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Progression of liver disease and portal hypertension in dyskeratosis congenita and related telomere biology disorders

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ABSTRACT

Background & Aims: Dyskeratosis congenita (DC) and related telomere biology disorders (TBD) are characterized by very short telomeres and multi-system organ involvement including liver disease. Our study aimed to characterize baseline hepatic abnormalities in patients with DC/TBD and determine risk factors associated with liver disease progression.

Approach and Results: Retrospective review of a cohort of 58 patients (39 males) with DC/TBD who were prospectively evaluated at a single institute from 2002-2019. The median age at initial assessment was 18 (1.4-67.6) years and median follow-up duration was 6 (1.4-8.2) years. Patients with autosomal or X-linked recessive inheritance and those with heterozygous *TINF2* DC were significantly younger, predominantly male, and more likely to have DC-

associated mucocutaneous triad features and severe bone marrow failure compared with autosomal dominant-non-*TINF2* DC/TBD patients. Liver abnormality (defined at baseline assessment by laboratory and/or radiological findings) was precent in 72.4% of patients with predominantly cholestatic pattern of liver enzyme elevation. Clinically significant liver disease and portal hypertension developed in 17.2% of patients during the six-year follow-up, this progression was mainly seen in patients with recessive or *TINF2*-associated DC. Significant risk factors associated with progression included the presence of pulmonary or vascular disease.

Conclusion: Our experience shows a high prevalence of cholestatic pattern of liver abnormality with progression to portal hypertension in patients with DC/TBD. Presence of pulmonary and/or vascular disease in patients with recessive or *TINF2* DC were important predictors of liver disease progression suggesting the need for increased vigilance and monitoring for complications in these patients.

INTRODUCTION

Dyskeratosis congenita (DC) and related telomere biology disorders (TBD) include a spectrum of illnesses characterized by very short telomeres (<1st percentile for age). Telomeres, the nucleoprotein complex located at chromosome ends, are essential for maintaining genome stability. Pathogenic or likely pathogenic germline variants in at least 18 genes important in telomere biology (*DKC1, TERC, TERT, NOP10, NHP2, ACD, TINF2, POT1, CTC1, STN1, WRAP53, RTEL1, PARN, NAF1, ZCCHC8, RPA1, DLCRE1, NPM1*) have been reported to date (1). These variants can be inherited either in X-linked recessive (XLR), autosomal recessive (AR), or autosomal dominant (AD) patterns or may occur *de novo* (2, 3). The classical DC-phenotype consists of the mucocutaneous triad of dysplastic finger- and toenails, oral leukoplakia, and lacy reticular skin pigmentation. However, the mucocutaneous triad may be subtle, absent, and/or progressive with age (4). Recent advances in understanding the pathophysiology of telomere-associated disease have led to the recognition of a spectrum of related clinical features affecting multiple organ systems, complicating the diagnosis of DC/TBD (2). Notably, young patients may present with bone marrow failure (BMF) and classic DC mucocutaneous changes, whereas adults may be identified with isolated BMF, pulmonary fibrosis (PF), or liver disease (5-8). Family members with mutations in TBD-associated genes may display genetic anticipation, with the disease presentation at an earlier age in subsequent generations (9, 10).

The prevalence of liver disease in patients with known DC is estimated to be approximately 5-10%, but this has not been systematically studied (11, 12). Hepatic complications such as liver

fibrosis, cirrhosis, nodular regenerative hyperplasia leading to non-cirrhotic portal hypertension have been described in patients with DC/TBD depending on the mode of disease inheritance, gene mutation, other organ system involvement and possible interplay of TBD with exogenous factors (7, 12, 13). We recently analyzed the phenotypic spectrum of DC/TBD and noted severe liver disease (defined as fibrosis/cirrhosis, and/or portal hypertension) in predominantly young patients with AR, XLR and *TINF2*-associated DC/TBD (10). Liver fibrosis mainly reported in adult patients with DC/TBD often co-exists with pulmonary fibrosis (7) and may be associated with heterozygous variants in *TERT, TERC*, or *RTEL1* (7, 8, 13, 14) or hemizygous *DKC1* mutations (15). The disease may progress over time and require liver transplantation (7, 16-18). There are also reports of younger DC patients presenting with hepatopulmonary syndrome (HPS) (12), or life-threatening gastrointestinal bleeding in the context of liver dysfunction (19).

A study by Kapuria *et al* screened 100 patients (mostly adults) with DC/TBD for liver disease and reported liver enzyme elevations and ultrasound abnormalities in 40 patients. The pathogenic or likely pathogenic (P/LP) germline variants identified in these patients were *TERT*, *TERC* and *TINF2* (13). Another study that included patients with end-stage liver disease awaiting liver transplantation, identified heterozygous P/LP variants in *RTEL1, TERT, TINF2,* or *NHP2* in 18 (20%) of the 86 patients studied (20).

