Hashimoto F, Oka M, Ninchoji T, Ishimori S, Morisada N, Matsunoshita N, Kamiyoshi N, Yoshikawa N, Iijima K. Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV α5 chain. Kidney Int. 2013 Dec 4. [Epub ahead of print]

37. Iijima K, Sako M, Oba MS, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, Yoshikawa N. : Japanese Study Group of Kidney Disease in Children. Cyclosporine C2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children: A Multicenter Randomized Phase II Trial. Clin J Am Soc Nephrol. 2013 Dec 5. [Epub ahead of print]

(分担研究者：中西浩一)

1. Nakayama M, Nozu K, Goto Y, Kamei K, Ito S, Sato H, Emi M, Nakanishi K, Tsuchiya S, Iijima K. HNF1B alterations associated with congenital anomalies of the kidney and urinary tract. Pediatr Nephrol. 2010 Jun;25(6):1073-9.
2. Iijima K, Nozu K, Kamei K, Nakayama M, Ito S, Matsuoka K, Ogata T, Kaito H, Nakanishi K, Matsuo M. Severe Alport syndrome in a female caused by a t(X;1)(q22.3;p36.32) balanced translocation. Pediatr Nephrol. 2010 Oct;25(10):2165-70.
3. Ishikura K, Yoshikawa N, Hattori S, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Yata N, Ando T, Honda M; for Japanese Study Group of Renal Disease in Children. Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome. Nephrol Dial Transplant. 2010 Dec;25(12):3956-62.
4. Shima Y, Nakanishi K, Kamei K, Togawa T, Nozu K, Tanaka R, Sasaki S, Iijima K, Yoshikawa N. Disappearance of Glomerular IgA Deposits in Childhood IgA Nephropathy showing Diffuse Mesangial Proliferation after Two Years of Combination/Prednisolone Therapy. Nephrol Dial Transplant. 2011 Jan;26(1):163-9.
5. Nozu K, Iijima K, Kanda K, Nakanishi K, Yoshikawa N, Satomura K, Kaito H, Hashimura Y, Ninchoji T, Komatsu H, Kamei K, Miyashita R, Kugo M, Ohashi H, Yamazaki H, Mabe H, Otsubo A, Igarashi T, Matsuo M. The Pharmacological Characteristics of Molecular-Based Inherited Salt-Losing Tubulopathies. J Clin Endocrinol Metab. 2010 Dec;95(12):E511-8.
6. Ninchoji T, Kaito H, Nozu K, Hashimura Y, Kanda K, Kamioka I, Shima Y, Hamahira K, Nakanishi K, Tanaka R, Yoshikawa N, Iijima K, Matsuo M. Treatment strategies for Henoch-Schönlein purpura nephritis by histological and clinical severity. Pediatr Nephrol. 26:563-569, 2011 2011 Apr;26(4):563-9.
7. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, Hataya H, Honda M, Iijima K, Yoshikawa N; for the Japanese Pediatric IgA Nephropathy Treatment Study Group. Long-Term Results of a Randomized Controlled Trial in Childhood IgA Nephropathy. Clin J Am Soc Nephrol. 2011 Jun;6(6):1301-7.
8. Shima Y, Nozu K, Nozu Y, Togawa H, Kaito H, Matsuo M, Iijima K, Nakanishi K, Yoshikawa N. Recurrent EIARF and PRES with Severe Renal Hypouricemia by Compound Heterozygous *SLC2A9* Mutation Pediatrics. 2011 Jun;127(6):e1621-5.
9. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Hashimura Y, Kaito H, Sako M, Iijima K, Yoshikawa N. Validity of the Oxford classification of IgA nephropathy in children. Pediatr Nephrol. 2011 Dec 2. [Epub ahead of print]
10. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Sako M, Kaito H, Nozu K, Tanaka R, Iijima K, Yoshikawa N. Spontaneous remission in children with IgA nephropathy. Pediatr Nephrol. 2012;28:71-76
11. Ishikura K, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K,

