypes as well as implementing preventive measures to avoid ionizing radiation and adopt reduced intensity conditioning or non-myeloablative regimens. At the time our patient was transplanted, these practices were not widely employed and no clinically available test for telomere function was available. Because DC is often inherited, first-degree relatives should be screened for the pathogenic gene mutation (if one has been identified), and is especially critical when selecting related donors for bone marrow transplant. Management of DC requires a comprehensive clinical assessment and interdisciplinary care given the complex and evolving multisystem nature of this disease.

The biological consequences of mutations in telomerase and telomere-associated proteins (hTR, TERT, DKC1, NHP2, Nop10, TCAB1, PARN, TINF2, TPP1, POT1, CTC1, RTEL1, NAF1) are not completely understood but the systematic collection of tissues for histologic and functional abnormalities as we have described here are important steps towards understanding the diverse, multi-organ manifestations of this condition. While there has been much progress in the past decade towards recognizing the diverse and multi-organ manifestations of DC affecting virtually every major organ system [23], many important questions remain for every organ targeted by telomere dysfunction. Studying cell-type specific differences within each organ would provide a window into tissue-specific normal progenitor cell biology and how short telomere syndromes cause progressive disease with aging. Although we found that telomere lengths were compromised in every organ we examined, there is heterogeneity in the tolerance of short telomeres in each organ given the dominant manifestation of clinical disease in this patient in pulmonary and dermatologic systems. At the organ level, although there is less than a few kilobases of difference between bulk tissues, there may be large differences at the level of less abundant tissue specific progenitor cells. This study raises the possibility of additional disease manifestations in organs not typically associated with DC as patients are treated with bone marrow, lung, and kidney transplants, and have extended lifespans. Thus, special consideration must be given to such patients via thorough multisystem clinical histories and examinations at interval screening visits to identify early signs of involvement. Elucidating diversity of cell types within each tissue at single-cell resolution is a high priority for future studies because sub-populations could represent novel targets for corrective treatment.

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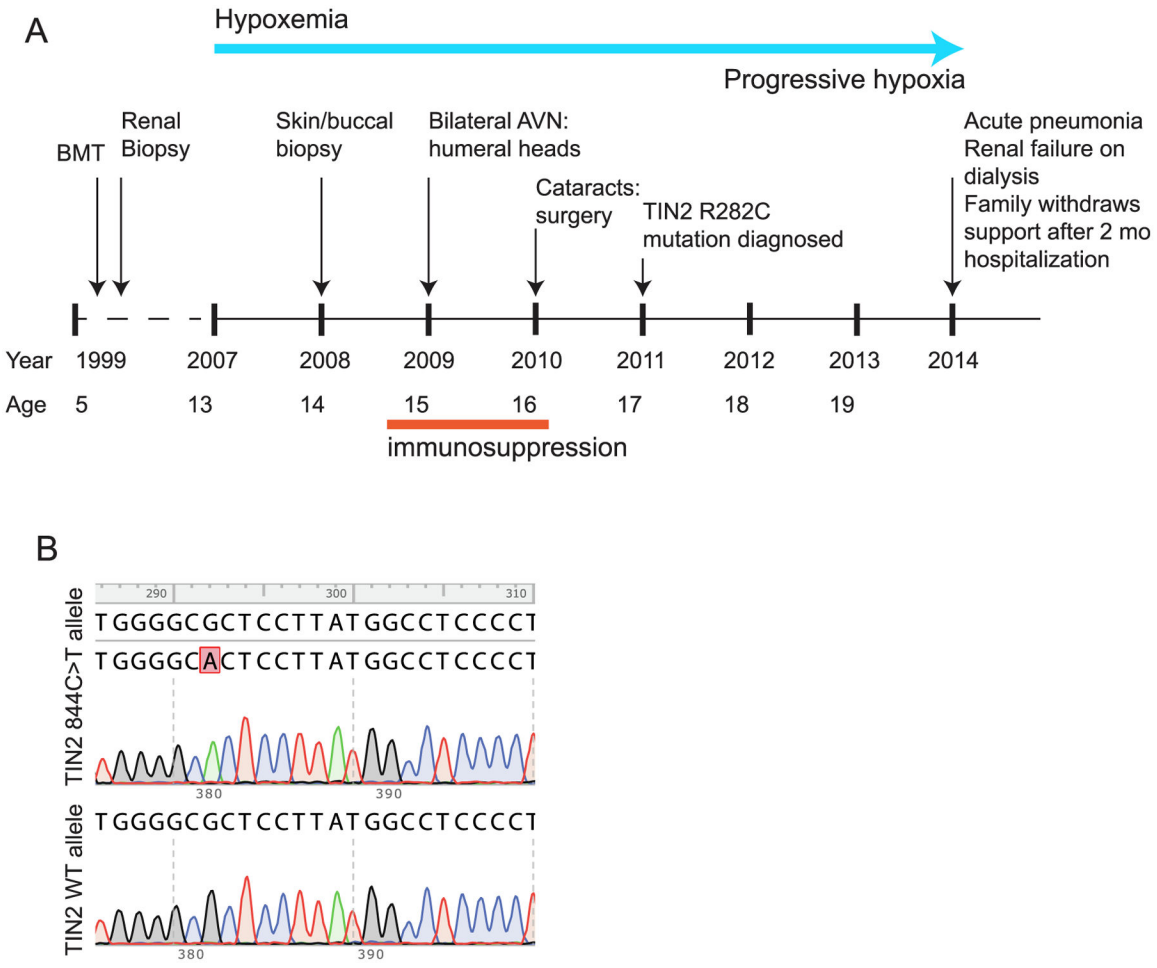