The aim of our present study is to characterize baseline hepatic abnormalities in a uniformly evaluated cohort of patients with DC/TBD, examine the trends in relation to the genetic background, and characterize the risk factors associated with liver disease progression.

METHODS

Study cohort and design

This is a retrospective and prospective observational study of individuals participating in the National Cancer Institute's (NCI) Institutional Review Board-approved longitudinal cohort study "Etiological Investigation of Cancer Susceptibility in Inherited Bone Marrow Failure Syndromes (IBMFS)" (ClinicalTrials.gov identifier: NCT00027274; https://marrowfailure.cancer.gov). The IBMFS study enrolls patients with DC/TBD and follows their clinical course longitudinally (21). All patients or their legal guardians sign informed consent at enrolment in accordance with the Health and Human Services regulation 45 CFR 46. A subset of participants is invited to the National Institutes of Health Clinical Center (NIH CC) to undergo detailed clinical evaluations. For the current report, all patients with DC/TBD evaluated at the NIH CC between January 2002 and November 2019 were included. All patients underwent laboratory testing including complete blood count, hepatic panel, creatinine, albumin, international normalized ratio (INR) as part of their evaluations at the NIH CC. All patients had ultrasound of the liver and spleen at baseline as part of their clinical evaluation and then as clinically indicated. Patients were cared for and followed locally by their primary teams. We obtained and reviewed biennial follow-up forms completed by patients or their proxies, and medical records through December 2020. We recorded data on demographics, clinical and genetic characteristics, medications, laboratory and imaging findings, liver biopsies, and clinical outcomes.

Definition of liver abnormality

We defined liver abnormality as the presence of elevated liver enzymes and/or abnormal findings on liver imaging at the time of initial evaluation. Liver enzyme elevation was defined by alanine aminotransferase (ALT) levels above the sex-related upper limit of normal (ULN). We used 25U/L and 33U/L as ULN for ALT for women and men, respectively (22). Patients with suspected drug-induced liver injury were not considered to have liver enzyme elevation as those changes are transient and usually resolve following discontinuation of the drug.

Imaging studies included ultrasound (US) of the liver in all participants and computed tomography (CT) or magnetic resonance imaging (MRI) when clinically indicated. All US, MRI and CT images were reviewed by an expert radiologist. Liver size, contour, echogenicity, echotexture, portal vein flow and velocity, and spleen size were recorded for US images. CT and MRI were used to identify collateral circulation around the spleen and to characterize any hepatic lesions seen on US.

Clinically significant liver disease or severe liver disease was defined as development of portal hypertension and its complications including splenomegaly, ascites, variceal hemorrhage, portal hypertensive gastropathy, spontaneous bacterial peritonitis, hepatorenal syndrome, hepatic hydrothorax, hepatopulmonary syndrome, and portopulmonary hypertension. A subset of patients underwent liver biopsies for clinically indicated reasons. All liver biopsies were reviewed by a single expert hepatopathologist (DK) for this study. The biopsies were scored for the degree of necroinflammation, fibrosis, and co-existence of other liver disease (23).

Definitions of DC/TBD and clinical features

The diagnosis of DC was based on the presence of at least two mucocutaneous triad features or one triad feature plus bone marrow failure (24), and/or very short telomeres (<1st percentile for

age) in peripheral blood lymphocyte subsets by flow cytometry with fluorescent *in situ* hybridization (flow FISH) (25), and confirmed by identification of germline pathogenic variants in DC/TBD genes in the majority of patients.

DC-associated mucocutaneous triad was defined as the presence of two or all three (2-3) features of dysplastic nails, lacy reticular pigmentation of the skin and oral leukoplakia. Lymphocyte telomere length was classified as very short if it was less than the 1st percentile and short if it was between the 1st and 10th percentile. Telomere length z-scores were calculated for comparison across ages (26).

Bone marrow failure (BMF) was diagnosed by the presence of bone marrow hypocellularity on bone marrow biopsy specimen and categorized as non-severe if peripheral blood absolute neutrophil count (ANC) was between 500 - <1500/mm^3, platelet count between 20,000 - <150,000/mm^3 and/or hemoglobin (Hb) ≥ 8g/dl, and severe if ANC was < 500/mm^3, platelets < 20,000/mm^3 and/or Hb < 8g/dL. BMF was classified as severe irrespective of laboratory values if a patient had received hematopoietic cell transplantation (HCT), regular red cell and/or platelet transfusions, if androgen treatment had been initiated for BMF, or if myelodysplastic syndrome or leukemia had been diagnosed.