Matsuyama T, Ito S, Yata N, Ando T, Honda M; for the Japanese Study Group of Renal Disease in Children. Two-Year Follow-Up of a Prospective Clinical Trial of Cyclosporine for Frequently Relapsing Nephrotic Syndrome in Children. Clin J Am Soc Nephrol. 2012;7:1576-1583
12. Iijima K, Someya T, Ito S, Nozu K, Nakanishi K, Matsuoka K, Ohashi H, Nagata M, Kamei K, Sasaki S. Focal Segmental Glomerulosclerosis in Patients With Complete Deletion of One WT1 Allele. Pediatrics. 2012;129:e1621-5
13. Konno K, Nakanishi K, Hishikawa S, Tanaka H, Yoshikawa N, Yasuda Y, Kobayashi E, Lefor A. Cryo-preserved porcine kidneys are feasible for teaching and training renal biopsy: "the bento kidney" Transplantation Research 2012;1:5
14. Hashimura Y, Kaito H, Nozu K, Shima Y, Nakanishi K, Iijima K, Yoshikawa N. Two cases of atypical membranoproliferative glomerulonephritis showing opposite clinical course. CEN Case Rep 2012;1:34-38
15. Ohtsubo H, Morisada N, Kaito H, Nagatani K, Nakanishi K, Iijima K. Alport-like glomerular basement membrane changes with renal-coloboma syndrome. Pediatr Nephrol. 2012;27:1189-92
16. Hama T, Nakanishi K, Shima Y, Mukaiyama H, Togawa H, Tanaka R, Hamahira K, Kaito H, Iijima K, Yoshikawa N. Renal biopsy criterion in children with asymptomatic constant isolated proteinuria. Nephrol Dial Transplant. 2012;27:3186-90
17. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Hashimura Y, Kaito H, Sako M, Iijima K, Yoshikawa N. Validity of the Oxford classification of IgA nephropathy in children. Pediatr Nephrol. 2012;27:783-92
18. Yoshihara D, Kugita M, Sasaki M, Horie S, Nakanishi K, Abe T, Aukema HA, Yamaguchi T, Nagao S. Telmisartan ameliorates fibrocystic liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. PLoS One. 2013 Dec 6;8(12):e81480
19. Iijima K, Sako M, Saito M, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, Yoshikawa N, for the Japanese Study Group of Kidney Disease in Children. Cyclosporine C2 monitoring for the treatment of frequently relapsing nephrotic syndrome in children: A multicenter randomized phase II trial. Clin J Am Soc Nephrol. 2013 Dec 5. [Epub ahead of print]
20. Hashimura Y, Nozu K, Kaito H, Nakanishi K, Fu XJ, Ohtsubo H, Hashimoto F, Oka M, Ninchoji T, Ishimori S, Morisada N, Matsunoshita N, Kamiyoshi N, Yoshikawa N, Iijima K. Genetic and clinical aspects of X-linked Alport syndrome in men positive for collagen IV α5 chain. Kidney Int. 2013 Dec 4. [Epub ahead of print]
21. Kaito H, Ishimori S, Nozu K, Shima Y, Nakanishi K, Yoshikawa N, Iijima K. Molecular Background of Urate Transporter Genes in Patients with Exercise-Induced Acute Kidney Injury. Am J Nephrol. 2013;38(4):316-20
22. Togawa H, Nakanishi K, Mukaiyama H, Hama T, Shima Y, Nakano M, Fujita N, Iijima K, Yoshikawa N. First Japanese case of Pierson syndrome with mutations in LAMB2. Pediatr Int. 2013 Apr;55(2):229-31
23. Nakanishi K, Iijima K, Ishikura K, Hataya H, Nakazato H, Sasaki S, Honda M, Yoshikawa N, for Japanese Study Group of Renal Disease in Children. Two-year outcome of the ISKDC regimen and frequent-relapsing risk in children with idiopathic nephrotic syndrome. Clin J Am Soc Nephrol 2013 May;8(5):756-62
24. Ishikura K, Uemura O, Ito S. Wada N, Hattori M, Ohashi Y, Hamasaki Y, Tanaka R, Nakanishi K, Kaneko T, Honda M; on behalf of The Pediatric CKD Study Group in Japan in conjunction with the Committee of

Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology: Pre-dialysis chronic kidney disease in children: results of a nationwide survey in Japan. Nephrol Dial Transplant 2013 Sep;28(9):2345-55
25. Hama T, Nakanishi K, Mukaiyama H, Shima Y, Togawa H, Sako M, Nozu K, Iijima K, Yoshikawa N. Endoplasmic reticulum stress with low-dose cyclosporine in frequently relapsing nephrotic syndrome. Pediatr Nephrol. 2013 Jun;28(6):903-9
26. Hamasaki Y, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ishikura K, Ito S, Kaneko T, Honda M; for Japanese Study Group of Renal Disease in Children. Prospective 5-year follow-up of cyclosporine treatment in children with steroid-resistant nephrosis. Pediatr Nephrol. 2013 May;28(5):765-71
27. Ishikura K, Uemura O, Hamasaki Y, Ito S, Wada N, Hattori M, Ohashi Y, Tanaka R, Nakanishi K, Kaneko T, Honda M; on behalf of the Pediatric CKD Study Group in Japan in conjunction with the Committee of Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology. Progression to end-stage kidney disease in Japanese children with chronic kidney disease: Results of a nationwide prospective cohort study. Nephrol Dial Transplant (in press)

（分担研究者：石倉健司）

1. Ishikura K, Yoshikawa N, Hattori S, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Yata N, Ando T, Honda M. Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome. Nephrol Dial Transplant, 2010, 25: 3956-3962
2. Tsurumi H, Ito M, Ishikura K, Hataya H, Ikeda M, Honda M, Nishimura G. Bifid epiglottis:
3. syndromic constituent rather than isolated anomaly. Pediatr Int, 2010, 52: 723-728
4. Ishikura K, Hamasaki Y, Sakai T, Hataya H, Goto T, Miyama S, Kono T, Honda M. Children with posterior reversible encephalopathy syndrome associated with atypical diffusion-weighted imaging and apparent diffusion coefficient. Clin Exp Nephrol, 2011, 15(2): 275-80
5. Wakaki H, Ishikura K. Henoch-Schonlein nephritis with nephrotic state in children: predictors of poor outcomes. Pediatr Nephrol, 2011, [Epub ahead of print]
6. Wakaki H, Ishikura K, Hataya H, Hamasaki Y, Sakai T, Yata N, Kaneko T, Honda M. Henoch-Schonlein purpura nephritis with nephrotic state in children: predictors of poor outcomes. Pediatr Nephrol, 2011, 26(6): 921-5
7. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K, Kitagawa T. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol, 2011, 15(5): 694-9
8. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, Hataya H, Honda M, Iijima K, Yoshikawa N. Long-term results of a randomized controlled trial in childhood IgA nephropathy. Clin J Am Soc Nephrol, 2011, 6(6): 1301-7
9. Ishikura K, Uemura O, Ito S, Wada N, Hattori M, Ohashi Y, Hamasaki Y, Tanaka R, Nakanishi K, Kaneko T, Honda M. Pre-dialysis chronic kidney disease in children: results of a nationwide survey in Japan. Nephrol Dial Trans in press
10. Ishikura K, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ito S, Yata N, Ando T, Honda M. for the Japanese Study Group of Renal Disease in Children. Two-Year Follow-Up of a Prospective Clinical Trial of Cyclosporine for Frequently Relapsing Nephrotic Syndrome in Children. Clin J Am Soc Nephrol 2012; 7: 1576-1583
11. Ishikura K, Hamasaki Y, Sakai T, Hataya H, Mak RH, Honda M. Posterior

