INTERNAL MEDICINE

Severe Glomerular Endothelial Injury Associated with a Short D4Z4 Repeat on Chromosome 4q35

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Abstract

The short D4Z4 repeat on chromosome 4q35 is a confirmatory genetic cause of facioscapulohumeral muscular dystrophy (FSHD), which presents with no renal complications. We herein report a five-year-old girl previously diagnosed with Coat's-like retinopathy, deafness, and mental retardation, who was found to have early-onset, severe FSHD. Despite the absence of muscle weakness, a Southern blot analysis showed a short D4Z4 repeat on chromosome 4q35. She presented with steroid-resistant nephrotic syndrome, and her renal histopathological findings were severe glomerular endothelial injury, which is a new complication associated with this genetic abnormality. Screening of renal complications may be necessary for FSHD patients. This patient requires close follow-up for her muscle symptoms.

Key words: glomerular endothelial injury, focal segmental glomerulosclerosis, facioscapulohumeral muscular dystrophy, Coats disease, D4Z4 repeat, chromosome 4

Introduction

The shortening of the D4Z4 repeat on chromosome 4q35 is a confirmatory genetic cause of facioscapulohumeral muscular dystrophy (FSHD) (1), which generally has no renal complications. Only one patient with shortening of the D4Z4 repeat and focal segmental glomerulosclerosis (FSGS) was previously reported, by Reynolds et al. (2). Despite the absence of muscle weakness, their patient had Coats'-like retinopathy, deafness, and mental retardation, which were often associated with early-onset, severe FSHD (2-4). Unfortunately, however, advanced sclerosed glomeruli prevented the definite clarification of the initial histological manifestation of FSGS in their patient.

We herein report the case of a five-year-old girl with shortening of the D4Z4 repeat on chromosome 4q35. Her clinical features were very similar to those of the patient reported by Reynolds (2). She is therefore the second case of FSGS with a short D4Z4 repeat. Furthermore, her renal histopathological findings revealed severe glomerular endo-

thelial injury, which to our knowledge has not yet been reported to be associated with genetic abnormalities on chromosome 4q35.

