Renal histopathological findings of retinal vasculopathy with cerebral leukodystrophy

Yutaka Tsubata¹ · Takashi Morita² · Tetsuo Morioka^{1,2} · Taiji Sasagawa¹ · Kouzo Ikarashi¹ · Noriko Saito¹ · Hisaki Shimada¹ · Shigeru Miyazaki¹ · Shinji Sakai¹ · Hajime Tanaka¹ · Rie Saito³ · Yasuko Toyoshima³ · Hiroaki Nozaki⁴ · Ichiei Narita⁵

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Abstract

Retinal vasculopathy with cerebral leukodystrophy (RVCL) is a rare autosomal dominant systemic microvascular disease. Neurological disorders and visual disturbance are highlighted as manifestations of RVCL; however, there are few reports focused on nephropathy. Herein, we describe detailed renal histopathological findings in a daughter and father with RVCL, proven by TREX1 genetic analysis. A kidney biopsy of the daughter, 35-year-old with asymptomatic proteinuria, revealed unique and various glomerular changes. Atypical double contour (not tram track-like) of the capillary wall was widely found, an apparent characteristic finding. Glomerular findings were varied due to a combination of new and old segmental mesangial proliferative changes, mesangiolysis, and segmental glomerulosclerosis-like lesions; these changes may be related to endothelial cell damage. Collapsed tufts were also found and thought to be the result of ischemia due to arterial changes. Glomerular findings in a kidney biopsy of the father revealed similarity to the daughter's glomerulus at a relatively advanced stage, but the degree of variety in the glomerular findings was much less. Kidney biopsy findings suggesting endothelial cell damage of unknown etiology need to be considered for possible RVCL.

Keywords Retinal vasculopathy with cerebral leukodystrophy · RVCL · TREX1 · Autosomal dominant disease · Kidney biopsy

Introduction

Retinal vasculopathy with cerebral leukodystrophy (RVCL) is a rare autosomal dominant systemic microvascular disease caused by mutations of the three-prime repair exonuclease

– 1 (TREX1) [1, 2]. TREX1 is a 3'–5' DNA exonuclease ubiquitously expressed in human organs, including kidney [1, 3]. Genetic analysis in three diseases: cerebroretinal vasculopathy [4], hereditary vascular retinopathy [5] and hereditary endotheliopathy, retinopathy, nephropathy and stroke (HERNS) [6], revealed these diseases are associated with C-terminal frame-shift mutations in TREX1, thus they were consolidated to a single entity named RVCL in 2007 [1]. RVCL patients typically exhibit visual disturbance and neurological disorders in middle age, often accompanied by renal and liver dysfunction. The disease is currently fatal within 5–10 years of diagnosis [2]. Neurological disorders and visual disturbance are highlighted as manifestations leading to the diagnosis of RVCL [7, 8]. According to a recent report [2], 61% of mutation carriers have features of nephropathy, but there are only brief descriptions on renal histopathological changes [6, 7, 9–11]. Herein, we describe two cases of RVCL and their unique and various histopathological findings found at renal biopsy.

✉ Yutaka Tsubata
tsubata.y@gmail.com

¹ Department of Internal Medicine, Shinrakuen Hospital, 3-3-11 Shindori-minami, Nishi-ku, Niigata 950-2087, Japan

² Department of Pathology, Shinrakuen Hospital, Niigata, Japan

³ Department of Pathology and Neurology, Brain Research Institute, Niigata University, Niigata, Japan

⁴ Department of Medical Technology, School of Health Sciences Faculty of Medicine, Niigata University, Niigata, Japan