reversible encephalopathy syndrome in children with kidney diseases. Pediatr Nephrol 2012; 27: 375-384
12. Shishido S, Satou H, Muramatsu M, Hamasaki Y, Ishikura K, Hataya H, Honda M, Asanuma H, Aikawa A. Combination of pulse methylprednisolone infusions with cyclosporine-based immunosuppression is safe and effective to treat recurrent focal segmental glomerulosclerosis after pediatric kidneytransplantation. Clin Transplant 2012 in press
13. Wakaki H, Ishikura K. Henoch-Schönlein nephritis with nephrotic state in children: predictors of poor outcomes. Pediatr Nephrol 2012;27: 335
14. Ikezumi Y, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Fujita N, Ito S, Iijima K, Kaneko T, Uemura O. Establishment of a normal reference value for serum β2 microglobulin in Japanese children:reevaluation of its clinical usefulness. Clin Exp Nephrol 2012; [Epub ahead of print]
15. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K; Japanese Society for Pediatric Nephrology, the Committee of Measures for Pediatric CKD. Is the new Schwartz equation derived from serum creatinine and body length suitable for evaluation of renal function in Japanese children? Eur J Pediatr 2012; 171: 1401-1404
16. Uemura O, Nagai T, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T, Honda M. Cystatin C-based equation for estimating glomerular filtration rate in Japanese children and adolescents. Clin Exp Nephrol. 2013; [Epub ahead of print]
17. Nagaoka Y, Ishikura K, Hamada R, Miyagawa T, Kono T, Sakai T, Hamasaki Y, Hataya H, Honda M. Severe posterior reversible encephalopathy syndrome resolved with craniectomy. Pediatr Int. 2013; 55: 644-646
18. Uemura O, Nagai T, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T, Honda M. Creatinine-based equation to estimate the glomerular filtration rate in Japanese children and adolescents with chronic kidney disease. Clin Exp Nephrol. 2013; [Epub ahead of print]
19. Nagai T, Uemura O, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T, Honda M. Creatinine-based equations to estimate glomerular filtration rate in Japanese children aged between 2 and 11 years old with chronic kidney disease. Clin Exp Nephrol. 2013; [Epub ahead of print]
20. Ishikura K, Uemura O, Ito S, Wada N, Hattori M, Ohashi Y, Hamasaki Y, Tanaka R, Nakanishi K, Kaneko T, Honda M. Pediatric CKD Study Group; Japan Committee of Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology. Pre-dialysis chronic kidney disease in children: results of a nationwide survey in Japan. Nephrol Dial Transplant. 2013; 28: 2345-2355
21. Ishikura K, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ito S, Yata N, Ando T, Honda M. Two-year follow-up of a prospective clinical trial of cyclosporine for frequently relapsing nephrotic syndrome in children. Clin J Am Soc Nephrol. 2013; 8: 756-62.
22. Yata N, Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K, Saito M, Keneko T, Kitagawa T. Reference ranges for serum cystatin C measurements in Japanese children by using 4 automated assays. Clin Exp Nephrol. 2013; 28. [Epub ahead of print].
23. Shishido S, Satou H, Muramatsu M, Hamasaki Y, Ishikura K, Hataya H, Honda M, Asanuma H, Aikawa A. Combination of pulse methylprednisolone infusions with cyclosporine-based immunosuppression is safe and

effective to treat recurrent focal segmental glomerulosclerosis after pediatric kidney transplantation. Clin Transplant 2013; 27: E143-E150.

24. Nakanishi K, Iijima K, Ishikura K, Hataya H, Nakazato H, Sasaki S, Honda M, Yoshikawa N. Two-year outcome of the ISKDC regimen and frequent-relapsing risk in children with idiopathic nephrotic syndrome. Clin J Am Soc Nephrol 2013; 8: 756-762.
25. Hamasaki Y, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ishikura K, Ito S, Kaneko T, Honda M. Prospective 5-year follow-up of cyclosporine treatment in children with steroid-resistant nephrosis. Pediatr Nephrol 2013; 28: 765-771.
26. Ikezumi Y, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Fujita N, Ito S, Iijima K, Kaneko T, Uemura O. Establishment of a normal reference value for serum beta2 microglobulin in Japanese children: reevaluation of its clinical usefulness. Clin Exp Nephrol 2013; 17: 99-105.
27. Honda M, Iijima K, Ishikura K, Kaneko K The problem of transition from pediatric to adult healthcare in patients with steroid- sensitive nephrotic syndrome (SSNS): a survey of the experts. Clin Exp Nephrol in press
28. Ishikura K, Uemura O, Hamasaki Y, Ito S, Wada N, Hattori M, Ohashi Y, Tanaka R, Nakanishi K, Kaneko T, Honda M. Progression to end-stage kidney disease in Japanese children with chronic kidney disease: Results of a nationwide prospective cohort study. Nephrol Dial Transplant in press
29. Iijima K, Sako M, Saito M, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, Yoshikawa N. for the Japanese Study Group of Kidney Disease in Children. Cyclosporine C2 monitoring for the treatment of frequently relapsing nephrotic syndrome in children: A multicenter randomized phase II trial. Clin J Am Soc Nephrol in press

(分担研究者：伊藤秀一)

