Liver pathology and biochemistry in patients with mutations in TRIM37 gene (Mulibrey nanism)

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Abstract

Background and Aims: Mulibrey nanism (MUL) is a multiorgan disease caused by recessive mutations in the TRIM37 gene. Chronic heart failure and hepatopathy are major determinants of prognosis in MUL patients, which prompted us to study liver biochemistry and pathology in a national cohort of MUL patients.

Methods: Clinical, laboratory and imaging data were collected in a cross-sectional survey and retrospectively from hospital records. Liver histology and immunohistochemistry for 10 biomarkers were assessed.

Results: Twenty-one MUL patients (age 1–51 years) with tumour suspicion showed moderate congestion, steatosis and fibrosis in liver biopsies and marginally elevated levels of serum GGT, AST, ALT and AST to platelet ratio index (APRI) in 20%–66%. Similarly, GGT, AST, ALT and APRI levels were moderately elevated in 12%–69% of 17 MUL patients prior to pericardiectomy. In a cross-sectional evaluation of 36 MUL outpatients, GGT, total bilirubin and galactose half-life (Gal½) correlated with age (r = 0.45, p = .017; r = 0.512, p = .007; r = 0.44, p = .03 respectively). The frequency of clearly abnormal serum values of 15 parameters analysed, however, was low even in patients with signs of restrictive cardiomyopathy. Transient elastography (TE) of the liver revealed elevated levels in 50% of patients with signs of heart failure and TE levels correlated with several biochemistry parameters. Biomarkers of fibrosis, sinusoidal capillarization and hepatocyte metaplasia showed increased expression in autopsy liver samples from 15 MUL patients.

Conclusion: Liver disease in MUL patients was characterized by sinusoidal dilatation, steatosis and fibrosis with individual progression to cirrhosis and moderate association of histology with cardiac function, liver biochemistry and elastography.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; CK7, cytokeratin 7; EpCAM, epithelial cellular adhesion molecule; FV, coagulation factor V; Gal½, galactose half-life; GGT, gamma-glutamyltransferase; HSC, hepatic stellate cells; MUL, Mulibrey nanism; NYHA, New York Heart Association; proBNP, pro-B-type natriuretic peptide; PSGL-1, P-selectin glycoprotein ligand-1; SMA, alpha smooth muscle actin; TE, transient elastography; TT, partial thromboplastin time.

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1 | INTRODUCTION

Mulibrey nanism (MUL) is an autosomal recessive disease caused by mutations in the TRIM37 gene encoding the peroxisomal TRIM37 protein with ubiquitin ligase activity. MUL typically causes growth restriction of prenatal onset, typical craniofacial features, infertility and multiple organ manifestations, but no major neurological defects. The patients are thin and gracile in childhood, but from puberty, a tendency towards abdominal obesity, insulin resistance, metabolic syndrome and type 2 diabetes is observed. Moreover, an increased risk for tumours in several organs has been reported. The majority of known MUL cases have been reported from Finland. The Finnish patients are genotypically homogenous as over 90% of the patients carry the founder mutation (Fin-major, c. 493-2A>G) in a homozygous form. Cardiac abnormalities are major determinants of prognosis in MUL disease. Variable signs of pericardial constriction and myocardial restriction may lead to congestive heart failure and right heart disease already at young age. Some patients may benefit from pericardiectomy, but congestive heart failure nevertheless progresses in one-third. Thus, these patients are at a risk for developing liver failure. Clinical experience shows that some MUL patients have hepatomegaly already in early childhood and at the time of diagnosis, 45% of patients display liver enlargement. Moreover, steatosis, fibrosis and blood-filled cysts (peliosis) have been described in MUL livers.

Laboratory tests and non-invasive methods, such as liver stiffness measurement by elastography, give conflicting results of the liver status in patients with chronic heart disease and especially those with congestive heart failure. Also, little is known how the absence of TRIM37 protein affects liver pathology in general. These questions prompted us to study systematically hepatic findings in MUL patients, using microscopy and immunohistochemistry of liver biopsy and autopsy samples, blood biochemistry and transient elastography (TE). The liver findings were associated with the cardiac status of the patients.

2 | PATIENTS AND METHODS

2.1 | Patients

The study comprises four overlapping groups of the Finnish MUL patients. The first group includes 21 stable MUL patients (age range 0.9–59 years), who underwent liver biopsy owing to suspicious ultrasound findings (mostly tumorous lesions). These biopsy findings were retrospectively compared with contemporary liver biochemistry values. The second group comprises 17 patients who underwent pericardiectomy owing to constrictive pericarditis (age range 0.5–38 years). Preoperative liver biochemistry in these patients was retrospectively recorded. The third group is a cross-sectional cohort of 36 MUL patients (age range 0.21–51.1 years), who were clinically stable and had a routine visit at the outpatient clinics of the Helsinki University Hospital. The studies of these patients included blood biochemistry, galactose half-life measurement, liver ultrasound and transient elastography as well as cardiac evaluation. The fourth group includes 22 deceased patients (age at death 0.65–48.7 years), of whom original autopsy reports were available. In addition, 15 of these patients had liver tissue samples available for histological and immunohistochemical analyses.