Vascular disease was reported as present if pulmonary arteriovenous malformations (PAVMs) or gastrointestinal telangiectasias were noted. Pulmonary disease was classified as present if a patient had pulmonary fibrosis or PAVMs (Supplementary Table 1).

Statistical analysis

Statistical analysis was performed using GraphPad Prism Version 9.2.0 and SAS version 9.4. Descriptive statistics of baseline demographic data are presented as frequencies for categorical variables and mean with standard deviation for continuous variables. Comparison of clinical and biochemical characteristics of patients with and without complications was performed using Fisher's exact t-test for categorical variables and Mann-Whitney test for non-parametric continuous variables. Comparison of clinical and biochemical characteristics of patients with different types of inheritance was performed using Kruskal Wallis test and significant variables were further assessed by pairwise multiple comparison testing. Factorial logistic regression analysis using Fisher's exact test was performed to understand the association of risk factors for liver disease progression. P value <0.05 was considered as statistically significant.

RESULTS

Fifty-eight patients with DC/TBD were included in the analysis. Baseline characteristics of the patients are described in Table 1. The median age at study was 18 years with a range of 1.4 to 69 years. Patients were followed for a median of 6 years (interquartile range 1.4-8.2 years). Thirty-eight patients (65.5%) had at least two mucocutaneous triad features. Thirty-three patients (56.8%) had severe BMF, 15 (25.8%) had non-severe BMF and 10 (17.2%) did not have any hematological abnormality. A total of 26 patients received HCT for severe BMF: five had received HCT 3-7 years prior to NIH evaluation and 21 underwent HCT 0.2-6.9 years after their NIH visit. Lymphocyte telomeres were very short (<1st percentile for age) in 50 patients, short (1-10 percentile for age) in four patients and not tested in four patients. The most frequently

affected genes were *RTEL1, TINF2, DKC1, TERC*, and *TERT* with pathogenic variants in 20.7%, 19%, 17.2%, 15.5%, and 12.1% of the patients, respectively. Seventeen patients (29.3%) had AD DC/TBD due to a pathogenic variant in *TERC, TERT*, or *RTEL1*, 11 (19.03%) had *TINF2* associated DC/TBD, and 25 (43.1%) had AR/XLR disease due to biallelic pathogenic variants in *RTEL1, TERT, ACD, PARN* and *WRAP53* or hemizygous variants in *DKC1*. Five patients did not have a pathogenic variant in any known DC/TBD gene identified by clinical sequencing.

Liver abnormalities

Elevated liver enzymes and/or abnormalities on liver imaging studies were identified in 42 patients (72.4%) at the initial NIH evaluation, 18 of these patients had no or non-severe BMF and 20 had severe BMF.

Liver enzymes

Liver enzymes were elevated in 18 patients after excluding 6 patients who had elevated liver enzymes in association with anabolic steroid treatment for severe BMF. Five patients with liver enzyme elevation had received HCT prior to the study. Using R factor (27) to distinguish the pattern of liver enzyme elevation, 9 patients had a predominantly cholestatic pattern, four had hepatocellular pattern and five patients had a mixed pattern. Eleven patients with liver enzyme elevation had imaging abnormalities while seven patients had isolated liver enzyme elevation (Table 2).

Imaging

Thirty-four of the 42 patients with liver abnormality had positive findings on liver ultrasound which included patchy or diffuse heterogenous coarsened liver echotexture in 26 and increased hepatic echogenicity in 19 patients (Table 2); two of these patients had received HCT prior to the study. Eleven patients had both the findings of coarsened echotexture and increased echogenicity while 15 had only coarsened echotexture and 8 had only increased echogenicity. Twenty-three patients with imaging abnormality had normal liver enzymes. Lymphocyte telomeres were very short (<1st percentile) in 32 patients and short (1st-10th percentile) in two of 34 patients with imaging abnormalities.

Liver abnormalities associated with patient characteristics

We compared the demographic and clinical characteristics of patients with liver abnormality or no liver abnormality at the time of baseline evaluations at NIH and found that they were similar as shown in Table 3. Specifically, there was no difference in the age, presence, or absence of 2-3 mucocutaneous triad features, severe BMF or history of HCT, telomere length Z-score, and laboratory parameters such as AST, ALT, ALP, total bilirubin, direct bilirubin, albumin, GGT and AFP between the two groups (Table 3).