1. Machida H, Ito S, Hirose T, Takeshita F, Oshiro H, Nakamura T, Mori M, Inayama Y, Yan K, Kobayashi N. Yokota S. Expression of Toll-like receptor 9 in renal podocytes in childhood-onset active and inactive lupus nephritis. Nephrol Dial Transplant 25:2530-537, 2010
2. Ito S, Machida H, Harada T, Teranishi J, Aihara Y, Yokota S. Extended-specdtrum beta-lactamase-producing bacteria and vesicoureteral reflux in children. Pediatr Int. 52 :134-7, 2010
3. Iijima K, Nozu K, Nakayama M, Ito S, Matsuoka K, Ogata T, Kaito H, Nakanishi K, Matsuo M. Severe Alport syndrome in a young woman caused by a t(X;1)(q22.3;p36.32)balanced translocation. Pediatr Nephrol 25 :2165-70, 2010
4. Nakayama M, Nozu K, Goto Y, Kamei K, Ito S, Sato H, Emi M, Nakanishi K, Tsuchiya S, Iijima K. HNF1B alterations associated with congential anomalies of the kidney and urinary tract. Pediatr Nephrol 25:1073-9, 2010
5. Ito S, Tsutsumi A, Harada T, Inaba A, Fujinaga S, Kamei K. Long-terma remission of nephritic syndrome with etanercept for concomitant juvenile idiopathic arthritis. Periatr Nephrol 25:2157-80, 2010
6. Ito S, Kamei K, Ikoma M. Primary Sjogren syndrome that developed after lgA nepjropathy. Pediatr Nephrol 25:1579-80, 2010
7. Kikuchi E, Kubota M, Kamei K, Ito S. Critical illness polyneuropathy after septic peritonitis in a boy with nephritic syndrome. Pediatr Nephrol 25:1771-2, 2010
8. Fujinaga S, Hirano D, Nishizaki N, Kamei K, Ito S, Ohtomo Y, Shimizu T, Kaneko K. Single infusion of rituxibmab for persistent steroid-dependent minimal-charge nephritic syndrome after long-term cyclosporine. Pediatr

Nephrol. 25:539-44, 2010
9. Ogura M, Kikuchi E, Kaito H, Kamei K, Matsuoka K, Tanaka H, Kuroda T, Sekine T, Ito S. ABO-incompatible renal transplantation in Epstein syndrome. Clin Transplant. Suppl 22: 31-4, 2010
10. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, Hataya H, Honda M, Iijima K, Yoshikawa N; for the Japanese Pediatric IgA Nephropathy Treatment Study Group. Long-TermResults of a Randomized Controlled Trial in Childhood IgA Nephropathy. Clin J Am Soc Nephrol. 2011, 6:1301-7
11. Ito S, Kamei K, Ogura M, Sato M, Fujimaru T, Ishikawa T, Udagawa T, Iijima K. Maintenance therapy with MMF after rituximab in pediatric patients with steroid-dependent nephrotic syndrome. Pediatr Nephrol. 2011, 26:1823-8
12. Kamei K, Ito S, Shigeta T, Sakamoto S, Fukuda A, Horikawa R, Saito O, Muguruma T, Nakagawa S, Iijima K, Kasahara M. Preoperative dialysis for liver transplantation of methylmalonic acidemia. Ther Apher Dial, 2011, 15:488-92
13. Harada T, Kaneko T, Ito S, Hataya H, Nariai A, Mori M, Yokota S. Superior mesenteric artery syndrome : risk factor for duodenal involvement in Henoch-Schönlein purpura. Pediatr Int. 2011, 53:630-3
14. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K, Kitagawa T. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol, 2011, 15:694-9
15. Sakamoto S, Kasahara M, Fukuda A, Tanaka H, Kakiuchi T, Karaki C, Kanazawa H, Kamei K, Ito S, Nakazawa A. Pediatric liver-kidney transplantation for hepatorenal fibrocystic disease from a living donor. Pediatr Transplant. 16 : 99-102, 2012
16. Iijima K, Someya T, Ito S, Nozu K, Nakanishi K, Matsuoka K, Ohashi H, Nagata M, Kamei K, Sasaki S. Focal segmental glomerulosclerosis in patients with complete deletion of one WT1 allele. Pediatrics. 129:e1621-5, 2012
17. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K; The Japanese Society for Pediatric Nephrology, the Committee of Measures for Pediatric CKD. (9/11) Is the new Schwartz equation derived from serum creatinine and body length suitable for evaluation of renal function in Japanese children? Eur J Pediatr. 171:1401-1404, 2012
18. Ishikura K, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ito S, Yata N, Ando T, Honda M; for the Japanese Study Group of Renal Disease in Children. (8/12) Two-Year Follow-Up of a Prospective Clinical Trial of Cyclosporine for Frequently Relapsing Nephrotic Syndrome in Children. Clin J Am Soc Nephrol. 7:1576-1583, 2012
19. Udagawa T, Kamei K, Ogura M, Tsutsumi A, Noda S, Kasahara M, Fukuda A, Sakamoto S, Shigeta S, Tanaka H, Kuroda T, Matsuoka K, Nakazawa A, Nagai T, Uemura O, Ito S. Sequential liver-kidney transplantation in a boy with congenital hepatic fibrosis and nephronophthisis from a living donor. Pediatr Transplant. 16:E275-80, 2012.
20. Noda S, Ogura M, Tsutsumi A, Udagawa T, Kamei K, Matsuoka K, kitamura H, Atsumi T, Ito S. Thrombotic microangiopathy due tomultiple autoantibodeies related to antiphospholipid syndrome . Pediatr Nephrol 27:681-5, 2012
21. Ito S, Kemei K, Ogura M, Udagawa T, Fujinaga S, Saito M, Sako M, Iijima K. Survey of rituximab treatment for childhood-onset refractory nephrotic syndrome. Pediatr Nephrol. 28:257-64, 2012
22. Ito S. Disease Course in Childhood Steroid Sensitive Nephrotic Syndrome: Is it Changeable? Ind Pediatr. 49:868-9, 2012