⁵ Divisions of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Fig. 1 **a** The glomeruli appears almost normal but there were several tiny segments showing double contour of the capillary wall (thick arrows) and wrinkling of the glomerular membrane (thin arrow), while another section of the same glomerulus had no such, even tiny, changes (PAM). **b** The glomeruli had a relatively diffuse double contour of the capillary wall, but without marked mesangial change (PAM). **c** This glomerulus has several regions of mesangial change, among them two regions are fairly expansive, one without an increase in the mesangial matrix (arrow M1), and the other with an increased mesangial matrix (arrow M2), in addition to a relatively diffuse double contour of the capillary walls (PAM). **d** There were PAS-positive droplets (arrows), which were not distinctly located as intra- or extracellular. The sizes of the droplets varied and some were larger ordinary phagosomes (PAS). **e** CD-68 positive granules were found near PAS-positive granules and some PAS positive granules were discolored (arrows) (CD68, PAS). **f** In relatively advanced diffuse sclerotic glomerulus with small capillary lumen, many immunostained CD68 + PAS cells were also found in the mesangial area (CD68, PAS). **g** There were three cystic lesions, one with mesangiolytic changes (arrow) and two containing eosinophilic droplets (PAM + HE). **h** At the hilar region segmental sclerotic changes with hyaline deposits and an increase in epithelial cells (thick arrows) were observed, there was a swollen large epithelial cell (thin arrow) containing PAS-positive droplets, one of which was fairly large. The afferent arteriole had mild hyalinosis (PAS). **i** The glomerulus had collapsed tufts without mesangial expansion, while the surrounding interstitium was fibrotic and infiltrated with mononuclear cells (PAM). **j** The glomeruli had collapsed tufts, apparent expansion of the mesangium, and adhesion to Bowman's capsule (PAM). IgG (**k**) and C4 (**l**) were weakly positive with a similar pattern (**l**)

Case 1

The patient was a 35-year-old Japanese woman who came to our hospital with a chief complaint of asymptomatic proteinuria. The patient had exhibited proteinuria since 25 years old, but did not undergo further examination for proteinuria. The past medical history included temporary depression at the age of 30 and a family history of kidney disease; her father (case 2) had chronic renal failure and her sister has exhibited asymptomatic proteinuria. Her paternal grandmother was blind and died of a brain tumor in her thirties. Her mother is healthy. On examination, the patient was normotensive and had a regular pulse. Laboratory investigations were remarkable only for abnormalities in urinalysis. Urinalysis showed urine protein excretion of 0.7 g/day and β 2-microglobulin of 300 μ g/L. Urine sediment showed 1–4 red blood cells/high-power field, and 10–19 hyaline casts/whole field. Creatinine clearance was 121 ml/min/1.73 m². Full blood count, urea and electrolytes, liver function, complement and immunoglobulin were normal. Antinuclear antibodies and anti-neutrophil cytoplasmic antibody were negative. CT scan of the chest–abdomen–pelvis showed no abnormality. A biopsy specimen of her right kidney showed various findings as follows.

Light microscopic (LM) findings The average number of glomeruli per section was 12, but we could observe at least 25 glomeruli using serial sections. Glomerular histological

findings were various in quality and distribution among glomeruli, and even among segments. The most widely found findings were an apparent double contour of the capillary walls, but a tram track-like structure composed of two simple parallel lines was rare. Most parts of the walls appeared to have spindle-shaped or vesicular spaces between the two lines (Fig. 1a, b). Mesangial expansion, with or without an increase in the mesangial matrix, was frequently observed, but not diffuse (Fig. 1c), and sometimes PAS-positive droplets were found (Fig. 1d); some were larger than ordinary phagosomes, and CD68-positive granules lay nearby, the PAS-positive granules became discolored in a section of double stain with CD68 and PAS (Fig. 1e). CD68-positive cells were found also in the mesangial area without PAS-positive granules (Fig. 1f). In some glomeruli cystic lesions were found, and occasionally the cystic lesions had apparent mesangiolytic changes (Fig. 1g). Segmental sclerotic lesions with hyaline deposits accompanied by epithelial cell increase and adhesion to Bowman's capsule were also found (Fig. 1h). Collapsed tufts with or without mesangial expansion were also relatively frequent (Fig. 1i, j). There was a range from almost normal glomerulus to obsolescing glomerulus with collapsed tufts, but the glomeruli from each stage of the histological findings were evenly distributed and not biased to a specific stage. Expansion of the interstitium with mild fibrosis and mild infiltration by mononuclear cells (Fig. 1i) were found in about 10% of the cortex. Mild arteriosclerosis (intimal fibro-elastosis) and mild arteriolar hyalinosis, but no otherwise specific findings, were found in the light microscopic observation specimen.

Immunofluorescent study findings IgG and C4 were weakly positive (IgG < C4) (Fig. 1k, l).

Electron microscopic (EM) findings Four glomeruli were examined by electron microscopy. Even in tufts with normal appearing epithelial basement membrane covered foot processes, irregular widening of the subendothelial space was frequently observed. In such areas the subendothelial basement membrane was not distinct, and if anything was very thin and short (Fig. 2a). At the widespread double contour stage of the capillary wall without mesangial area expansion, the epithelial basement membrane was markedly wrinkled, and effacement of the foot processes and epithelial cell villi formation were found (Fig. 2b, d).