**Fig. 1. Summary of clinical history and genetic diagnosis.**

A. Timeline of patient's clinical course with the year and the age of patient indicated. BMT, bone marrow transplant; AVN, avascular necrosis; red bar, indicates timing and duration of immunosuppression. B. DNA chromatogram obtained by Sanger sequencing. Representative traces from segments of patient's wild-type and mutant alleles are shown for the TINF2 locus.

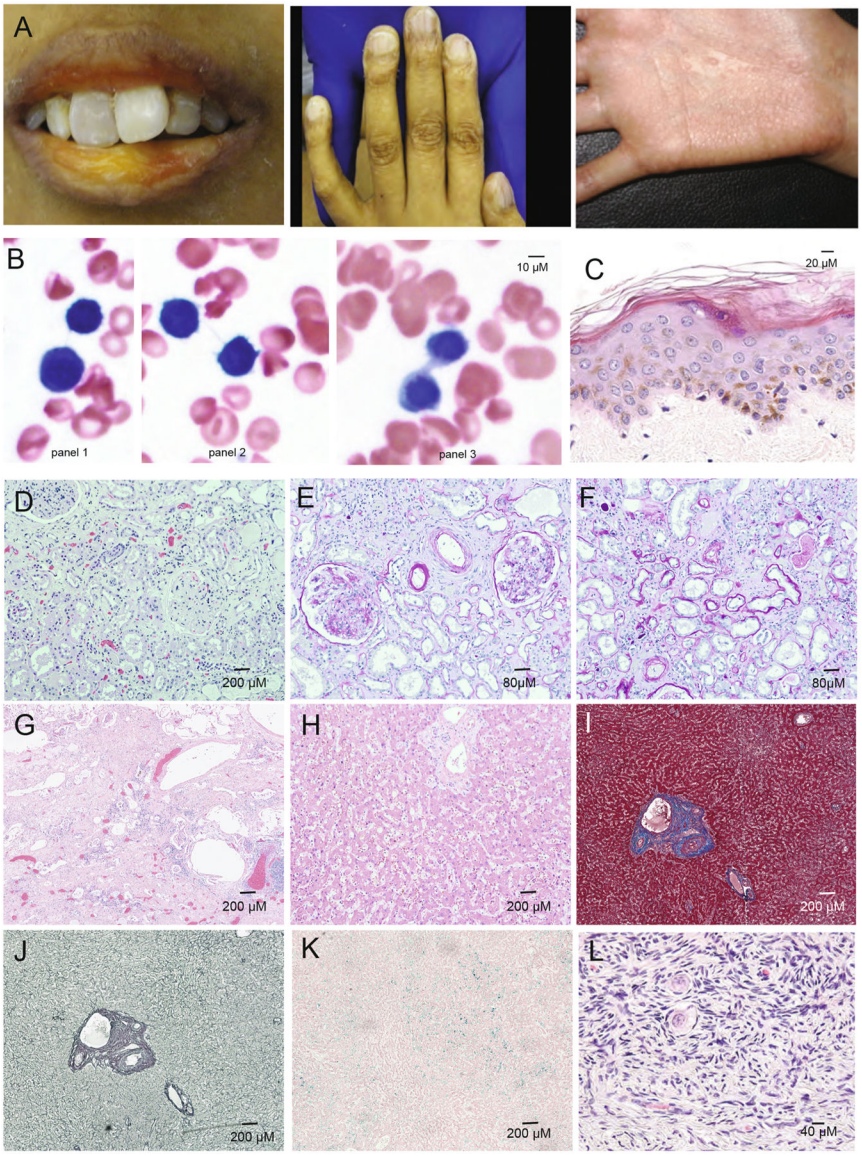


**Fig. 2. Clinical and histologic features.**

A. Photographs taken at autopsy of front incisors (left), nails (center), and palmar skin (right panel). B. Blood smears from bone marrow aspirate showing nuclear (panels 1,2) and cytoplasmic (panel 3) bridging between erythrocytes (scale bar, 10 μm in panels 1–3). C. Hematoxylin & eosin (H&E) stain of skin showing hyperkeratosis with hyperpigmentation of the basal keratinocytes (bar, 20 μm). D. H&E stain of kidney at autopsy showing tubular atrophy (bar, 200 μm). E, F. Periodic acid-schiff (PAS) stain of kidney at autopsy showing intimal sclerosis (bar, 80 μm). G. H&E stain of lung (right upper lobe) at autopsy showing fibrosis (bar, 200 μm). H. H&E stain of liver at autopsy showing mild expansion of the sinusoidal spaces (bar, 200 μm). I, J. Trichrome (I) and Reticulin (J) stains of liver at autopsy showing mild to minimal centrilobular fibrosis (bar, 200 μm). K. Iron stain of liver at autopsy showing moderate iron deposition (bar, 200 μm). L. H&E stain of ovary at autopsy showing sparse follicles with limited follicle maturation (bar, 40 μm).