Case Report

A five-year-old Japanese girl with a complex medical background was referred to us for an approximately two-year history of marked proteinuria. Her parents were not consanguineous, and her family history included no individuals with similar symptoms or terminal renal failure. A physical examination showed a height of 100.0 cm (-1.3 SD), a body weight of 17.2 kg (-0.1 SD), a body temperature of 36.2℃, a pulse rate of 105 beats/min, and a blood pressure of 94/62 mmHg. She showed no edema. Laboratory tests suggested nephrotic syndrome with microhematuria. Her serum albumin and total cholesterol level were 1.85 g/dL and 408 mg/dL, respectively. Her blood urea nitrogen level was 10.9 mg/dL, her β2 microglobulin level was 1.2 mg/dL, and her creatinine level was 0.22 mg/dL. Her hemoglobin level was 14.0 g/dL, and her platelet count was
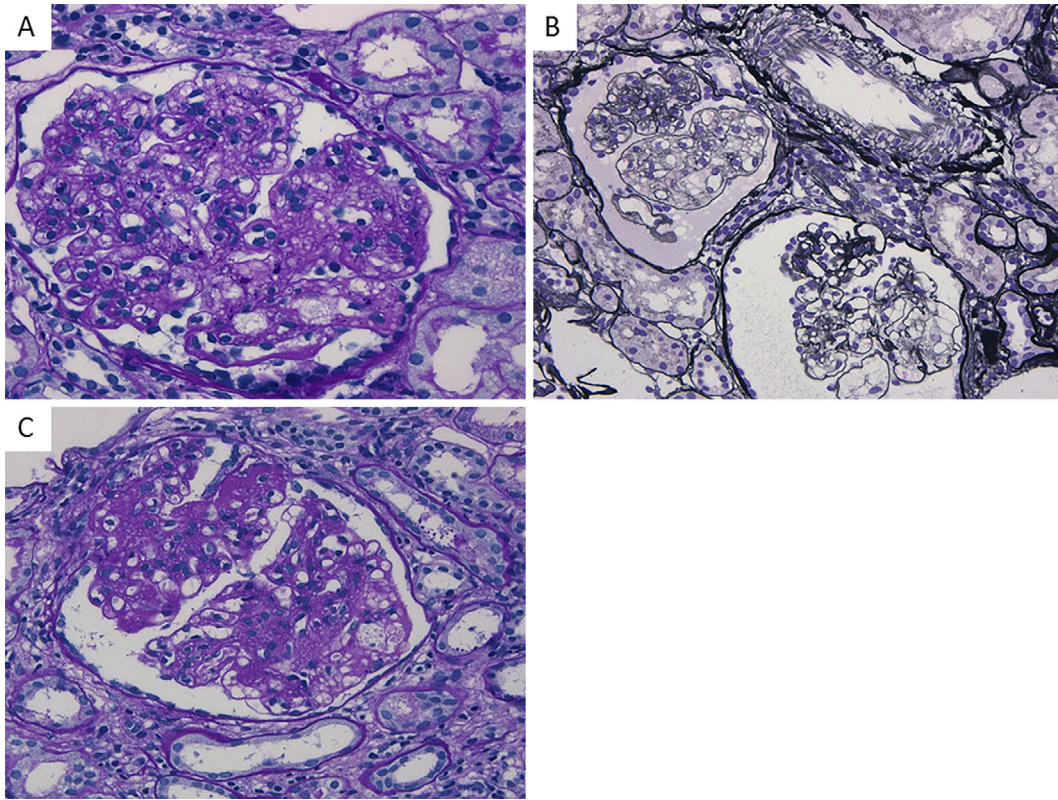
Figure.   The renal biopsy findings in our patient. (A) Membranoproliferative glomerulonephritis-like lobulation, thrombotic microangiopathy-like lesions, and diffuse mesangiolysis (periodic acid Schiff staining; original magnification). (B) Diffuse mesangiolysis in glomeruli (periodic acid methenamine silver staining; original magnification). (C) Hyalinosis and segmental sclerotic lesion (periodic acid Schiff staining; original magnification).

431,000/µL. A urinalysis revealed 19 red blood cells per high-power field and a protein level of 3+. Her spot urine protein/creatinine ratio was 8.8. Her serum C3 and C4 levels were within normal ranges, and she was negative for antinuclear antibody. Her serum vascular endothelial growth factor (VEGF) level was 35.6 pg/mL (normal range: <38.3 pg/mL). Renal ultrasonography showed normal kidneys.

Optical microscopy of a renal biopsy specimen showed 25 glomeruli; of these, only 2 were almost normal, and 5 showed global sclerosis. The remainder showed a membranoproliferative glomerulonephritis (MPGN)-like lobulation with mesangiolysis and thrombotic microangiopathy (TMA)-like lesions characterized by severe glomerular endothelial injury (Figure A and B). Red blood cells were observed in the expanded subendothelial area. Endocapillary foam cells and insudative lesions were also detected (Figure A and B). Two glomeruli showed segmental sclerosis with capping and severe alternation of epithelial cells (Figure C). Mild renal tubular atrophy, interstitial fibrosis, and mononuclear cell invasion around sclerotic glomeruli were observed (Figure C), but injury to the endothelial cells in the peritubular capillaries and small arterioles was not observed. Immunofluorescence showed IgM and slight segmental C4 deposition, but staining for C3, C1q, IgG, and IgA was negative. The patient was histopathologically diagnosed with severe glomerular endothelial injury.

A 4-week trial of prednisolone (2 mg/kg/day) for her nephrotic syndrome was unsuccessful in reducing her proteinuria. She began treatment with the angiotensin receptor blocker valsartan to reduce her proteinuria and support her renal function. Over the next five months, her nephrotic proteinuria persisted, but her renal function did not deteriorate further.