The inclusion criteria comprised adequate clinical findings and TRIM37 gene mutations. One patient had limited clinical data and was excluded from the analyses. In clinical studies, all except one patient were Fin major (c.493-2A>G) homozygotes. One patient had Fin minor (c.2212delG)/Fin-major genotype. Of the deceased patients, 17 were Fin-major homozygotes, one had Fin-minor/Fin-major genotype, three sisters had c.1166A>T/Fin-major genotype and one had c.227 T>C/Fin-major mutations. All mutations led to truncated TRIM37 protein.

2.2 | Clinical evaluation

MUL patients were clinically followed by experienced paediatricians and paediatric and adult cardiologists, as previously described. The patients were mostly treated and followed at the Helsinki University Hospital, forming the biggest MUL cohort worldwide. Liver ultrasound and transient elastography (TE) (FibroScan®; Echosens) were performed according to standard methods. TE measurement values over 7 kPa were regarded as increased in all age groups. Cardiac evaluation was performed by an experienced cardiologist (EJ). The assessment of heart function was based on clinical signs (hepatomegaly and filling level of the jugular veins), impaired ejection fraction or signs of restrictive physiology on echocardiography. These signs included impaired collapsing of inferior vena cava, retrograde flow of hepatic veins and breathing variation of tricuspid E wave exceeding 30%. Besides echocardiography findings, the plasma proBNP levels were used in the assessment of cardiac dysfunction.
2.3 | Liver biochemistry

Liver biochemistry included blood values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, conjugated bilirubin, albumin, fibrinogen, partial thromboplastin time (TT), coagulation factor V (FV), prealbumin, haemoglobin, leucocytes and thrombocytes. AST to platelet ratio index (APRI) was calculated using a standard formula with a cut-off value of 0.5.17 Galactose half-life test (Gal½) was performed as previously reported, with a cut-off value of 12 min.18 The normal range for each parameter was defined by the HUS Clinical Laboratory, Helsinki University Hospital. The cut-off values were adjusted for age and sex when needed.

2.4 | Liver histology and immunohistochemistry

Liver specimens from MUL patients were obtained by percutaneous core-needle biopsies on clinical indications (21 patients) as well as from autopsies (15 patients). For immunohistochemistry, six samples from liver transplant donors were used as normal controls. These specimens were taken as wedge biopsies at the operation.

Haematoxylin-eosin and Mason's trichrome stainings were used for histological analysis. Immunohistochemical stainings on formalin-fixed autopsy samples were performed as previously reported.19 Immunohistochemical grading was done semiquantitatively by the first authors on a scale of 0–3 for each marker. At least 20 fields per sample were analysed. Central, lobular (sinusoidal) and portal areas were separately scored, except in the staining for CK7, where bile ducts and parenchyma (hepatocytes) were scored separately. Also, search for malignant lesions was performed using EpCAM and glycopen-3 stainings in 15 MUL autopsy livers (and 5 controls). The primary antibodies used for stainings were as follows: alpha smooth muscle actin (SMA) (1:400, Agilent Dako), vimentin (1:100, Agilent), collagen 1 (1:1000, Abnova), CD34 (1:100, Agilent), CD68 (1:400, Cell Signalling Technology), P-selectin glycoprotein ligand-1 (PSGL-1) (1:500, Santa Cruz Biotechnology), cytookeratin 7 (CK7) (1:100, DakoCytomation Norden A/S), EpCAM (BER-EP4, 1:100, Dako) and glypcan-3 (1:100, Invitrogen, Thermo Fisher). Microscopy was performed with Zeiss microscope (Carl Zeiss AG) using 10–40× magnification.

2.5 | Statistics

Statistical analyses were performed by IBM SPSS Statistics 25 (IBM). The normality of the distribution among different groups was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. For comparisons, T-test was used for normally distributed parameters and Mann–Whitney U or independent samples Kruskal–Wallis test for not normally distributed parameters. Correlations were calculated with Spearman’s rho. Two-tailed p-values <.05 were considered statistically significant.

2.6 | Ethical issues

This study was approved by the Institutional Ethical Review Board at the University of Helsinki (approval numbers 420/E7/2001 and HUS/1538/2016/38). Informed consent was obtained from all patients or their guardians. The procedures involving human subjects were in accordance with the Helsinki Declaration of 1975 as revised in 1983 or comparable ethical standards.

3 | RESULTS

3.1 | Liver pathology and biochemistry in patients undergoing liver biopsy

Twenty-one clinically stable MUL patients underwent liver biopsy owing to abnormal ultrasound findings in the clinical follow-up. The indications included suspicion of tumour (n = 13), hepatomegaly (n = 5), abnormal blood biochemistry (n = 1) and suspicion of cirrhosis (n = 1). Eight of the patients were <10 years of age, five between 10 and 20 years and eight over 20 years.