Liver abnormalities and clinical characteristics in relation to DC/TBD inheritance

The clinical features at the time of evaluation at NIH in relation to the mode of inheritance as AR/XLR, *TINF2*, AD-non-*TINF2* or gene unknown DC/TBD, are described in Table 4. Patients with *TINF2* DC were analyzed separately from the AD non-*TINF2* DC/TBD because *TINF2* variants which can be inherited as AD or occur *de novo* are associated with more severe phenotypes than AD-non-*TINF2* disease (4, 28). We compared the presence of liver abnormality

and other clinical characteristics between the groups. The five patients with unknown genetic etiology were excluded from the analyses based on inheritance pattern. There was no association between liver abnormality and inheritance pattern. In the univariate analysis, we noted significant differences in age, gender, follow up duration, presence of 2-3 DC triad features and severe BMF between patients with *TINF2* or AR/XLR DC compared with AD-non-*TINF2* DC/TBD. Patients with *TINF2* and AR/XLR DC were significantly younger, predominantly male, had 2-3 DC triad features and severe BMF compared with patients with AD-non-*TINF2* DC/TBD. These differences persisted in pairwise multiple comparison testing between AD-non-*TINF2* DC/TBD and the other two groups comprising *TINF2* and AR/XLR DC.

Progression of liver disease

Imaging abnormalities

During the follow-up of over 8 years, 10 patients reported having an MRI scan of the liver for clinical suspicion of liver disease; four of these patients had evidence of liver cirrhosis or advanced fibrosis and two had evidence of hemosiderosis on MRI scans.

Development of clinically significant liver disease

Ten of the 58 patients (17.2%) developed portal hypertension at a median follow-up of 7.2 years (range 0-12.8 years) from the initial evaluation. Detailed description of these 10 patients is provided in Table 5. The median age of the patients at baseline was 15.45 years (range 3.7-46.3 years), and six were male. All 10 patients had a clinical diagnosis of DC and had the presence of 2-3 mucocutaneous triad features and severe BMF. Two patients had received HCT prior to NIH evaluation (UPN 204, UPN 231) while six underwent HCT during follow up. Six of the 10

patients had XLR/AR inheritance due to pathogenic variants in *DKC1* (n=2), *RTEL1* (n=2) or *PARN* (n=2), three had *TINF2* associated DC while the gene was unknown in one patient. One patient had no evidence of pre-existing liver abnormality at baseline evaluation, six had both liver enzyme elevation and ultrasound abnormality including splenomegaly suggestive of portal hypertension in one (UPN 167), and one had histological evidence of cirrhosis (UPN 288). Seven patients developed complications from portal hypertension: 5 patients developed HPS, one patient developed variceal hemorrhage and one patient had spontaneous bacterial peritonitis. Of the 5 patients who developed HPS, two died before referral for liver transplant evaluation, three were referred for liver transplant evaluation, two of whom underwent orthotopic liver transplant, the third was alive at last follow-up and had not yet been transplanted. In addition to portal hypertension, seven patients developed pulmonary disease with evidence of pulmonary fibrosis on CT scan. Seven patients had associated vascular complications, 2 of these patients had gastrointestinal arteriovenous malformation, 3 had pulmonary arteriovenous malformation and 2 had esophageal varices. Two patients with HPS who had received HCT 5 years (UPN 279-2) and 10 years (UPN 216) prior, **and had undergone** liver transplantation, were alive without complications at 22- and 12-months post-liver transplant, respectively. One other patient with HPS who has not received liver transplant was alive at last follow up (UPN 291). Seven of the 10 patients with severe liver disease have died, one from complications of spontaneous bacterial peritonitis while waiting for liver transplant (UPN 382), two patients died due to HPS, two patients developed oral squamous cell carcinoma and died, one died from pulmonary fibrosis and one from complications related to HCT.

Histological findings

Eleven patients underwent a liver biopsy for clinical diagnostic purposes (Table 5). Four patients had Knodell 1 fibrosis at initial biopsy (mainly periportal and focal pericellular fibrosis), one had Knodell 0 fibrosis, and one had cirrhosis (the liver biopsy slides on this patient were not available for review at NIH), two patients had hemosiderosis, and three patients did not have any concerning findings. One patient who had mild fibrosis on initial biopsy, died two years later from progressive HPS (UPN 145). Liver histopathology on the autopsy specimen showed obliterative portal venopathy with mild inflammatory activity and bridging fibrosis associated with features of nodular regenerative hyperplasia (Figure 1). Liver histopathology of explant specimen on two patients (UPN 297-2 and UPN 216) who had liver transplant for HPS also showed nodular regenerative hyperplasia with mild inflammation and perisinusoidal fibrosis.

