Once-Daily Low-Dose Cyclosporine A Treatment with Angiotensin Blockade for Long-Term Remission of Nephropathy in Frasier Syndrome

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Cyclosporine A is known to be effective in some genetic podocyte injury. However, the efficacy of cyclosporine A depends on the degree of histopathological findings, and the relationship between long-term use and renal prognosis remains unknown. Frasier syndrome is a rare genetic disorder caused by intronic mutations in \textit{WT1}, and is characterized by progressive glomerulopathy, a 46,XY disorder of sex development, and an increased risk of gonadoblastoma. We report here a 16-year-old phenotypically female patient with Frasier syndrome. A renal biopsy at the age of seven years showed segmentally effaced podocyte foot processes with no evidence of glomerulosclerosis. Steroid-resistant proteinuria progressed to the nephrotic range at the age of 10 years, which responded to once-daily administration of cyclosporine A with low two-hour post-dose cyclosporine A (C2) levels; she then achieved stable partial remission in combination with renin-angiotensin system (RAS) blockade. At the age of 12 years, examinations for delayed puberty confirmed the diagnosis of Frasier syndrome. The second renal biopsy showed widespread foot process effacement and a minor lesion of segmental glomerulosclerosis without findings suggestive of cyclosporine A nephropathy. She maintained partial remission and normal renal function with the continuation of once-daily low-dose cyclosporine A. The C2 levels required for the remission were between 212 and 520 ng/ml. Cyclosporine A dosages sufficient for maintaining the C2 levels were 1.1-1.2 mg/kg per day. In conclusion, the long-lasting treatment of once-daily low-dose cyclosporine A with RAS inhibition was effective for induction and maintenance of partial remission in Frasier syndrome.

Keywords: cyclosporine A; Frasier syndrome; lisinopril; podocytes; telmisartan


Introduction

The podocyte cytoskeleton is maintained by the actin system. In podocyte foot processes, synaptopodin is an actin binding protein that plays a crucial role in maintaining the cytoskeleton. The direct action of cyclosporine A (CsA) on podocytes, which suppresses synaptopodin degradation by calcineurin activation, is a convincing mechanism for CsA efficacy in genetic podocyte injury (Faul et al. 2008). However, the efficacy has only been explained for some genetic abnormalities such as \textit{WT1} mutations (Büscher et al. 2016).

\textit{WT1} continues to be expressed throughout the life of the podocyte and is responsible for regulating the expression of various genes involved in the maintenance of the cytoskeleton and the slit diaphragm (Dong et al. 2015). Hence, the mutation of \textit{WT1} exerts a great influence on the podocyte function. For \textit{WT1} mutations, there are few studies on the difference in the effect of CsA according to the mutation site or histopathological findings (Lehnhardt et al. 2015). The relationship between long-term use and renal prognosis remains unknown. Establishing a safer administration method to prevent the progression of chronic CsA nephrotoxicity is also an intractable problem.

Frasier syndrome is a rare genetic disorder caused by heterozygous splice donor site mutations in intron 9 of \textit{WT1} (OMIM 136680). The syndrome is characterized by progressive glomerulopathy, a 46,XY disorder of sex development and an increased risk of gonadoblastoma from streak gonads. Because most patients are raised as girls, Frasier syndrome is often suspected when affected children are examined for delayed puberty (Niaudet and Gubler 2006). Patients usually present with asymptomatic proteinuria in early childhood, then gradually develop steroid-resistant nephrotic syndrome that progresses to chronic kidney disease (Niaudet and Gubler 2006). Renal biopsy findings...
often represent focal segmental glomerulosclerosis (FSGS) (Ruf et al. 2004). Lipska et al. (2014) showed that the age at 50% kidney survival for Frasier syndrome was 13.6 years. Here we report a case of Frasier syndrome maintaining partial remission and normal renal function by continuing once-daily low-dose CsA treatment with renin-angiotensin system (RAS) inhibition at the age of 16 years.

**Case Report**

At the age of four years, this phenotypically normal female patient was noticed to have proteinuria (1+ by dipstick) in a routine urine screening at kindergarten. Her family history and past medical history were unremarkable. She had no hematuria, and serum creatinine (SCr) was 0.38 mg/dl.