Moderate fibrosis (62%) and steatosis (43%) were observed in all age groups. Two adults (ages 41 and 48 years) had advanced cirrhosis. Congestion or sinus dilatation (38%), biliary tract proliferation (24%) and cholestasis (14%) were present in a minority of the samples. Neoplasia or dysplasia was not found in any of the biopsies, indicating that ultrasound revealed vascular lesions which are common in MUL. The benign nature of the lesions was supported by the fact that none of the patients undergoing biopsy developed liver carcinoma during the follow-up of 2–26 years.

Data on ALT, AST, GGT, total bilirubin, APRI and TT levels were available in 16 patients (Table 1). Half of the patients with fibrosis had moderately elevated levels of GGT, ALT and AST (up to 411, 63 and 71 U/L respectively). All patients with congestion had slightly increased AST levels (up to 71 U/L). APRI score was slightly increased (0.5–0.9) in half of the patients with steatosis.

3.2 | Liver biochemistry in patients before and after pericardiectomy

Seventeen MUL patients underwent pericardiectomy owing to severe congestive heart failure at a median age of 8 years (0.5–38 years; five patients <2 years). The GGT level was moderately increased (up to 411 U/L) in 9/13 (69%) patients before the operation. Similarly, AST levels were abnormal (up to 84 U/L) in 9/16 (56%) patients. On the other hand, ALT values were marginally elevated (up to 50 U/L) in only 4/17 (24%) patients. APRI was abnormal (0.8 and 1.4) in two (12%) patients.
MUL children (Table 1). Data on the liver parameters after pericardiectomy were limited (Table 1). Before and after the operation, the median values of ALT (32 vs 27 U/L) and AST (49.5 vs. 39 U/L) were quite similar with a maximal individual decrease of 10 and 31 U/L respectively.

### 3.3 Blood biochemistry in a cross-sectional survey of clinically stable MUL patients

Liver function of 36 stable MUL patients was evaluated in a cross-sectional analysis. Ten of the patients were under 10 years of age, 12 were 10–20 years and 14 were over 20 years. Liver values in the three age groups were mostly normal or only marginally elevated as shown in Tables 2 and 3. Overall, transaminase (ALT and AST) levels were elevated above the normal range in 10–36% of the patients, cholestatic markers (GGT, bilirubin and bile acids) in 0%–27%, synthetic markers (prealbumin, albumin, TT and fibrinogen) in 0%–29% and APRI-score (0.5–1.2) in 17% of the patients (Table 3). Values exceeding two times the normal range were, however, recorded in only seven measurements: ALT 108 and 130 U/L; GGT 109 and 128 U/L; bile acids 47.6 and 16.8 μmol/L and TT 25%.

In Spearman’s bivariate analysis, GGT and total bilirubin values increased with age ($r = 0.45, p = .017$; $r = 0.512, p = .007$ respectively), which was not seen with the other markers (Figure 1). Galt showed an association with ALT ($r = 0.53, p = .008$), GGT ($r = 0.63, p = .001$) and APRI ($r = 0.65, p = .002$) in the Spearman analysis.

#### TABLE 1 Liver parameters in MUL patients undergoing liver biopsy or pericardiectomy (see the text)

| Biopsy finding | ALT, U/L | AST, U/L | GGT, U/L | Bilirubin, μmol/L | TT, % | APRI |
|----------------|---------|---------|----------|-------------------|------|------|
| Fibrosis       | 40.5 (13–63)<sup>a</sup> | 40 (22–71) | 59.5 (10–411) | 11 (2–18) | 87.5 (52–118) | 0.4 (0.3–1.2) |
|                | 5/12 (42)<sup>b</sup> | 6/10 (60) | 5/10 (50) | 0/9 (0) | 4/12 (33) | 4/9 (44) |
| Congestion     | 26 (17–43) | 60.5 (38–71) | 159 (21–411) | 8 (3–16) | 99 (59–118) | 0.45 (0.3–0.7) |
|                | 1/5 (20) | 4/4 (100) | 2/3 (66) | 0/5 (0) | 1/5 (20) | 2/4 (50) |
| Steatosis       | 43 (25–58) | 40 (33–71) | 28 (10–159) | 6.5 (2–8) | 90.5 (52–101) | 0.5 (0.3–0.9) |
|                | 4/8 (50) | 3/7 (42) | 2/7 (29) | 0/4 (0) | 2/8 (25) | 4/7 (57) |

Pericardiectomy

|                      | Preoperative value | Postoperative value |
|----------------------|-------------------|---------------------|
|                      | 32 (12–50)       | 4 (2–8)             |
|                      | 4/17 (24)        | 9/16 (56)           |
|                      | 13 (12–44)       | 14 (10–18)          |
|                      | 1/12 (8)         | 2/14 (14)           |
|                      | 7 (4–13)         | 0/7 (0)             |
|                      | 0/12 (0)         | 2/14 (14)           |
|                      | 1/12 (8)         | 3/12 (25)           |
|                      | 0/12 (0)         | 2/14 (14)           |
|                      | 1/12 (8)         | 2/14 (14)           |
|                      | 2/12 (17)        | 2/14 (14)           |
|                      | 1/12 (8)         | 3/12 (25)           |

<sup>a</sup>Median value, range.