Factors associated with the progression of liver disease

To further understand the association of risk factors in liver disease progression, we performed a univariate and multivariate analysis using logistic regression model. Since all patients with portal hypertension had severe BMF and 2-3 DC triad features, these factors were not evaluated as analysis covariates. Additionally, only patients with XLR/AR and *TINF2* DC were included in this analysis because none of the patients with AD DC/TBD had portal hypertension in our cohort. We analyzed the association between baseline liver abnormality and male gender (to discern the association with X-linked *DKC1* inheritance), pulmonary disease, vascular disease, and inheritance pattern in the development of portal hypertension among patients with *TINF2* versus XLR/AR DC. We found a statistically significant association between the presence of pulmonary disease, vascular disease, and development of portal hypertension in the univariate analysis (Table 6). No significant association was noted between other variables such as gender

or DC inheritance pattern in the progression of liver disease. Only vascular disease retained significance in the multivariate analysis.

Mortality

Overall, 23 of the 58 patients (39.6%) in our cohort have died during follow up. The most common causes of death were complications related to BMF or HCT in 7/23 (30.4%), pulmonary fibrosis in 6/23 (26%), solid tumor malignancies in 4/23 (17.3%) followed by deaths attributed to liver disease or complications related to liver disease in 3/23 (13%) patients as shown in Supplementary Table 2.

DISCUSSION

In this longitudinal cohort study, we comprehensively evaluated liver abnormalities and progression to liver disease in predominantly young patients with DC/TBD in relation to the genetic etiology and inheritance pattern. We found a high prevalence of liver abnormality in our cohort compared with other published studies although the largely cholestatic pattern of liver enzyme elevation was similar to that noted previously by Kapuria et al (13). Unlike the study by Kapuria et al which mainly included adult patients with TBD with a median age of 42 years, our cohort of primarily young patients (median age 18 years) was biased towards clinically severely affected children, which may explain a higher occurrence of liver abnormalities in the context of more severe organ involvement with multisystem disease characteristic of younger DC/TBD patients (10).

Given the higher prevalence of liver involvement in our cohort, we did not find any significant difference in demographic and laboratory parameters between patients with and without liver abnormality. It is possible that the presence of extremely short telomeres associated with *TINF2* or recessive inheritance likely contributes to the high prevalence of liver abnormalities/disease in this young cohort. Hepatic steatosis which was noted in 31% of our cohort, could be due to either metabolic-associated or alcohol-associated fatty liver disease. However, given the younger age (median age 18 years) and low mean BMI of the cohort, alcohol and metabolic associated fatty liver disease are unlikely. We cannot exclude the contribution of iron overload in increased echogenicity as 6 of the 18 patients with increased echogenicity had received more than 20 red blood cell transfusions and had high serum ferritin levels (>1,000 ng/mL). Hemosiderosis was also noted on liver biopsy in 2 patients. Additionally, liver insults occurring in the lifetime of DC/TBD patients due to medications, chronic transfusions and HCT in the context of BMF may contribute to chronic liver injury which stimulates hepatocellular proliferation, cell turnover and progressive telomere loss, which in turn promotes cell proliferation arrest and apoptosis (29). For example, eight of the 10 patients with liver disease in our cohort had received HCT, a procedure often complicated with liver toxicity. Another possibility may be vascular disease, which has recently been recognized as a major complication in TBDs (19), however the underlying pathophysiology remains unclear to date.

Portal hypertension was found in 17.2% of patients. Of 10 patients with portal hypertension, 7 developed complications from portal hypertension. Of note, all 10 patients presented with a severe DC/TBD phenotype and 9 of them harbored underlying *TINF2* or AR/XLR disease. It has previously been shown that severe DC/TBD manifestations are associated with AR/XLR

inheritance and *TINF2* mutations (10). All 10 patients with portal hypertension had severe bone marrow failure and 8 of them received HCT. A statistically significant association was found between progression of liver disease (i.e., development of portal hypertension) and presence of pulmonary and vascular disease. We found severe liver disease predominantly in younger, severely clinically affected DC/TBD patients. This suggests that liver disease severity may be correlated to the overall phenotype involvement in DC/TBD. However, we lack the numbers to verify this statement statistically. In patients with BMF and TBDs, loss-of-function mutations correlate with a high prevalence of severe hepatic disease, mainly represented by cirrhosis and nodular regenerative hyperplasia (7). Calado et.al. also described telomerase complex mutations associated with shorter telomeres occurred more commonly in sporadic cirrhosis than in age matched controls, suggesting telomere dysfunction as a molecular event in the pathophysiology of cirrhosis (14).