She was admitted to our hospital at the age of seven years, because proteinuria progressed asymptomatically to an early-morning urinary protein/creatinine ratio of over 1.0 g/g. Her height was 136.0 cm (+2.56 SD for standard Japanese females) and her weight was 31.0 kg (+1.52 SD). Physical examination showed no edema with normal blood pressure (100/59 mmHg). Laboratory tests demonstrated blood urea nitrogen 9.8 mg/dl; SCr 0.40 mg/dl; total protein 6.8 g/dl; albumin 4.5 g/dl; total cholesterol 179 mg/dl. Total blood cell count, and complement components C3 and C4 were normal. Urinalysis revealed an osmolality of 1.025, a pH of 6.0, and proteinuria (2+; 100 mg/dl) without hematuria (<1 red blood cells per high powered field). Abdominal ultrasound demonstrated normal-sized kidneys.

A renal biopsy was performed. Light microscopy showed 20 glomeruli with a mild diffuse mesangial proliferation. No segmental sclerosis or adhesion was observed. Tubulointerstitial and capillary areas were normal. On immunostaining, a mesangial deposition of C3 (3+) and C1q (2+) was detected, but IgA was negative. Electron microscopy showed mild to moderate mesangial proliferation. Podocyte foot processes were segmentally effaced (Fig. 1a). Intravenous methylprednisolone (mPSL) pulse therapy followed by alternative day oral administration of prednisolone (PSL) was performed with a diagnosis of mild mesangial proliferative glomerulonephritis. Low-dose (0.8 mg/kg per day) telmisartan (an angiotensin receptor blocker, ARB) was started for renal protection purposes.

Fig. 2 demonstrates the changes in the amount of urinary protein/creatinine ratio (g/g) and SCr during our patient’s course of treatment. Proteinuria showed resistance to steroids and urinary protein reached 1.5 g/g at the age of eight years. CsA was initiated once daily before breakfast (2.0 mg/kg per day), adjusted to achieve two-hour post-dose CsA (C2) levels of approximately 700 ng/ml. Although urinary protein showed a mild decrement to nearly 0.8 g/g, complete remission was not achieved after continuing for six months. We decided to taper the CsA off. PSL was also tapered and stopped because of the potential side effects of long-time use.

At the age of 10 years, proteinuria significantly progressed to 3.8 g/g and serum albumin gradually decreased to 3.8 g/dl. Because of concern over the onset of overt

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**Fig. 1.** Histological findings of the initial renal biopsy at the age of seven years and the second renal biopsy at the age of 12 years and 10 months.

(a) Electron microscopy of the initial renal biopsy specimen shows segmental foot process effacement (arrows) (original magnification × 3,000).

(b) Light microscopy of the second renal biopsy specimen shows glomerulus with a minor perihilar lesion of segmental sclerosis (white arrowhead) (Periodic acid silver methenamin staining; original magnification × 400).

(c) Light microscopy of the second renal biopsy specimen shows mild lesions of tubular atrophy and interstitial proliferation (Azan staining; original magnification × 200).

(d) Electron microscopy of the second renal biopsy specimen shows widespread effacement of foot processes with microvillous transformation (white arrow), and thickening of glomerular basement membrane (original magnification × 1,500).
nephrotic syndrome, we resumed CsA with once-daily administration. Low-dose (0.1 mg/kg per day) lisinopril (an angiotensin-converting enzyme inhibitor, ACEI) was also started in combination with telmisartan. Five weeks after resumption, proteinuria decreased to 0.65 g/g at the C2 level of 320 ng/ml as shown in Fig. 2. The CsA dose during this period was 1.2 mg/kg per day. Then partial remission was obtained with urinary protein between 0.3 and 0.8 g/g. At the age of 12 years and five months, proteinuria increased to 1.88 g/g. Because the C2 level proved to be low (129 ng/ml), we raised the CsA doses from 0.9 mg/kg per day to 1.1 mg/kg per day. Three months later, it was confirmed that the C2 level had risen to 427 ng/ml and the urinary protein had decreased to 0.65 g/g. Proteinuria was reduced to 1.0 g/g or less by maintaining the C2 levels in the range of 212 to 520 ng/ml. CsA dosages sufficient for maintaining the C2 levels were 1.1-1.2 mg/kg per day.