<sup>b</sup>Number of patients with abnormal value/number of patients in the group (percentage).

#### 3.4 Liver biochemistry and cardiac function in clinically stable patients

Cardiac evaluation was performed in 26 MUL patients concurrently with liver biochemistry analyses. Echocardiography showed signs of restrictive physiology in 11/22 (50%) patients, and proBNP levels were above the normal range in 16/26 (62%) patients. The liver values in these patient groups were not very different (Tables 3 and 4). GGT levels were more often above the normal range in patients with restriction (25%) or elevated proBNP level (33%) as compared to those with normal heart findings (14% in both). The TT value was slightly lower in patients with signs of heart disease ($p = .047$, median 97% vs. 126%). Similarly, median prealbumin value was marginally decreased (190 vs. 240 mg/L: $p = .031$) in patients with elevated proBNP levels. The Spearman analysis showed a negative correlation between proBNP and prealbumin levels ($r = -0.63, p = .003$), but no association with other parameters was found.

#### 3.5 Transient elastography (liver stiffness) in comparison with hepatic and cardiac functions

Transient elastography (TE) of the liver showed elevated values (>7 kPa) in 14/29 (58%) patients who underwent a cross-sectional survey of liver and heart functions. Six patients (20%) had a highly elevated TE value (21.4–26.3 kPa). TE levels did not correlate significantly with the age of the patients ($r = 0.266, p = .164$) (Figure 1). However, they are associated with several biochemical parameters. In Spearman’s bivariate analysis, positive association was strong with ALT ($r = 0.65, p < .001$), GT ($r = 0.65, p = .001$) and moderate with Galt1/2 ($r = 0.48, p = .033$), FV ($r = 0.52, p = .012$) and APRI ($r = 0.58, p = .005$). Thrombocytes and TT showed a strong negative correlation with TE ($r = -0.64, p = .001$ and $r = -0.68, p < .001$ respectively).

Patients with and without signs of heart disease as indicated by restrictive physiology on echocardiography (Table 4) had a median TE value of 6.9 kPa (4.0–26.1 kPa) and 5.4 (3.8–21.4 kPa) ($p = .28$).
respectively. TE levels were above 7 kPa in 8/16 (50%) and 3/9 (33%) in the two groups. Patients with and without an elevated proBNP level had an increased TE value in 5/10 (50%) and 5/11 (45%) patients respectively. TE values did not associate with proBNP levels in the Spearman analysis ($r = 0.198, p = .391$).

### 3.6 Liver pathology and biomarker expression in autopsy samples

Original autopsy reports on liver pathology were available from 22 MUL patients who deceased between the years 1970 and 2020. The major finding in 10 children (0.65–14.6 years) was congestion with variable amount of fibrosis, but no cirrhosis. In seven young adults (22.3–39.3 years), advanced fibrosis was reported in all samples with cirrhosis in three (youngest 24 years). Five deceased patients were over 40 years (41.7–48.7 years), and all had severe cirrhosis.

Liver specimens from 15 deceased MUL patients (age 0.65 to 31.1 years and in autopsy samples, their liver histology was not significantly different from that of Fin-major homozygotes (Table 5).

The amount of fibrosis was scored (0–3) using Masson’s trichrome staining in MUL and control livers (Table 6). The staining, indicating fibrosis, was significantly increased in central ($p < .001$), sinusoidal ($p < .001$) and portal ($p < .001$) areas of MUL livers. The immunohistochemical expression of type 1 collagen, the main marker for fibrosis, was clearly elevated in the portal and sinusoidal areas ($p < .001$, $p < .001$) and SMA, the marker for activated HSC, was increased in portal areas ($p = .019$). Scores for endothelial cell marker CD34 were higher in the portal and sinusoidal areas of MUL livers as compared to controls ($p < .001$ and $p = .004$). Scores for epithelial-mesenchymal transformation marker vimentin, Kupffer cell (activation) marker CD68 and inflammation marker PSGL-1 were not increased in MUL livers as compared to controls. Finally, the levels of cytokeratin CK7 in bile ducts did not differ in MUL and control livers, whereas the aberrant expression of CK7 in hepatocytes was clearly increased in MUL livers ($1.08 \pm 0.65$ vs. $0.11 \pm 0.12$; $p < .001$) speaking for metaplastic changes in hepatocytes. In search for malignant lesions, EpCAM staining was strongly positive in the portal tracts but not in liver parenchyma of 5 control or 15 MUL livers. Two small clusters of EpCAM positive hepatocytes were observed in two liver noduli of a MUL patient with cirrhosis. Glypican-3 stainings remained negative for both control and MUL livers and did not reveal malignant lesions.