The patient was previously evaluated by a family doctor at eight months of age owing to floppy infant syndrome and psychomotor retardation. She became capable of holding up her head at 11 months and was able to stand at 2 years of age. At the age of 1 month, she was found to have myoclonus of the upper limbs and was treated with phenobarbital sodium. The genetic analysis of G-banding showed 46, XX. Her myoclonus and muscle weakness gradually disappeared, and a muscle biopsy was not performed. She was also found to have bilateral sensorineural deafness, about 50-70 dB. Due to her mental retardation and deafness, she could understand simple language but speak no meaningful words. Although a small nodular lesion in the pons was detected by brain magnetic resonance imaging, the causes of her neurological abnormalities were not determined. She was also diagnosed with Coat's disease at the age of 1 year and treated with multiple photocoagulations. Her vision was impaired bilaterally, especially in her left eye, which showed photesthesia with cataracts and retinal detachment.

Table. **Comparison of the Clinical Characteristics among This Case, a Similar Patient and Patients with Typical FSHD.**

	The patient	Reynolds et al. (2)	Typical FSHD (1,3,4)
Age at onset (year)	2	5	during the second decade
Gender	female	female	mostly male
Short D4Z4 repeat on chromosome 4q35	+	duplicated short D4Z4 repeat	+
Coats'-like retinopathy	+	+	60% patients early-onset and severe case
Deafness	+	+	~75% patients early-onset and severe case
Mental retardation	+	+	early-onset and severe case
Muscle weakness	during only infancy	-	+
Involuntary movement	myoclonus	myoclonic jerks	-
Lesion of brainstem	nodular lesion in pons	basal ganglia calcification	-
FSGS	+	+	-

FSHD: facioscapulohumeral muscular dystrophy, FSGS: focal segmental glomerulosclerosis

Coats'-like retinopathy, mental retardation, and deafness have been reported to be associated with severe, early-onset FSHD (3, 4). Although a muscle biopsy was not performed for our patient, a genetic analysis was performed. After obtaining informed consent from the patient's parents, a Southern blot analysis was performed using the probe p13E-11, which revealed a 10-kb *Eco*RI fragment. This fragment was further shortened to 7 kb by *Eco*RI/*Bln*I double enzyme digestion, confirming the shortening of the D4Z4 repeat on chromosome 4q35.

Discussion

In this report, we described the case of a 5-year-old girl with Coats'-like retinopathy, mental retardation, deafness, and steroid-resistant nephrotic syndrome, along with the histopathological appearance of severe endothelial injury. Despite the absence of muscle weakness, a Southern blot analysis showed a short *Eco*RI fragment, known as a D4Z4 repeat, on chromosome 4q35.

To our knowledge, there has only been one patient whose clinical characteristics including the absence of muscle weakness were very similar to those of our patient (2). Table compares the clinical characteristics of our patient with those of a similar patient (2), as well as with patients with typical FSHD (1, 3, 4). This finding supports the hypothesis that the novel association of these symptoms is caused by abnormalities in genes located on chromosome 4q35. Furthermore, our results indicate that FSGS in our patient was caused by severe glomerular endothelial injury, which has not been reported to be associated with genetic abnormalities on chromosome 4q35, including shortening of the D4Z4 repeat.

In most FSHD patients, a Southern blot analysis using the probe p13E-11 detects short (from 10 to 35 kb) *Eco*RI fragments on chromosome 4q35, which contains the 3.3-kb

*Kpn*I digestible tandem repeats known as D4Z4 (1). However, chromosome 10q26 also contains repeats with 98% nucleotide identity to D4Z4 on chromosome 4 (5). Nevertheless, the 10q26-derived fragment contains a unique *Bln*I restriction site not present in the 4q35-derived fragment (6). Therefore, double enzyme digestion with *Eco*RI and *Bln*I can distinguish between 4q35- and 10q26-derived units (6). Because the short 10-kb *Eco*RI fragment was further shortened to 7 kb by double digestion, the diagnosis of 4q35-FSHD was confirmed.