The endothelial basement membrane was thinner and segmentally disrupted (Fig. 2b, c), but lamellation of either basement membrane was not observed. In glomeruli in which changes were segmental, but more severe, there were several cells containing vacuoles and phagolysosomes in the mesangial area, and some of the phagolysosomes were fairly large. Tufts having only tiny capillary lumens due to widely expanded subendothelial space occupied by fluffy material were also found (Fig. 2e). Arterial and arteriolar changes in the electron microscopic specimens were more

Fig. 2 **a** Irregular widening of the subendothelial space containing fluffy material was found in tufts with normal appearing epithelial basement membrane and well preserved foot processes. A podocyte had large phagolysosomes (arrow). **b** This glomerulus had a wide-spread double contour of the capillary wall without expansion of the mesangial area. Some subendothelial spaces were relatively wide (arrows). **c** Enlargement of the white box in figure c. The epithelial basement membrane was markedly wrinkled, and endothelial basement membrane was thinner and occasionally disrupted (arrows). **d** Enlargement of the black box in c. Epithelial basement membrane was markedly wrinkled, and the endothelial basement membrane was thinner. **e** A glomerulus with relatively mild expansion of the mesangial area, three cells with numerous vacuoles and phagosomes (M1–3) and a cell with large phagolysosome (thin arrow) were found. A tuft had a tiny capillary space due to the widely expanded subendothelial space being occupied by fluffy material (thick arrow). **f** Small arteries with marked hyalinosis (H1 and H2), the lumen of H1 was almost occluded. Changes were more severe in the right lower H2 than in the left upper H2. **g** Arrow indicated portion in a, the subendothelial basement membrane-like material appeared to be multilayered. **h** Rectangular portion in a, lamina elastic (E) was irregularly thinned and interrupted (arrows), and media cells had numerous vacuoles and osmium-stained granules. **i** Basement membrane of the peritubular capillary was duplicated

severe than those in the light microscopic specimens. Hyalinosis was marked, and one arteriolar lumen appeared almost occluded, possibly exaggerated due to the plane of the sections. Arterial changes were also segmentally various, and in more severe segments media cells were vacuolated and contained osmium-stained granules (phago-lysosome) (Fig. 2f, h), while hyalinosis was marked. The lamina elastica was irregularly thinned and segmentally disappeared, and subendothelial basement membrane-like material appeared segmentally multi lamellar (Fig. 2g). Occasionally, the basement membrane of the peritubular capillary was duplicated (Fig. 2i).

The possibility of hereditary disease emerged since glomerular changes in kidney biopsy of the patient's father (case 2) revealed glomerulus similarities at a certain advanced stage. Additionally, the family history such as brain pseudotumor and visual disturbance, led to suspected RVCL. Written informed consent for genetic screening was obtained from the patient. Screening of the TREX1 gene revealed a heterozygous 4-bp insertion (3727_3730dupGTCA) resulting in a frame-shift at T249, the same as a mutation in HERNS, thus confirming the diagnosis of RVCL. Fundoscopy revealed grade 3 retinopathy, according to the Keith–Wagener classification. Brain MRI showed no abnormalities.

Case 2 (father of case 1)

At the age of 26, asymptomatic proteinuria was detected. A kidney biopsy was done at age 29 in another hospital and diagnosed as membranoproliferative glomerulonephritis

(MPGN), but histological findings were not typical of MPGN, and a descriptive diagnosis of MPGN-like lesions was apparently more suitable. The patient's left eye went blind at age 30, and he underwent maintenance hemodialysis, exhibiting left hemiplegia at age 31. Brain CT revealed a ring-enhancing lesion in the left frontal lobe. The patient died of hepatic encephalopathy at age 37. Detailed central nervous system histological findings will be reported in another paper. A PAM-stained section of the kidney biopsy was available. Six glomeruli were found, one obsolescing with collapsed tufts, and the other five showing a relatively diffuse double contour of the capillary walls and segmental expansion of the mesangial areas, although the degree varied among the glomeruli. However, the variety of glomerular changes was much smaller than the daughter, apparently due to the disappearance of changes observed in earlier stages. An afferent arteriole had marked hyalinosis (Fig. 3a). The kidneys on autopsy were 110 and 80 g, with numerous small cysts. There were global and segmental sclerotic glomeruli and the remaining tufts were collapsed. Arterial sclerosis was marked (Fig. 3b). The liver was small, weighting 705 g, the surface was smooth, and the regenerative nodules were indistinct. Fibrosis was marked, but the degree varied among segments (Fig. 3c), and there were also fresh central necroses (Fig. 3d).