At the age of 12 years and eight months, it was noticed that she had delayed puberty. Breast development was at Tanner stage 2 and development of neither axillary-hair or pubic-hair was observed. Her height was 164.3 cm (+2.02 SD) and weight was 53.2 kg (+0.89 SD). Hormonal analyses revealed high follicle-stimulating hormone 120.37 mIU/ml (reference range 3.4-9.8 mIU/ml); high luteinizing hormone 41.8 mIU/ml (reference range 1.2-10.2 mIU/ml); extremely low estradiol < 10 pg/ml levels, indicating hypergonadotropic hypogonadism. Pelvic magnetic resonance imaging showed a small uterus with no detection of ovaries. Chromosomal analysis of peripheral blood (the G-band method) revealed a 46,XY karyotype, suggesting that she had a disorder of sex development. Because patients with a 46,XY disorder of sex development are at increased risk for developing germ cell tumors, a laparoscopic bilateral gonadectomy was carried out at the age of 12 years and 10 months. Histological evaluation of resected streak gonads confirmed no presence of gonadoblastoma.

A second renal biopsy was subsequently performed laparoscopically at the age of 12 years and 10 months. Light microscopy showed eight glomeruli with a mild diffuse mesangial proliferation, and one glomerulus had a minor perihilar lesion of segmental sclerosis (Fig. 1b). Mild lesions of tubular atrophy and interstitial proliferation were detected (Fig. 1c), but there were no findings of vascular changes or striped interstitial fibrosis suggestive of CsA nephropathy. ImmunoFluorescence investigation revealed mesangial deposition of C3 (2+), IgM (1+), C1q (+ –). Electron microscopy demonstrated widespread effacement of foot processes, accompanied by microvillous transformation, and thickening of glomerular basement membrane (Fig. 1d).

Disorder of sex development-related genes were analyzed using next-generation sequencing technology, which demonstrated a heterozygous mutation at the splice donor site of WTI intron 9 (IVS9+4 C>T), confirming a diagnosis
of Frasier syndrome. Hormone replacement with estrogen and prevention of osteoporosis with alfacalcidol were started. At the age of 16 years, she maintained partial remission with once-daily CsA administration, preserving normal renal function (SCr 0.61 mg/dl). She had no hypertension or electrolyte abnormalities during the course of treatment.

**Discussion**

We present a case of Frasier syndrome with nephrotic-range proteinuria, in which the patient showed a favorable response to long-term CsA therapy in combination with oral ACEI and ARB, maintaining partial remission with normal renal function for many years. By repeated renal biopsies before and after CsA administration, we could observe electron microscopic histological changes in the affected podocytes in this patient. Both clinical features and electron microscope findings during the course of the disease support the view that CsA plays a key role in inhibiting deterioration of podocyte function, resulting in maintaining partial remission for a long time. We speculate that the following three factors are attributable to the beneficial effects of CsA on glomerulopathy in the patient.

First, early administration and continuous use of CsA seem to contribute to delaying the progression of podocyte injury. In our patient, the initial renal biopsy was performed at the age of seven, before the treatment with CsA. At that time, although the urinary protein/creatinine ratio exceeded 1.0 g/g, renal biopsy specimen showed only mild diffuse mesangial proliferation without overt lesions indicative of glomerulosclerosis (Fig. 1a). The characteristic electron microscope finding in the affected podocytes was principally segmental foot process effacement. This finding suggested that podocyte injury was extremely localized and mild. Lehnhardt et al. (2015) reported that 56% of patients with Frasier syndrome present renal histological FSGS, whereas 33% show minimal change disease, suggesting that some patients with Frasier syndrome can be identified before FSGS develops. Our patient seems to belong to such a case because she was diagnosed by chance at the earliest stage of the disease.

When actin filament abnormalities occur within podocytes, the first detectable pathological change of podocyte injury can be foot process effacement (Kriz et al. 2013). When the influence of the abnormality extends to the slit membrane molecules, overt proteinuria occurs. If the injury further progresses, podocyte detachment advances as the injury progresses, causing a certain degree of podocyte depletion, which leads to the development of FSGS lesions and massive proteinuria (Wharram et al. 2005). Our patient showed only segmental foot process effacement, an early stage of podocyte injury, in the first biopsy specimen. Similar foot process effacement lesions have been reported previously in Frasier syndrome (Li et al. 2007). Conversely, in the second biopsy specimen which was evaluated five years after the first biopsy during the CsA therapy, the foot process effacement lesions became widespread with microvillous transformation (Fig. 1d), indicating that podocyte injury had expanded within almost the whole glomerulus. However, lesions of segmental glomerulosclerosis were still restricted, suggesting that the progress of podocyte depletion was still limited (Wharram et al. 2005). This phenomenon might explain why the degree of proteinuria remained stable in our patient at the age of 12 years.