In our cohort, 2 patients received liver transplant following HCT and were alive at last contact, however the post-transplant follow up period was less than 2 years in both cases. The literature on liver transplantation in DC/TBD patients is currently still sparse with limited follow up data. There are few case reports describing the success of liver transplantation in patients with DC and cirrhosis, HPS, and numerous co-morbid conditions (30-33). However, they only reported the short-term outcomes of liver transplant with no follow up after. In a population of patients with liver cirrhosis awaiting liver transplantation, P/LP TBD gene variants were more prevalent than general population and was found to be associated with lower baseline platelet and WBC counts and a longer posttransplant length of stay (20).

To our knowledge, we here present the largest study of liver disease in a cohort of young DC/TBD patients to date. A strength of our study is that we followed patients over several years (median period of 6 (1.4-8.2) years), thus were able to evaluate liver disease progression over time. While providing extensive medical data, the limitations of our study include its retrospective nature, referral bias, small sample size, and partly missing/incomplete data received during follow up. We found severe liver disease specifically in patients with distinct DC/TBD clinical features and underlying AR/XLR or *TINF2* inheritance pattern. We suggest carefully monitoring this patient subgroup for worsening of liver disease and progression to portal HTN. Moreover, we found a common occurrence of any liver abnormalities in all DC/TBD patients, irrespective of age. This warrants the need for large prospective studies to better understand the nature of liver affections and its progression to clinically significant liver disease in DC/TBD individuals and explore treatment options.

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Figure 1: Representative hepatic histological changes

Fibrosis and nodular regeneration observed at autopsy in a 16-year-old white male patient with de novo TINF2 mutation who progressed to portal hypertension. A. A small regenerative nodule (arrows) consistent with nodular regenerative hyperplasia (H&E, 100x). B. Many portal areas lacked a normal portal vein, as in this portal area, where only small vein profiles are seen within the portal area. Larger veins which may either represent abnormally placed portal or central veins are seen outside the portal area in the adjacent parenchyma. (H&E, 200x). C. Irregular periportal fibrosis that focally extended between portal area is seen. The fibrosis also extended outward to involve the sinusoids, trapping the periportal hepatocytes. (Masson trichrome, 40x). D. The reticulin stain highlights the regenerative nodularity of the parenchyma (Reticulin, 100x).

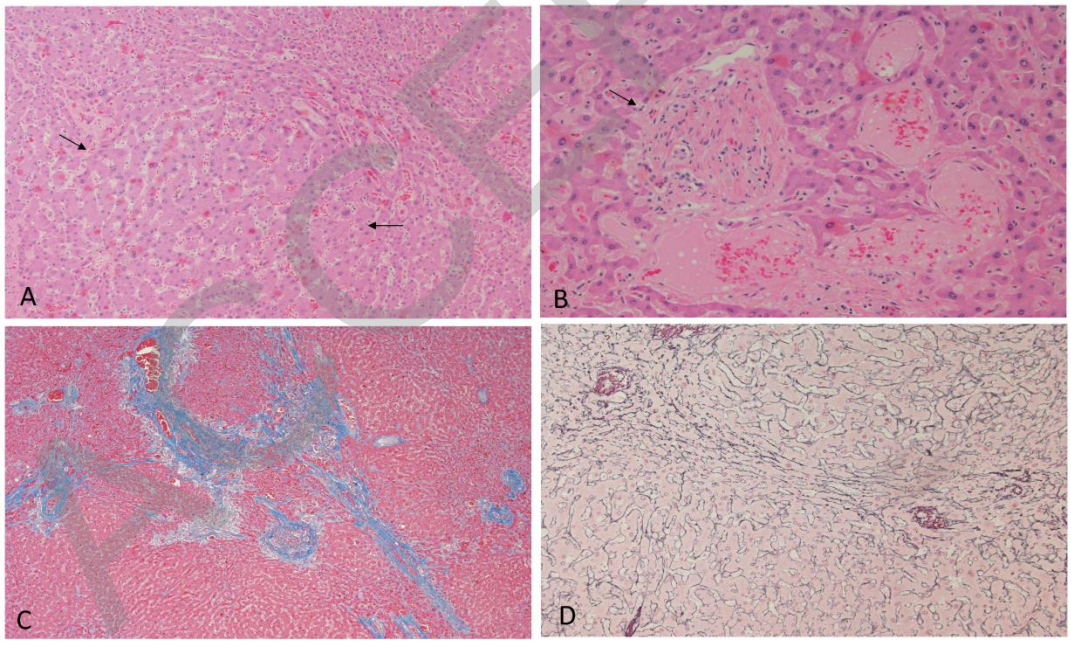

Table 1: Baseline demographics of the cohort

Number of patients (n)	**58**
Age at study (years)	18 (10-28)[1]
Follow up duration (years)	6 (1.4-8.2)
Males [n (%)]	39 (67.2%)
Race [n (%)]	
White	50 (86.2%)
Non-white	6 (10.3%)
Unknown	2 (3.4%)
Number of DC triad Features	
0	7
1	13
2	18
3	20
Number with BMF	
No BMF	10
Non-severe BMF	15
Severe BMF	33
Number with Lymphocyte Telomeres[2]	
Telomere length Z score	-4.2 (3.2-5.6)[1]
Short (1st-10th percentile)	4
Very short (<1st percentile)	50