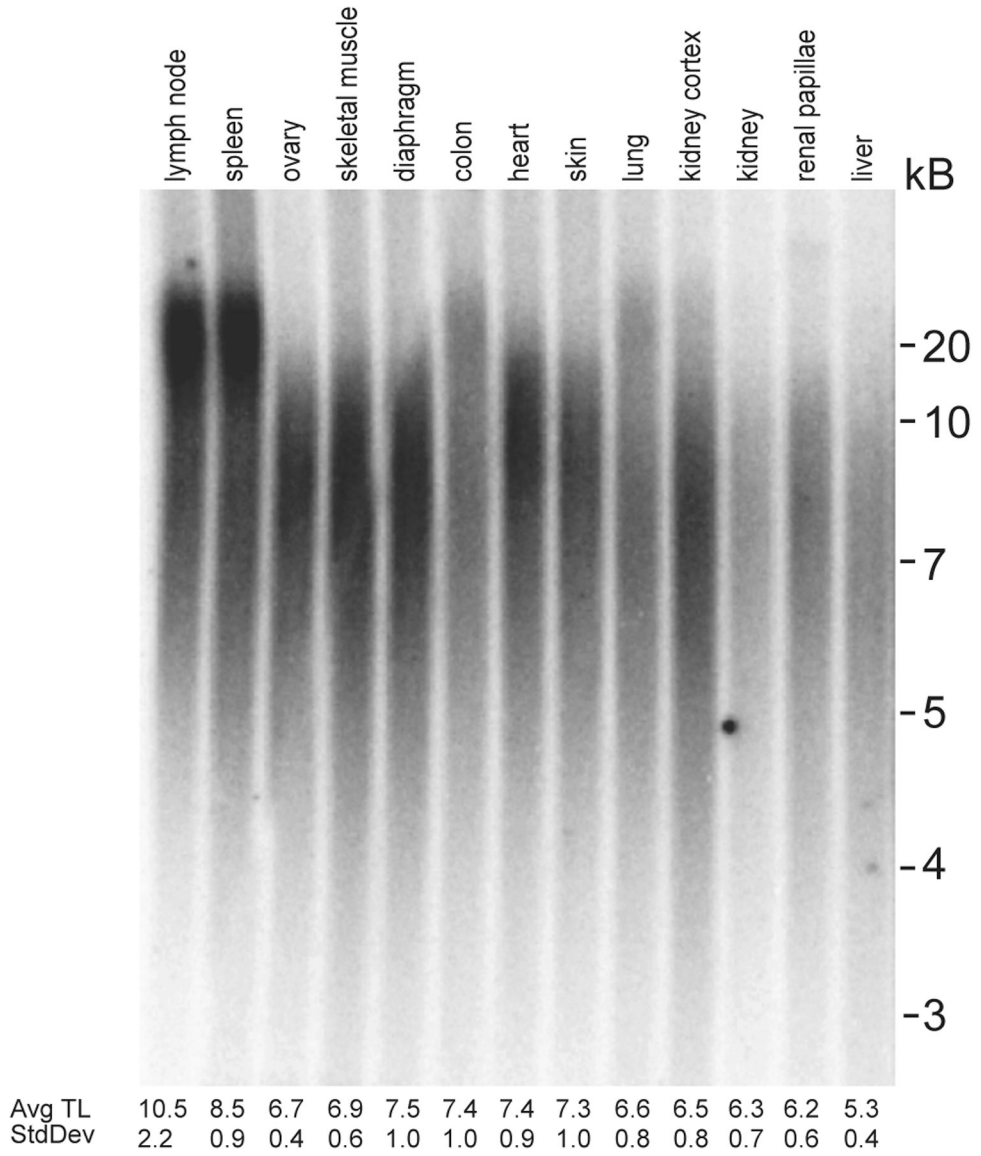


**Fig. 3. Telomere restriction fragment (TRF) analysis of patient DNA.**

A. Average telomere length (TL) is derived from two technical replicates and expressed in kilobases (kB).

## Table 1

Summary of histopathologic findings including pre-autopsy clinical findings and autopsy findings.

| **Pre-Autopsy Findings** | |
|---|---|
| Bone Marrow | MDS *, aplastic anemia, erythroid cytoplasmic bridging |
| Skin | Hyperkeratosis, hyperpigmentation of the basal keratinocytes, areas of netlike pigmentation with melanophages in the upper dermis, atrophy of the epidermis |
| Autopsy Findings Colon | Thinned mucosa, shortened crypts, hypoplasia (total length ~ 30% of normal) without villous atrophy |
| Small intestine | Shallow crypts with increased Paneth cells and lack of intervening crypt progenitor cells |
| Esophagus | Minimal chronic esophagitis |
| Stomach | No significant abnormality |
| Bone | Pagetoid growth pattern; abnormal lamellar pattern in vertebral bone with immature osteogenesis |
| Lung | Extensive diffuse alveolar damage, patchy fibrosis, increased numbers of dilated thin walled pulmonary vessels |
| Ovaries | Occasional primordial follicles without folliculogenesis |
| Heart | LVH ** with Kawasaki-like coronary arteries |
| Skeletal muscle | No significant abnormality |
| Liver | Centrilobular fibrosis and mild to moderate iron deposition |
| Kidney | Glomerular mesangiolysis and sclerosis, Tubular atrophy and fibrosis, blood vessel intimal sclerosis and arterial hyalinosis |
| Pancreas | Normal acini and ducts without inflammation or fibrosis |
| Adrenal | No significant abnormality |
| Lymphoid and Spleen | Spleen with rare extramedullary hematopoiesis; lymph nodes normal |

* MDS: myelodysplastic syndrome.

** LVH: left ventricular hypertrophy.