23. Sato M, Ito S, Ogura M, Kamei K, Miyairi I, Miyata I, Higuchi M, Matsuoka K. Atypical Pneumocystis jiroveci pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome. Pediatr Nephrol. 28:145-9, 2013
24. Miyazono A, Abe J, Ogura M, Sato M, Fujimaru T, Kamei K, Ito S. Successful remission induced by plasma exchange combined with leukocytapheresis against refractory systemic juvenile idiopathic arthritis. Eur J Pediatr. Jul 13. 2013[Epub ahead of print]
25. Kamei K, Ogura M, Ishimori S, Kaito H, Ito S Acute kidney injury after acute gastroenteritis in an infant with hereditary hypouricemia. Eur J Pediatrl May 8 2013[Epub ahead of print]
26. Kaito H, Kamei K, Ogura M, Kikuchi E, Hoshino H, Nakagawa S, Matsuoka K, Abe J, Ito S. Acute encephalopathy and tubulointerstitial nephritis associated with Yersinia pseudotuberculosis. Pediatr Int. 54:926-8, 2013.
27. Kamei K, Okada M, Sato M, Fujimaru T, Ogura M, Nakayama M, Kaito H, Iijima K, Ito S. Rituximab treatment combined with methylprednisolone pulse therapy and immunosuppressants for childhood steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2014 Feb 6. [Epub ahead of print]
28. Sato M, Ito S, Ogura M, Kamei K. Impact of rituximab on height and weight in children with refractory steroid-dependent nephrotic syndrome. Pediatr Nephrol. 2014. [Epub ahead of print]
29. Hattori M, Matsunaga A, Akioka Y, Fujinaga S, Nagai T, Uemura O, Nakakura H, Ashida A, Kamei K, Ito S, Yamada T, Goto Y, Ohta T, Hisano M, Komatsu Y, Itami N. Darbepoetin alfa for the treatment of anemia in children undergoing peritoneal dialysis: a multicenter prospective study in Japan. Clin Exp Nephrol. 17:582-8, 2013
30. Yata N, Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Nagai T, Ikezumi Y, Fujita N, Ito S、Iijima K, Saito M, Kaneko T, Kitagawa T. Reference ranges for serum cystain C measurements in Japanese children by using 4 automated assays. Clin Exp Nephrol. 2013 Feb 28. [Epub ahead of print]
31. Hamasaki Y, Yoshikawa N, Nakazato H, Sasaki S, Iijima K, Nakanishi K, Matsuyama T, Ishikura K, Ito S, Keneko T, Honda M;for Japanese Study group of Renal Disease in Children Prospective 5-year follow-up of cyclosporine treatment in Children with wteroid-resistant nephrosis. Pediatr Nephrol 28: 765-71, 2013.
32. Ikezumi Y, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Fujita N, Ito S, Iijima K, Kaneko T, Uemura O. Establishment of a normal reference value for serum β2 microglobulin in Japanese children: reevaluation of its clinical usefulness. Clin Exp Nephrol. 17:99-105, 2013
33. Nagai S, Saito Y, Endo Y, Saito T, Sugai K, Ishiyama A, Komaki H, Nakagawa E, Sasaki M, Ito K, Saito Y, Sukigara S, Ito M, Goto Y, Ito S, Matsuoka K. Hypoalbuminemia in early onset dentatorubral-pallidoluysian atrophy due to leakage of albumin in multiple organs. J Neurol. 260:1263-71, 2013.
34. Ishikura K, Uemura O, Ito S, Wada N, Hattori M, Ohashi Y, Hamasaki Y, Tanaka R, Nakanishi K, Kaneko T, Honda M; on behalf of the Pediatric CKD Study Group in Japan in conjunction with the Committee of Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology. Pre-dialysis chronic kidney disease in children: results of a nationwide survey in Japan. Nephrol Dial Transplant. 28:2345-55, 2013
35. Nagai T, Uemura O, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T, Honda M. Creatinine-based equations to estimate glomerular filtration rate in Japanese children aged between 2 and 11 years old with chronic kidney

disease. Clin Exp Nephrol. 2013 Apr 6. [Epub ahead of print]

36. Itoh M, Iwasaki Y, Ohno K, Inoue T, Hayashi M, Ito S, Matsuzaka T, Ide S, Arima M. Nationwide survey of Arima syndrome: Revised diagnostic criteria from epidemiological analysis. Brain Dev. 2013 Jul 8. doi:pii: S0387-7604(13)00195-2. 10.1016/j.braindev.2013.06.005. [Epub ahead of print]
37. Ishikura K, Uemura O, Hamasaki Y, Ito S, Wada N, Hattori M, Ohashi Y, Tanaka R, Nakanishi K, Kaneko T, Honda M; on behalf of the Pediatric CKD Study Group in Japan in conjunction with the Committee of Measures for Pediatric CKD of the Japanese Society of Pediatric Nephrology. Progression to end-stage kidney disease in Japanese children with chronic kidney disease: results of a nationwide prospective cohort study. Nephrol Dial Transplant. 2014 Feb 9. [Epub ahead of print]
38. Ijima K, Sako M, Saito M, Ito S, Hataya H, Tanaka H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, Yoshikawa N, for the Japanese Study Group of Kidney Disease in Children. Cyclosporine C2 monitoring for the treatment of freqauently relapsing nephritic syndrome in children: A multicenter randomized phase II trial. Clin J of the Amecican Society of Nephrol. Sept. 25,2013 [Epub ahead of print]
39. Uemura O, Nagai T, Ishikura K, Ito S, Hataya H, Gotoh Y, Gujita N, Akioka Y, Kaneko T, Honda M. (4/10) Cystatin C-based equation for estimating glomerular filtration rate in Japanese children and adolescents. Clin Exp Nephrol. Nov. 20, 2013 [Epub ahead of print]