Discussion

At the time of the case 1 renal biopsy, the patient had no physical symptoms at all, but the histological findings were varied and unique. The biopsy was done at a good time because the specimen ranged from almost normal to sclerotic glomeruli. Glomerular changes apparently begin sporadically in space and time, and then insidiously and diffusely advance with the various combinations of new and old changes resulting in a variety of glomerular changes. The main changes were a widening subendothelial space and atypical double contour of the capillary wall, suggesting the results were due to endothelial damage. Segmental mesangial changes, mesangiolysis, and proliferation of mesangial cell may be the result of severe endothelial damage and repair processes. Previously reported RVCL histological findings resembled those at a certain stage of case 1 [6, 7, 9–11].

The significance of IgG and C4 deposits in glomeruli is not clear, as there were too few to cause such severe mesangial changes. Electron dense deposits, suggesting immune-mediated nephropathy, were not found electron microscopically.

PAS-positive granules in the mesangial area were not distinct, whether intra- or extra-cellular, by light microscopy, but they were considered intracellular due to the

Fig. 3 **a** A representative glomerulus in the biopsy specimen revealed a diffuse double contour of the capillary wall, and two expanded mesangial areas, one (M1) with mild, and the other (M2) with a marked increase in the mesangial matrix. The afferent arteriole showed severe hyalinosis and the lumen of an interstitial small artery (A) appeared very narrow (PAM). **b** In an autopsied kidney there

were global and segmental sclerotic glomeruli and the remaining tufts were collapsed, apparently due to ischemia (PAM). Marked arteriosclerosis (arrow) was also found. **c** Relatively mild fibrotic area of the liver. The degree of fibrosis differed by area; the lower right region was more severe than the upper left (silver stain). **d** In some areas fresh central necroses (N) were also found (HE)

coexistence of CD68-positive granules, while the cytoplasm of the swollen degenerative epithelial cells, containing large PAS-positive granules, was not stained clear in the HE- or PAS-stained sections (Fig. 1h). It was not directly confirmed electron microscopically, but some cells in the mesangial area contained many, and occasionally large, phagolysosomes. These findings suggest impairment of the phago-lysosome system in this disease. Reports on electron microscopic study of RVCL are insufficient; double contour of the capillary wall and widening subendothelial spaces seem common findings. However, a multilayered endothelial basement membrane of the glomerulus was described in one report [6], although we, and two other reports, [9, 10] did not confirm this finding.

Arterial findings such as multi-lamellation of subendothelial membrane-like material and basement membrane duplication of the peritubular capillary are the same as reported arterial changes in the brain and other organs [6, 9]. In

segmentally severely changed areas, arterial media cells also contained many phagosomes and vacuoles. The liver fibrosis in case 2 is compatible with that induced by ischemia. The varied distribution and degree of change and the simultaneous existence of fresh necrosis suggest arterial changes start sporadically in space and time and advance steadily.

In our two cases, careful exclusion of other renal disease, such as MPGN, thrombotic microangiopathy and chronic humoral rejection, is needed. But these renal diseases are distinguished by their clinical course and histological findings.

The kidney biopsy findings and family history in case 1 led to an early diagnosis of RVCL before the patient had subjective symptoms and brain lesions. A review of 78 mutation carriers of a C-terminal frame-shift in TREX1 from 11 unrelated families reported the manifestations in RVCL [2]. At diagnosis, 92% of mutation carriers presented with retinopathy. In the patients with retinopathy, neuroimaging

abnormalities were found in 97% and nephropathy was exhibited in 61%. One was detected as nephropathy prior to retinopathy. It differs by individual in terms of how the manifestations present [2, 4–6, 8] and nephropathy can be the first manifestation found, as in this case. Therefore, clarifying the histopathology of the kidney assists in providing early RVCL diagnosis.

In conclusion, kidney biopsy findings suggesting endothelial cell damage of unknown etiology need to be considered for possible RVCL.

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Compliance with ethical standards

Conflict of interest All the authors have declared no competing interests.

Ethical statement This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained.

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