| TABLE 2 | Liver biochemistry and transient elastography (TE) in 36 clinically stable MUL patients |
|----------|----------------------------------------|
| Normal value | 0–10 years (n = 10) | 11–20 years (n = 12) | ≥21 years (n = 14) | p |
| TE, kPa | <7 | 6.0 (3.8–25.3) | 6.8 (4.0–26.1) | 9.7 (4.7–26.3) | .30 |
| Galactose t1/2, min | <12 | 9.5 (6.5–10.5) | 9.5 (7.5–12.5) | 10.0 (8.0–12.5) | .33 |
| APRI | <0.5 | 0.3 (0.2–0.6) | 0.3 (0.1–0.6) | 0.3 (0.2–1.2) | .74 |
| ALT, U/L | <35–50 | 28 (18–43) | 32 (14–130) | 29.5 (13–67) | .65 |
| AST, U/L | <36–50 | 44.5 (30–60) | 34 (20–53) | 33.5 (26–46) | .10 |
| GGT, U/L | <40–60 | 16 (9–80) | 36 (12–128) | 33 (19–93) | .14 |
| Bilirubin, μmol/L | <20 | 7 (3–9) | 9.5 (5–31) | 10 (5–22) | .049 |
| Bile acids, μmol/L | <6.0 | 4.4 (0.0–11.1) | 3.3 (0.0–6.6) | 3.7 (0.0–47.6) | .15 |
| TT, % | ≥70 | 104.5 (65–155) | 97 (55–136) | 108 (25–156) | .97 |
| Fibrinogen, g/L | ≥2 | 2.6 (1.9–4.5) | 3.0 (2.2–5.0) | 2.6 (2.2–3.3) | .41 |
| FV,% | ≥65 | 95 (83–143) | 109 (66–126) | 96.5 (47–128) | .61 |
| Albumin, g/L | ≥35–37 | 40.9 (37.8–44.8) | 39.5 (36.5–44.7) | 41.55 (36.3–46.4) | .65 |
| Prealbumin, mg/L | ≥95–200 | 190 (134–261) | 240.5 (167–429) | 233 (127–388) | .30 |
| Haemoglobin, g/L | ≥94–134 | 123.5 (94–146) | 139.5 (124–172) | 152 (137–170) | .001 |
| Leucocytes, E9/L | ≥3.4–6.0 | 5.45 (4.3–15.0) | 6.15 (4.1–12.1) | 5.6 (4.0–6.6) | .80 |
| Thrombocytes, E9/L | ≥150–200 | 261.5 (176–526) | 249 (183–361) | 232 (106–280) | .34 |

Note: Values are in median (range). Significant $p$ values ($<.05$) are bolded.

* Depending on age and/or gender.

† Between first and third age group $p = .001$.

‡ Between first and third age groups $p = .040$, between first and second groups $p = .026$. 


DISCUSSION

Mulibrey nanism (MUL) is a rare genetic disorder with prenatal onset growth failure and multiorgan manifestations, including perimyocardial heart disease, primary hypogonadism, insulin resistance and a susceptibility for tumours. In this work, we characterized liver pathology and assessed liver biochemistry in MUL patients in relation to cardiac status. The results indicate that MUL patients develop sinusoidal dilatation, congestion, nodularity, steatosis and increased fibrosis in periportal, sinusoidal and central vein areas of liver lobuli. While these lesions can be seen in early childhood, the development of frank cirrhosis is often delayed until middle age. Non-invasive assessment of liver status by transient elastography (TE) and blood biochemistry revealed moderately abnormal values in up to half of the MUL children and adults. The tests, however, were insensitive in revealing the congestive and fibrotic liver processes.

Congestive heart failure is a major determinant of prognosis in MUL patients. It is caused by pericardial constriction and myocardial restriction-related diastolic dysfunction. Pericardiectomy may provide clinical benefit, but heart failure progresses in at least a third of patients following pericardiectomy. In general, right heart failure leads to congestive hepatopathy owing to increased hepatic venous pressure, decreased hepatic blood flow and decreased arterial oxygen saturation as observed in other forms of right heart disease. In later stages, central-to-circular and central-to-portal bridging fibrosis with nodularity as well as frank cirrhosis may develop. In our study, autopsy liver samples showed congestion, sinus dilatation and fibrosis already in young MUL children who had died of heart failure. Interestingly, early fibrosis was evident not only in the central vein area but also in periportal and sinusoidal areas, which are regarded as more advanced right heart disease. Importantly, both signs were more advanced right heart failure. Centrilobular fibrosis and sinusoidal dilatation, atrophy of liver cell plates and fibrosis are common in congestive heart failure. Centrilobular congestion and sinusoidal dilatation, atrophy of liver cell plates and fibrosis are common in congestive heart failure. Centrilobular congestion and sinusoidal dilatation, atrophy of liver cell plates and fibrosis are common in congestive heart failure.