（分担研究者：佐古まゆみ）

1. Kamei K, Nakanishi K, Ito S, Saito M, Sako M, Ishikura K, Hataya H, Honda M, Iijima K, Yoshikawa N. Japanese Pediatric IgA Nephropathy Treatment Study Group. Long-term results of a randomized controlled trial in childhood IgA nephropathy. Clin J Am Soc Nephrol, 2011, 6:1301-7.
2. Togawa H, Nakanishi K, Mukaiyama H, Hama T, Shima Y, Sako M, Miyajima M, Nozu K, Nishii K, Nagao S, Takahashi H, Iijima K, Yoshikawa N. Epithelial-to-mesenchymal transition in cyst-lining epithelial cells in an orthologous PCK rat model of autosomal-recessive polycystic kidney disease. Am J Physiol Renal Physiol, 2011, 300:F511-520.
3. Hama T, Nakanishi K, Mukaiyama H, Shima Y, Togawa H, Sako M, Nozu K, Iijima K, Yoshikawa N. Endoplasmic reticulum stress with low-dose cyclosporine in frequently relapsing nephrotic syndrome. Pediatr Nephrol. 2013 Jan 15. [Epub ahead of print]
4. Ito S, Kamei K, Ogura M, Udagawa T, Fujinaga S, Saito M, Sako M, Iijima K. Survey of rituximab treatment for childhood-onset refractory nephrotic syndrome. Pediatr Nephrol. 2013;28:257-264.
5. Shima Y, Nakanishi K, Hama T, Mukaiyama H, Togawa H, Sako M, Kaito H, Nozu K, Tanaka R, Iijima K, Yoshikawa N. Spontaneous remission in children with IgA nephropathy. Pediatr Nephrol. 2013 ;28:71-76.
6. Iijima K, Sako M, Saito M, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, Yoshikawa N, for the Japanese Study Group of Kidney Disease in Children. Cyclosporine C2 monitoring for the treatment of frequently relapsing nephrotic syndrome in children: A multicenter randomized phase II trial. Clin J Am Soc Nephrol. 2013 Dec 5. [Epub ahead of print]

（分担研究者：中村秀文）

1. H. Nakamura : Current situation and critical points for peadiatric research in Japan. Innovative Medicine: The Science and the Regulatory Framework.

Pharmaceuticals Policy and Law Volume 12. pp 117-124. 2010
2. M Mori, H Kawashima, H Nakamura, M Nakagawa, S Kusuda, T Saji, H Tsutsumi, S Yokota, S Itoh. Nationwide survey of severe respiratory syncytial virus infection in children who do not meet indications for palivizumab in Japan. J Infect Chemother., 2011, Apr; 17(2): Page 254-63.
3. K Hoppu, G Anabwani, F Garcia-Bournissen, M Gazarian, GL Kearns, H Nakamura, RG Peterson, S Sri Ranganathan, SN de Wildt. The status of paediatric medicines initiatives around the world-what has happened and what has not?. Eur J Clin Pharmacol, 2012, 68:1-10
4. Inoue Y, Otsuki T, Nakamura H, Nakagawa E, Usui N; Efficacy, safety, and pharmacokinetics of fosphenytoin injection in Japanese patients. 臨床医薬 2012; 28:623-632.
5. Nakamura H, Kawashima H, Azuma R, Sato I, Nagao K, Miyazawa K: Pharmacokinetics of the H2 blocker roxatidine acetate hydrochloride in pediatric patients, in comparison with healthy adult volunteers. Drug Metab. Pharmacokinet 2012;27:422-429.
6. Tahara T, Asano Y, Mitamura K, Nakamura H, Itoh S: Safty of oseltamivir in infants less than one year old:Prospective surveillance during the 2004-2005 influenza season in Japan. Journal of Pediatric Infectious Diseases 2013;8(2):71-81
7. Iijima K, Sako M, Saito Oba M, Ito S, Hataya H, Tanaka R, Ohwada Y, Kamei K, Ishikura K, Yata N, Nozu K, Honda M, Nakamura H, Nagata M, Ohashi Y, Nakanishi K, and Yoshikawa N, Japanese Study Group of Kidney Disease in Children: Cyclosporine C2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children:A Multicenter Randomized Phase II Trial. Clinical Journal of the American Society of Nephrology 2014;9(2):1-8

G. 知的所有権の取得状況

なし

III.　研究成果の刊行に関する一覧表

IV.　研究成果の刊行物

研究成果の刊行に関する一覧表

書籍

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ishikura K, Yoshikawa N, Iijima K, Nakanishi K, Honda M, et al	Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome	Nephrol Dial Transplant	25	3956-62	2010
Kanda S, Igarashi T, et al	Tyrosine phosphorylation-dependent activation of TRPC6 regulated by PLC-γ1 and nephrin: effect of mutations associated with focal segmental glomerulosclerosis	Mol Biol Cell	22	1824-35	2011
Ishikura K, Yoshikawa N, Iijima K, Nakanishi K, Ito S, Honda M, et al.	Two-year follow-up of a prospective clinical trial of cyclosporine for frequently relapsing nephrotic syndrome in children.	Clin J Am Soc Nephrol	7	1576-1583	2012
Yoshikawa N, Iijima K, Nakanishi K, Ishikura K, Ito S, Honda M, et al.	Prospective 5-year follow-up of cyclosporine treatment in children with steroid-resistant nephrosis.	Periatr Nephrol	28	765-771	2013
Nakanishi K, Iijima K, Ishikura K, Honda M, Yoshikawa N, et al.	Two-year outcome of the ISKDC regimen and frequent-relapsing risk in children with idiopathic nephrotic syndrome.	Clin J Am Soc Nephrol	8	756-762	2013
Iijima K, Sako M, Ito S, Ishikura K, Honda M, Nakamura H, Nakanishi K, Yoshikawa N et al.	Cyclosporine C2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children: A Multicenter Randomized Phase II Trial.	Clin J Am Soc Nephrol	9	Epub ahead of print	2014

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Original Article

Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome

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Abstract

Background. We previously established a treatment protocol for conventional cyclosporine (Sandimmune, Novartis, Basel, Switzerland) in children with frequently relapsing nephrotic syndrome; ~50% of patients remained relapse free for 2 years, without serious adverse events. Recently, microemulsified cyclosporine (Neoral, Novartis), which has a more stable absorption profile than conventional cyclosporine, has been developed. We tested the hypothesis that microemulsified cyclosporine is at least as effective as conventional cyclosporine.

Methods. To evaluate the safety and efficacy of microemulsified cyclosporine, a prospective, multicentre trial was conducted according to the previously established protocol, using microemulsified cyclosporine instead of conventional cyclosporine. The duration of treatment was 24 months. During the first 6 months, patients received microemulsified cyclosporine in a dose that maintained the trough level between 80 and 100 ng/mL of cyclosporine. For the next 18 months, the dose was adjusted to maintain a level between 60 and 80 ng/mL.