Several reports have suggested an inverse correlation between the clinical severity of FSHD and the size of the *Eco*RI fragment, with the smallest fragments found in patients with very-early-onset FSHD, accompanied by epilepsy, deafness, Coats'-like retinopathy, and mental retardation (3, 4). The small *Eco*RI fragment size (10 kb) of our patient corresponds to the fifth percentile among patients with FSHD (1); however, no muscle weakness was observed. Other studies have detected the same short fragment in asymptomatic individuals and affected family members, suggesting no correlation between the fragment size and disease severity (7). Recent studies have suggested that FSHD is caused primarily by the inappropriate expression of the double homeobox protein 4 gene (DUX4), which lies within each D4Z4 repeat on chromosome 4q35 (8). Plural pathways have been reported to lead to D4Z4 chromatin relaxation and the abnormal expression of DUX4, including D4Z4 repeat contractions and hypomethylation of the 4q35 region associated with SMCHD1 gene mutations (1, 9). Our patient was not examined for these genetic abnormalities, and she should be monitored periodically for the development of a muscle phenotype.

The patient was histopathologically diagnosed with severe glomerular endothelial injury with TMA-like lesions. Representative nephropathy due to TMA includes hemolytic uremic syndrome and thrombotic thrombocytopenic pur-

pura (10). However, the endothelial injury of small arterioles and thrombi were not observed, and her laboratory data did not indicate hemolytic anemia or thrombocytopenia. There is no evidence of any relationship between chromosome 4g35 and genetic complement abnormalities leading to atypical hemolytic uremic syndrome (10). To our knowledge, there are no reports of genetic abnormalities with endothelial injury limited to glomeruli. Interestingly, her pathological findings are similar to the glomerulopathy associated with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS) syndrome (11). In the kidney of POEMS syndrome patients, continuous endothelial injury causes mesangiolysis with mesangial interposition, mesangial proliferation, duplication of glomerular basement membrane, and finally mesangial sclerosis (11). The mechanism of podocyte injury and glomerular sclerosis associated with endothelial injury has also been reported for other diseases, such as membranous nephropathy and primary FSGS (12, 13). Although the present patient's pathological diagnosis was not confirmed specifically, the pathological findings suggest that her proteinuria and secondary glomerular sclerotic lesion were caused by severe endothelial injury.

There is probably a common mechanism underlying the symptoms in different organs, namely glomerular endothelial injury, Coats-like retinopathy, deafness, and muscle weakness. Dysregulation of VEGF and Wnt signaling may be involved in this mechanism. VEGF is an important protein for angiogenesis, and similar glomerular endothelial injury with mesangiolysis caused by its dysregulation has been observed in patients receiving anti-VEGF therapy or in patients with POEMS syndrome with elevated VEGF concentrations due to plasma cell dyscrasia (14, 15). An elevated VEGF level has also been found in the ocular fluid of patients with Coats' disease, a lesion in which retinol vasculopathy is characterized by bilateral retinal telangiectasias and microaneurysms (16). Although the serum VEGF level of our patient was not increased, VEGF is produced mainly by glomerular podocytes and plays a crucial role in maintaining the glomerular endothelial cells (17). While we wanted to evaluate the expression of VEGF in glomeruli in the present report, we did not have the means; this is a limitation of this study.

Wnt signaling is a highly conserved signaling pathway that regulates cellular interactions at many stages of embryonic development in many organs, including skeletal myogenesis, retinol angiogenesis, cochlear angiogenesis, and glomerular regeneration (18, 19). The regulation of VEGF may be one way in which Wnt signaling controls angiogenesis (20). There is no evidence that the regulation of VEGF and the Wnt signaling pathway are associated with chromosome 4g35 or whether they work tissue-specifically in the muscles, retina, kidneys, and cochlea. One or more mechanisms associated with chromosome 4q35 may be responsible for the symptoms in our patient, although the reason for the muscle sparing remains unclear.

In conclusion, in this report, we described a patient with severe glomerular endothelial injury, which is a new renal complication associated with shortening of the D4Z4 repeats on chromosome 4q35. We suggest that screening for renal complications be performed for patients with FSHD.

The authors state that they have no Conflict of Interest (COI).

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