TABLE 3 Frequency of abnormal values and TE (%) in MUL patients

| Age       | ALT    | AST    | GGT     | Bilirubin | Bile acids | Albumin | Prealbumin | TT      | FV      | Gal%     | APRI     | TE      |
|-----------|--------|--------|---------|-----------|------------|---------|------------|---------|---------|----------|----------|---------|
| 0–10      | 2/8 (25) | 3/8 (38) | 2/8 (25) | −/8       | 3/7 (43)  | −/8    | 1/7 (14)   | −/8 | −/7     | −/5      | 2/7 (29) | 3/9 (33) |
| 11–20     | 4/11 (36) | 1/10 (10) | 3/11 (27) | 2/10 (20) | 1/11 (9)  | −/12   | 1/10 (10)  | −/11   | −/11    | 1/10 (10) | 5/10 (50) |
| >20       | 4/12 (33) | 1/8 (13) | 1/8 (13) | 2/7 (29) | −/10      | 2/7 (29) | −/9       | 1/8 (13) | −/8     | 1/8 (13) | 6/10 (60) |

Echocardiography

| Restriction | ALT    | AST    | GGT     | Bilirubin | Bile acids | Albumin | Prealbumin | TT      | FV      | Gal%     | APRI     | TE      |
|-------------|--------|--------|---------|-----------|------------|---------|------------|---------|---------|----------|----------|---------|
| Restriction | 5/17 (29) | 3/16 (19) | 4/16 (25) | 2/15 (13) | 4/15 (27) | 1/17 (6) | 3/14 (21)   | 2/16 (13) | −/14   | −/14     | 2/16 (13) | 8/16 (50) |
| No restriction | 3/8 (38) | 1/6 (17) | 1/7 (14) | −/7       | 1/7 (14)  | −/9    | 0/7        | −/7     | −/7     | −/6      | 1/5 (20)  | 3/9 (33) |

ProBNP

| Elevated | ALT    | AST    | GGT     | Bilirubin | Bile acids | Albumin | Prealbumin | TT      | FV      | Gal%     | APRI     | TE      |
|----------|--------|--------|---------|-----------|------------|---------|------------|---------|---------|----------|----------|---------|
| 4/12 (33) | 2/9 (22) | 3/9 (33) | 1/9 (11) | 3/8 (34) | −/9       | 3/8 (34) | 1/9 (11)   | −/7    | −/8     | 2/9 (22) | 5/10 (50) |

Normal

| 4/14 (29) | 2/13 (15) | 2/14 (14) | 1/13 (8) | 2/13 (15) | 1/15 (7) | −/12     | 1/14 (7)   | −/14   | −/12    | 1/12 (8) | 5/11 (45) |

TE

| ≥7 kPa | ALT    | AST    | GGT     | Bilirubin | Bile acids | Albumin | Prealbumin | TT      | FV      | Gal%     | APRI     | TE      |
|--------|--------|--------|---------|-----------|------------|---------|------------|---------|---------|----------|----------|---------|
| 4/13 (31) | 2/11 (18) | 4/11 (36) | 3/11 (27) | 3/10 (30) | 1/12 (8) | 2/11 (18) | 4/12 (33)   | 1/12 (8) | −/10    | 3/10 (30) | 14/14    |

<7 kPa

| 2/12 (17) | 2/12 (17) | 1/2 (8) | 3/2 (25) | −/12 | 2/10 (20) | −/12 | −/10 | −/12 | −/12 |

a Signs of restrictive physiology in echocardiography.

b Children and adolescents >178, adult males >50, adult females >180.
study showed increased liver stiffness values in Fontan patients and in patients with right heart failure. In our study, TE showed elevated levels (>7 kPa) in half of the patients studied, with six patients having a value over 20 kPa. TE levels did not correlate with patient age, nor with the cardiac findings or proBNP levels. Of the liver biochemistry values, TT, FV, ALT, GT and APRI showed statistically significant correlations with TE.

Liver biochemistry values in patients with chronic heart disease and especially those with congestive heart failure are conflicting. In a study of 2679 patients with symptomatic chronic heart failure,
Note: Semi-quantitative scoring from − to +++. Fin-major mutation = c.493-2A > G; Fin-min mutation = c.2212delG. Staining, haematoxylin-eosin.

TABLE 5 Liver histology in 15 autopsy samples of MUL patients deceased at the age of 0.65–48.7 years