Gene Affected [n (%)]	
***RTEL1* (AD=3 AR=9)**	12 (20.7%)
***TINF2* (AD)**	11 (19.0%)
***DKC1* (XLR)**	10 (17.2%)
***TERC* (AD)**	9 (15.5%)
***TERT* (AD=5, AR=2)**	7 (12.1%)
***PARN* (AR)**	2 (3.4%)
***ACD* (AR)**	1 (1.7%)
***WRAP53* (AR)**	1 (1.7%)
Unknown	5 (8.6%)

[1]Median with Interquartile range

[2]4 patients did not have telomere testing

DC: dyskeratosis congenita; BMF: bone marrow failure; AD: autosomal dominant; XLR: X-linked recessive; AR: autosomal recessive; n: number

Table 2: Characterization of liver enzymes and imaging abnormalities

Liver abnormalities	**Number (%)**
Number of patients with liver abnormality[$]	42 (72.4%)
Number with liver enzyme elevation	18 (31%)
Pattern of elevation	
Cholestatic [n]	9
Hepatocellular [n]	4
Mixed [n]	5
Number with liver enzyme elevation and imaging abnormalities	11
Number with liver imaging abnormalities on ultrasound	34 (58.6%)
Hepatic steatosis alone [n]	8
Coarsened echotexture alone [n]	15
Both steatosis and coarsened echotexture [n]	11

[$] Liver abnormality indicates either liver enzyme elevation or imaging abnormalities or both

Table 3: Comparison of baseline patient characteristics in relation to liver abnormality

Parameter	**Liver abnormality**	**No liver abnormality**	**P-value**
Number	42	16	
Age (years)	21±15	23±18	0.88

Male, [n (%)]	29 (69)	10 (88)	0.75
Follow up (years)	8.5±16	4.2±3.9	0.1
2-3 DC triad features [n (%)]	26 (61.9)	10 (62.5)	0.52
Severe BMF [n (%)]	20 (47.6)	5(31.3)	0.37
Telomere length Z-score (mean ± SD)	-4.2±1.5	-4.6±1.4	0.36
AST (U/L) (mean ± SD)	39±29	30±17	0.5
ALT (U/L) (mean ± SD)	59±137	45±41	0.25
ALP (U/L) (mean ± SD)	156±114	131±88	0.66
Total Bilirubin (mg/dL) (mean ± SD)	0.7±0.5	0.8± 0.4	0.58
Direct Bilirubin (mg/dL) (mean ± SD)	0.2±0.1	0.2±0.2	0.53
GGT (U/L) (mean ± SD)	38±34	45±63	0.56
Albumin (g/dL) (mean ± SD)	4.0±0.4	4.0±0.7	0.3
Alpha-fetoprotein (ng/ml) (mean ± SD)	2.2±1.8	1.5±0.6	0.21

BMF: bone marrow failure; U/L: units per liter; AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase; GGT: gamma glutamine transferase; SD: standard deviation

Table 4: Comparison of clinical characteristics based on inheritance pattern

Parameters	XLR/AR	*TINF2*	AD-non-*TINF2*	Unknown[1]	P value
Number (%)	25 (43.1%)	11 (18.9%)	17 (29.3%)	5 (8.6%)	
Age at study (mean ± SD)	17.2±10.7	16.6±9.6	31.3± 19.9	11±4.1	0.02*
Males	20 (80%)	9 (81.8%)	7 (41.1%)	3 (60%)	0.01*
Follow up in years (mean ± SD)	5.3±4	9.2±4.8	3.4±3.5	7.2±1.1	0.004*
Deceased at last follow up	11 (44%)	5 (45.4%)	6 (35.2%)	1 (20%)	0.97
Liver abnormality	18 (72%)	10 (90.9%)	10(58.8%)	4 (80%)	0.83
Severe DC triad features	17 (68%)	10 (90.9%)	6 (35.3%)	5 (100%)	0.007*
Pulmonary disease	6 (24%)	6 (54.5%)	7 (41.2%)	1 (20%)	0.18
Vascular disease	6 (24%)	2 (18.2%)	1 (5.8%)	1 (20%)	0.31
Severe BMF	18 (72%)	8 (72.7%)	6 (35.3%)	1 (20%)	0.04*

[1] Unknown group was excluded from the analysis due to small sample size.