Results. A total of 62 patients (median age, 5.4 years; 48 males, 14 females) were studied. The frequency of relapse decreased from 4.6 ± 1.4 to 0.7 ± 1.5 times per year ($P<0.0001$). The probability of relapse-free survival at Month 24 was 58.1% (95% confidence interval, 45.8–70.3%). The probability of progression (to frequently relapsing nephrotic syndrome)-free survival at Month 24 was 88.5% (95% confidence interval, 80.4–96.5%). Cyclosporine nephrotoxicity was detected in only 8.6% of patients who underwent renal biopsy after 2 years of treatment. Antihypertensive agents were administered to 12.9% of the patients to control hypertension without severe sequelae.

Conclusions. Microemulsified cyclosporine administered according to our treatment protocol is safe and effective in children with frequently relapsing nephrotic syndrome.

Keywords: clinical trial; microemulsified cyclosporine; nephrotic syndrome; paediatric nephrology

Introduction

Managing frequently relapsing nephrotic syndrome (FRNS) in children remains challenging despite progress in treatment. The development of immunosuppressive therapies other than corticosteroids has been enthusiastically attempted to date [1–4] because repeated treatment with corticosteroids can lead to serious adverse events.

Cyclosporine is one treatment of choice for children with FRNS or steroid-dependent nephrotic syndrome [5–10]. For such patients, we have already established a safe and effective protocol for treatment with conventional cyclosporine, Sandimmune (Novartis, Basel, Switzerland), in a prospective, randomized, multicentre trial [11]. With our protocol, the dose of cyclosporine is titrated on the basis of the whole-blood trough level. Approximately 50% of children with FRNS treated according to this protocol are expected to remain relapse free for 2 years, without serious adverse events.

Microemulsified cyclosporine, Neoral (Novartis, Basel, Switzerland) is a newer formulation of cyclosporine, designed to promote stable absorption and improved bioavailability [12–15]. Several small studies have compared safety and efficacy between conventional cyclosporine and microemulsified cyclosporine in children with nephrotic syndrome [16] and recipients of renal transplants [17,18];

microemulsified cyclosporine has consistently been suggested to be more effective, without compromising safety.

Because cyclosporine is stably absorbed after administration of microemulsified cyclosporine, the dose of microemulsified cyclosporine can be titrated on the basis of the area under the concentration-time curve during the first 4 h after treatment (AUC_{0-4h}) or the 2-h post-dose cyclosporine level (C2) in children who receive kidney transplants [19,20]. The clinical efficacy of microemulsified cyclosporine titrated by monitoring AUC_{0-4h} or C2 in patients with nephrotic syndrome is also expected but remains to be confirmed.

We performed a prospective, single-arm, multicentre trial according to our previously established protocol, using microemulsified cyclosporine instead of conventional cyclosporine. The principal aim of this trial was to evaluate the efficacy in terms of relapse-free survival probability and the safety of microemulsified cyclosporine in children with FRNS. The benefits of AUC_{0-4h} and C2 monitoring in this clinical setting were also assessed.

Materials and methods

Patients

The study group comprised children (1–18 years of age) with FRNS who had idiopathic nephrotic syndrome. Patients were excluded if they had any of the following conditions: (i) other renal or systemic forms of nephrotic syndrome diagnosed on the basis of renal biopsy, clinical features or serology; (ii) poorly controlled hypertension; (iii) chronic renal dysfunction (creatinine clearance of ≤60 mL/min/1.73 m^2); (iv) active infectious disease; (v) severe liver dysfunction; (vi) a history of treatment with cyclosporine; or (vii) pregnancy.

The criteria for and definitions of nephrotic syndrome, remission, and relapse were in accordance with the International Study of Kidney Disease in Children [21]. FRNS was defined as two or more relapses of nephrotic syndrome within 6 months after the initial episode, three or more relapses within any 6-month period, or four or more relapses within any 12-month period. Steroid dependence was defined as the occurrence of two consecutive relapses on tapering the steroid dosage or within 14 days after the termination of steroids.

'Ethical Guidelines for Clinical Research', requiring that all protocols for clinical studies are reviewed by an external ethics committee, were issued by the Japanese Ministry of Health, Labour and Welfare in 2003. At the start of our trial (January 2000), the study protocol was approved by the director or other responsible person at each participating centre and was not reviewed by an external review board. Therefore, at the time of the submission for publication, a retrospective approval by an ethical committee was performed. The ethical standards laid down in the Declaration of Helsinki were applied accordingly in the design and execution of this study. Informed consent was obtained from all patients or their parents.

Protocol

The total duration of treatment was 24 months. For the first 6 months, all patients received microemulsified cyclosporine in a dose that maintained a whole-blood trough level between 80 and 100 ng/mL of cyclosporine; for the next 18 months, the dose was adjusted to maintain a trough level between 60 and 80 ng/mL. Maintenance prednisolone was not prescribed. After 2 years of treatment, all patients were scheduled to undergo renal biopsy, and the dose of cyclosporine was tapered by 0.5–1.0 mg/kg per day every week. The concomitant use of drugs other than corticosteroids and immunosuppressants was not restricted. Antihypertensive agents, including angiotensin-converting enzyme inhibitors, and HMG-CoA reductase inhibitors (statins) were also permitted.

Blood analysis (complete blood cell count and blood chemistry) and urine tests (urinalysis and quantitative proteinuria) were performed monthly during follow-up. The trough level of cyclosporine was measured monthly by monoclonal radioimmunoassay. In addition to the trough level, other indices of cyclosporine absorption (i.e. AUC_{0-4h} and C2) were examined at Month 1. For cyclosporine AUC_{0-4h} and C2 sampling, time lags of ±5 mins were allowed. AUC_{0-4h} was calculated by the linear trapezoidal method.

Patients in whom FRNS or steroid-resistant nephrotic syndrome developed during treatment received off-protocol therapy, left to the discretion of the physician in charge.