| Patient | Age  | Genotype               | Steatosis | Congestion | Sinus dilatation | Hepatocyte atrophy | Bridging fibrosis | Nodularity |
|---------|------|------------------------|-----------|------------|------------------|---------------------|-------------------|------------|
| 1       | 0.65 | Fin-maj/c.1166A > G    | −         | +          | +                | −                   | −                 | −          |
| 2       | 1.1  | Fin-maj/c.1166A > G    | +         | +++        | +                | +++                 | +                 | −          |
| 3       | 1.3  | Fin-maj/Fin-maj        | −         | +++        | ++                | ++                  | ++                | −          |
| 4       | 2    | Fin-maj/Fin-maj        | +         | +++        | ++                | ++                  | +++               | +          |
| 5       | 2.1  | Fin-maj/c.1166A > G    | −         | ++         | −                 |−                   | −                 |−           |
| 6       | 2.6  | Fin-maj/Fin-maj        | +         | ++         | +                 |−                   | −                 |−           |
| 7       | 3.5  | Fin-maj/c.227T > C     | −         | ++         | ++                |−                   | −                 |−           |
| 8       | 12.6 | Fin-maj/Fin-maj        | −         | ++         | +                 |−                   | −                 |−           |
| 9       | 13.9 | Fin-maj/Fin-maj        | +         | +++        | ++                |++                  | +                 |+          |
| 10      | 14.5 | Fin-maj/Fin-maj        | +         | ++         | +                 |++                  | +                 |+          |
| 11      | 31.1 | Fin-maj/Fin-min        | +         | +++        | +                 |++                  | +                 |+          |
| 12      | 31.6 | Fin-maj/Fin-maj        | +         | +          | −                 |−                   | −                 |−           |
| 13      | 39   | Fin-maj/Fin-maj        | +++       | ++         | −                 |−                   | −                 |−           |
| 14      | 41.3 | Fin-maj/Fin-maj        | −         | +          | +                 |+++                 | +++               |++          |
| 15      | 48.7 | Fin-maj/Fin-maj        | ++        | +++        | ++                |+++                 | +++               |+++         |

Note: Scoring range 0–3, over 20 visual fields per sample analysed. Values are in mean ± SD.

Abbreviations: CK7, cytokeratin 7; PSGL-1, p-selectin glycoprotein ligand-1; SMA, alpha smooth muscle actin;

In the present study, liver values were available in 17 MUL patients with significant pericardial constriction referred for pericardectomy. Although many patients had life-threatening congestion, the liver values were mostly near the normal range. Elevated GGT values were observed in 67% of the patients and in four cases, the values were significantly elevated (116–420 U/L). The other markers were moderately abnormal (<2 times of normal) in 12–56% of the patients. Our results do not support the findings that abnormal liver values are associated with the degree of congestion rather than the liver changes.31

TABLE 6 Scoring of Masson trichrome staining and 7 immunohistochemical biomarkers in autopsy liver samples of 14 MUL patients and 6 control specimens

| Marker     | Central vein region | Portal space | Sinusoids |
|------------|---------------------|--------------|-----------|
|            | MUL | Control | p    | MUL | Control | p    | MUL | Control | p    |
| Trichrome  | 1.45 ± 0.57        | 0.31 ± 0.32 | <.001 | 2.16 ± 0.53 | 1.07 ± 0.10 | <.001 | 1.50 ± 0.66 | 0.28 ± 0.20 | <.001 |
| SMA        | 1.23 ± 0.38        | 1.02 ± 0.04 | ns   | 1.43 ± 0.40 | 1.02 ± 0.04 | .019  | 1.29 ± 0.43 | 1.14 ± 0.30 | ns   |
| Vimentin   | 0.37 ± 0.37        | 0.52 ± 0.26 | ns   | 1.61 ± 0.81 | 1.06 ± 0.06 | ns   | 1.25 ± 0.32 | 1.01 ± 0.03 | ns   |
| Collagen I | 0.89 ± 0.41        | 1.00 ± 0.37 | ns   | 1.62 ± 0.48 | 1.00 ± 0.00 | .001  | 1.22 ± 0.51 | 0.54 ± 0.58 | .033 |
| CD34       | 0.60 ± 0.36        | 0.90 ± 0.09 | .008  | 1.25 ± 0.29 | 1.01 ± 0.02 | .004  | 1.21 ± 0.67 | 0.20 ± 0.15 | <.001 |
| CD68       | 0.49 ± 0.93        | 0.11 ± 0.11 | ns   | 1.05 ± 0.82 | 1.06 ± 0.18 | ns   | 1.53 ± 0.62 | 1.05 ± 0.07 | ns   |
| PSGL-1     | 0.24 ± 0.16        | 0.72 ± 0.33 | .014  | 1.12 ± 0.43 | 1.20 ± 0.13 | ns   | 1.11 ± 0.17 | 1.00 ± 0.00 | ns   |

| Marker     | Ductules | Parenchyma |
|------------|----------|------------|
|            | MUL | Control | p    | MUL | Control | p    |
| CK7        | 1.17 ± 0.26 | 1.03 ± 0.05 | ns   | 1.08 ± 0.65 | 0.11 ± 0.12 | <.001 |

Note: Scoring range 0–3, over 20 visual fields per sample analysed. Values are in mean ± SD.

moderate elevation of serum ALT was seen in 3.1% of patients, AST in 4.1%, ALP in 14%, total bilirubin in 13% and low albumin in 18.3%. Several studies have shown that GGT and bilirubin are prognostic factors in predicting mortality in patients with chronic heart failure.30 A cholestatic liver chemistry profile is commonly encountered also in patients with hepatic venous outflow obstruction owing to right heart failure.29,30 On the other hand, liver tests, including serum AST, ALT, ALP, GGT and total and direct bilirubin, were not significantly different in congestive patients with various amount of hepatic fibrosis.11
Liver and cardiac assessment of clinically stable MUL patients (age range 0.2–51 years) visiting the outpatient clinic was performed cross sectionally. Over 400 values of 15 parameters were obtained from 36 patients. Marginally abnormal values were common, but clinically relevant results were rare. The situation was similar in patient groups divided by age, heart function (restriction in echocardiography and serum proBNP levels) and TE findings. The results indicate that conventional liver tests as well as Gαl½ and APRI are insensitive in the follow-up of patients with MUL.