Our immunofluorescent microscopic observations of mesangial C1q (2+) and C3 (3+) deposits and light microscopic findings of mild diffuse mesangial proliferation in the initial biopsy were similar to C1q nephropathy (Vizjak et al. 2008). Although we could not find other reports of Frasier syndrome with positive C1q staining, the pathophysiology of C1q deposition in podocyte injury is intriguing. C1q may fix to immunoglobulins that become trapped nonspecifically in the mesangium as a result of increased mesangial trafficking during glomerular proteinuria in podocyte injury (Markowitz et al. 2003). Furthermore, some patients with C1q nephropathy have reportedly lost C1q deposits during the follow-up period, whereas FSGS progression and increased urinary protein levels were observed (Hisano et al. 2008). In our patient, although we observed attenuated C1q deposition in repeated biopsy during the CsA therapy, most of the glomeruli showed no sclerosis and the degree of proteinuria remained stable.

Second, continuous use of once-daily, low-dose CsA was effective for the induction and maintenance of partial remission. This treatment also seems to avoid eliciting adverse effects of CsA, known as CsA nephropathy (Fujinaga et al. 2012). For our patient, considering the possibility of long-term use, once-daily administration of CsA was selected (Suzuki et al. 2010). It is difficult to administer appropriate amounts of CsA because there are no reliable indexes for individual dosage determination. Thus, target blood concentrations are usually set based on consideration of both therapeutic effects and adverse effects. In pediatric steroid-dependent nephrotic syndrome, Fujinaga et al. (2012) administered CsA once-daily at a target C2 level of approximately 700 ng/ml, attaining a mean C2 level of 670 ± 64 ng/ml at a dose of 2.8 ± 0.6 mg/kg per day. With reference to their study, we initially prescribed 2.0 mg/kg per day of CsA to attain C2 levels of around 700 ng/ml in the patient. However, in our experience, lower C2 levels between 212 and 520 ng/ml seem to be sufficient to maintain stable partial remission. Such a CsA blood concentration was achieved by oral once-daily intake of 1.1 to 1.2 mg/kg per day.

It should be noted that we must not discontinue CsA to avoid a relapse of proteinuria. Several papers have reported that interruption of CsA treatment caused uncontrollable increment of nephrotic-range proteinuria or rapid deterioration of renal function in pediatric patients with Frasier syndrome (Gellermann et al. 2010; Sinha et al. 2010). CsA treatment was interrupted in our patient, who was treated with only ARB for one year, and her nephrotic-range pro-
teinuria was exacerbated. Fortunately, after we resumed CsA, the degree of proteinuria (3.7 g/g) drastically decreased to 0.65 g/g with a 320 ng/ml C2 level of CsA. To avoid or prevent CsA nephropathy, Kengne-Wafo et al. (2009) proposed that the C2 levels of CsA be maintained below 550 ng/ml in steroid-dependent nephrotic syndrome. Similarly, we estimated that the effective dose of CsA was between 212 and 520 ng/ml in treating Frasier syndrome. This dose was safe and tolerable for long-term use in pediatric patients.

Third, dual RAS blockade with low-dose ACEI (0.1 mg/kg per day of lisinopril) and ARB (0.8 mg/kg per day of telmisartan) may present a new therapeutic strategy not only to improve prognosis in proteinuric patients with Frasier syndrome.

In conclusion, continuous use of CsA by once-daily administration with low C2 levels was effective for the induction and maintenance of partial remission in Frasier syndrome. The beneficial effect of CsA is attributed to the early start of treatment before podocyte injury had accumulated, which further encourages us to emphasize the importance of early diagnosis of the disease. Based on electron microscopic evaluations, early administration and continuous use of CsA seem to contribute to delaying the progression of podocyte injury that may cause podocyte depletion, leading to glomerulosclerosis. We believe that this present case report is of high clinical importance. We suggest administration of once-daily, low-dose CsA with RAS inhibition may present a new therapeutic strategy not only to induce long-term stabilization of renal function but also to improve prognosis in proteinuric patients with Frasier syndrome.

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Conflict of Interest

The authors declare no conflict of interest.

References


Lipska, B.S., Ranchin, B., Iatroupolos, P., Gellermann, J., Melk, A., Ozaltin, F., Caridi, G., Seeman, T., Töry, K., Jankauskienė, A., Zurowska, A., Szczepanska, M., Wasilewska, A., Harambat, J.,