*Significant p-values for univariate analysis

XLR: X-linked recessive; AR: autosomal recessive; AD: autosomal dominant; SD: standard deviation; DC: dyskeratosis congenita; BMF: bone marrow failure

Table 5: Characteristics of the patients who developed progressive liver disease

Patient	Age at baseline, years	Gender	DC triad	BMF	Gene/ Inheritance	Liver enzyme elevation	Imaging: Liver abnormality	Total Follow-up, years	HCT Age	Pulmonary features	Liver Biopsy	LD complications (interval from baseline)	Outcome, Age, Death cause
UPN 145	9.5	F	severe	severe	*TINF2 /de novo*	Yes	Yes	6.8	10.87	HPS	Knodell-1 fibrosis; HS	pHTN + HPS (3.1 yrs)	D, 16.4 HPS
UPN 156	3.7	M	severe	severe	*TINF2 /de novo*	Yes	Yes	12.8	8.87	PF and HPS	Knodell-1 fibrosis	pHTN + HPS (10 yrs)	D, 16.5, HPS
UPN 167	29.2	M	severe	severe	*DKC1 /XLR*	Yes	Yes	0.3	29.41	PF	Knodell-0 fibrosis; HS	pHTN (baseline)	D, 29.6, HCT complications
UPN 204	12.7	M	severe	Severe, s/p HCT	*TINF2 /de novo*	Yes	No	6.5	4.19	PF, s/p lung transplant	NA	pHTN (5.6 yrs)	D, 19.3, HNSCC
UPN 231	9.8	F	severe	Severe, s/p HCT	*Unknown*	*No*	Yes	7.6	3.89	PF	Knodell-1 fibrosis	pHTN (5.8 yrs)	D, 17.6, PF
UPN 288	46.3	M	severe	severe	*DKC1 /XLR*	Yes	Yes	7.8	No HCT	DLCO-69-70%	Knodell 4 (Cirrhosi	pHTN (2.3 yrs)	D, 54.2, HNSCC

											s)		
UPN 297-2	18.2	F	severe	severe	*RTEL1 /AR*	No	No	8	19.28	PF+HPS	Knodell 1 fibrosis	pHTN+ HPS (6.8 yrs) s/p liver transplant	A, 26.4, s/p liver transplant
UPN 382	28.3	M	severe	severe	*PARN /AR*	Yes	Yes	5	No HCT	DLCO 45-60%	NA	pHTN (4 yrs)	D, 33.2, pHTN – bacterial peritonitis
UPN 291	19.2	M	severe	severe	*PARN /AR*	Yes	Yes	6.2	22.7	PF, PAVM	Knodell 0 fibrosis	pHTN + HPS (2 yrs)	A, 25.3
UPN 216	8.9	F	severe	severe	*RTEL1 /AR*	Yes	No	12.2	9.08	PF, PAVM, HPS	Knodell 3 fibrosis	pHTN+ HPS (5 yrs) s/p liver transplant	A, 21, s/p liver transplant

UPN: unique patient number; HPS: Hepatopulmonary syndrome; pHTN: portal hypertension; Male: M; Female: F; LD: Liver disease; D: Deceased; A: Alive; PF: Pulmonary fibrosis; PAVM: Pulmonary Arteriovenous malformations; HCT: hematopoietic stem cell transplant; HS: Hemosiderosis; HNSCC: Head and neck squamous cell carcinoma

Table 6: Factors associated with liver disease progression – Univariate and multivariate analysis

Factors evaluated	Univariate analysis		Multivariate analysis[1]	
	OR (95% CI)	**P Value**	**OR (95% CI)**	**P Value**
Liver Disease: Yes versus No	4.1 (0.5-35.3)	0.25	17.5 (0.46-667.2)	0.05
Gender: Male versus female	1.47 (0.36-5.97)	0.71	6.7 (0.53-83.8)	0.14
Presence of Pulmonary disease: Yes versus No	6.28 (1.41-28.1)	0.02*	1.2 (0.06-23.2)	0.91
Presence of Vascular disease: Yes versus No	35 (5.86-209.1)	0.0001*	40 (1.8-912.9)	0.02*

DC inheritance: **AR/XLR vs TINF2**[1]	0.84 (0.16-4.3)	1.0	0.9 (0.06- 14.4)	0.98

[1] Sample size of 36, as patients with AD (autosomal dominant) and unknown inheritance were excluded

*Significant p-values are marked with asterisk. OR: odds ratio; CI: confidence interval