Hepatic stellate cells (HSC) play a critical role in liver fibrosis. The actin network protein alpha-SMA and the intermediate filament protein, vimentin, are typically overexpressed in activated HSCs undergoing cytoskeleton remodelling.\(^3^{2,3}\) In the autopsy samples of MUL patients, immunohistochemical staining of both HSC markers showed a tendency for increased expression, especially in the sinusoidal and portal areas and less so around central veins. The same was seen with the fibrosis marker collagen type I. These results emphasize that the fibrotic process in MUL-related heart failure affects the whole liver lobule and not only the centrilobular zone. Inflammation precedes fibrosis in most liver diseases, but this does not seem to be the case among MUL patients with heart failure. Inflammatory cells were not abundant in MUL livers and the immunohistochemistry for Kupffer cells (CD68) showed marginally increased staining in sinusoidal and central vein areas. Also, the expression of inflammatory lectin, PSLG-1, located on white cells and endothelial cells, was comparable in MUL and control livers.

Sinusoidal dilatation (peliosis) and congestion are typical in congestive heart failure and are also commonly seen in MUL livers. Not surprisingly, the immunohistochemical score of CD34 was highly increased in the sinusoids of MUL livers, as compared to controls, which speaks for a reactive capillarization. Recently, increased endothelial CD34 positivity was reported especially in ‘centrilobal scars’ of livers with hepatic venous outflow obstruction.\(^3^{4}\) In MUL livers, the expression of CD34 pericentral was reduced rather than increased, which suggests a more diffuse pressure effect on liver endothelium. As peliosis is seen also in other organs in MUL, the CD34 expression may reflect other types of pathophysiology related to TRIM37 function.\(^3^{5}\) Besides heart failure, MUL patients suffer from insulin resistance and metabolic syndrome, which are typically associated with fatty liver. Somewhat surprisingly, steatosis was clearly a minor finding in the liver biopsy samples and a major finding in only two of the 22 original autopsy reports. Also, the amount of inflammation was low, which was evident, especially in immunohistochemistry with normal expression of CD68 and PSLG-1, and speaks against metabolic steatohepatitis.

Bile ductule proliferation often occurs in patients with congestive heart failure which can be visualized by staining for CK7, normally expressed only in biliary cells.\(^3^{0,3}\) However, in many liver disorders, the expression of CK7 is detected also in hepatocytes.\(^2^{2}\) Interestingly, the ductular proliferation was modest in autopsy samples of MUL livers as verified by the CK7 staining. On the other hand, hepatocyte CK7 staining was abundant in MUL livers indicating cellular metaplasia of these cells.

No hepatic neoplasia was found in the 22 autopsy samples or 21 biopsies taken from MUL patients with tumour suspicion. This was supported by the finding that biomarkers of liver carcinoma, EpCAM and glypican-3, did not reveal malignant lesions in fibrotic or cirrhotic MUL livers (with the exception of two small hepatocyte clusters positive for EpCAM in one patient). This is interesting since chronic hepatopathy may lead to hepatocellular carcinomas, and benign and malignant tumours have been reported in many non-hepatic organs in MUL patients.\(^6,3\) The Finnish MUL patients have truncating TRIM37 mutations with total loss of TRIM37 protein expression. In vitro, knockdown of TRIM37 inhibits tumorigenesis in cellular models.\(^3^{7,38}\) On the other hand, TRIM37 has profound effects in the cell cycle in various cell types (e.g. chondrocytes),\(^3^{9,40}\) and over-expression of TRIM37 has been reported in the progression of hepatic, colorectal and pancreatic carcinomas.\(^4^{1-4}\) The explanation for these somewhat conflicting observations is not clear.

Owing to the rarity of MUL, the main limitation of this study is the small number of patients and samples. In addition, liver biopsies were only obtained for clinical indications. Therefore, we assessed four overlapping patient groups to get maximal information. In the immunohistochemical assessment, the autopsy samples of MUL patients varied more in quality than those taken from normal control livers, which brought some challenges to the analyses.

The lack of TRIM37 protein causes a multiorgan syndrome and according to our previous studies and clinical findings, hepatopathy of different stages is one of the main findings in MUL. Our study suggests that the typical changes in MUL liver disease include congestion, fibrosis at different stages and steatosis. Despite the histological changes, most MUL patients do not seem to suffer from marked liver dysfunction at young age. However, liver cirrhosis is common in MUL patients after 40 years of age. No malignant liver tumours were found in biopsies. The histological and macroscopical changes speak for congestive hepatopathy. However, it cannot be excluded that the lack of TRIM37 protein modifies the liver pathology also independently of heart disease in these patients.

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CONFLICT OF INTEREST
None of the authors have to declare any conflict of interest.

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