Corticosteroid treatment

To treat relapses of nephrotic syndrome immediately before study entry, patients received 2 mg/kg/day of prednisolone in three divided doses (maximum dose, 80 mg/day) for 4 weeks, followed by a single dose of 2 mg/kg of prednisolone administered in the morning on alternate days for 2 weeks, 1 mg/kg on alternate days for 2 weeks and 0.5 mg/kg on alternate days for 2 weeks. Patients who had relapses of nephrosis during the study period received 2 mg/kg/day of prednisolone in three divided doses (maximum, 80 mg/day) until remission, followed by a single dose of 2 mg/kg of prednisolone administered in the morning on alternate days for 2 weeks, 1 mg/kg on alternate days for 2 weeks and 0.5 mg/kg on alternate days for 2 weeks.

Histopathological examination

A pathologist at each study centre examined each renal biopsy specimen. An independent investigator at the coordinating centre who was blinded to all patient data also reviewed the histologic sections. Arteriolar changes, tubular atrophy and interstitial fibrosis were graded semi-quantitatively on a scale of 0–3+ as follows: 0, none; 1+, mild; 2+, moderate; and 3+, intense.

Statistical analysis

The primary end point was the probability of relapse-free survival, based on the period until the first relapse. The secondary end point was the probability of progression-free survival, based on the period until the development of FRNS. Survival curves were estimated by the Kaplan–Meier method. Survival curves from our previous study of conventional cyclosporine are included in the figures of this study. Multivariate analyses using Poisson regression were performed to estimate the relations of AUC_{0-4h} or C2 to the incidence of relapse, adjusting for sex, age and steroid dependence. Data were analysed according to the intention-to-treat. A two-sided P-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed with the use of the software package SAS for Windows, release 9.13 (SAS Institute Inc., Cary, NC, USA).

Clinical trial registration

This study has been registered in a public trials registry, the University Hospital Medical Information Network (UMIN, ID C000000010, http://www.umin.ac.jp/ctr/index.htm).

Results

Data set

Between January 2000 and December 2005, a total of 66 children were enrolled at 21 institutions, and 4 patients were excluded from all analyses. Therefore, 62 children (59 with minimal change nephrotic syndrome and 3 with mesangial proliferative glomerulonephritis; 48 males and 14 females) received treatment and were included in analysis (Figure 1). Their median ages at diagnosis and at study entry were 3.0 years (range, 1.3–14.5) and 5.4 years (range, 1.7–15.3), respectively. The clinical characteristics of the patients at entry are shown in Table 1.

As for concomitant medications, antihypertensive agents were given to eight patients (angiotensin-converting enzyme inhibitors, 4 patients; calcium channel blockers, 3;

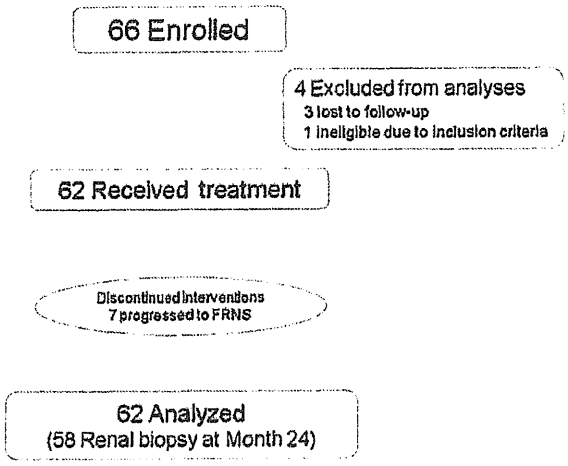

Fig. 1. Flow diagram. FRNS, frequently relapsing nephrotic syndrome.

and both drugs, 1). A HMG-CoA reductase inhibitor (statin) was given to one patient.

Cyclosporine dosage and trough level

The mean dose of cyclosporine required to maintain the whole-blood trough level between 80 and 100 ng/mL during the first 6 months of treatment was 5.1 mg/kg/day. During the next 18 months, the mean dose of cyclosporine required to maintain a trough level between 60 and 80 ng/mL was 4.5 mg/kg/day. The distributions of the trough level are shown in Figure 2.

Decreased frequency of relapses after treatment with cyclosporine

Before treatment, the mean number of relapses was 4.6 ± 1.4 times per year. During the 2 years of treatment with cyclosporine, the mean number of relapses decreased significantly to 0.7 ± 1.5 times per year (paired *t*-test, $P<0.0001$).

Probability of relapse-free and progression-free survival

The estimated relapse rate, defined as the total number of patients who had relapse during the trial divided by the duration of observation for all patients, was 0.28 (95% confidence interval, 0.17–0.39) per year. Figure 3 shows the results of Kaplan–Meier analysis. At Month 24, the probability of relapse-free survival was 58.1% (95% confidence interval, 45.8–70.3%). In this figure, the probability of relapse-free survival in the present study was compared with that in our previous trial [11]. The probability of relapse-free survival in Group A [24 patients (18 males); median age, 7.3 years], which received conventional cyclosporine according to the same protocol, was 50.0%, while that in Group B [20 patients (17 males); median age, 6.9 years old), which received a fixed dose of conventional cyclosporine (2.5 mg/kg) from Month 7 onwards, was 15.0%.

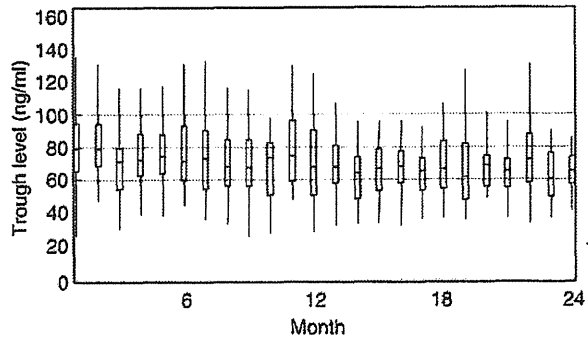

Fig. 2. Median trough level of cyclosporine during study period.

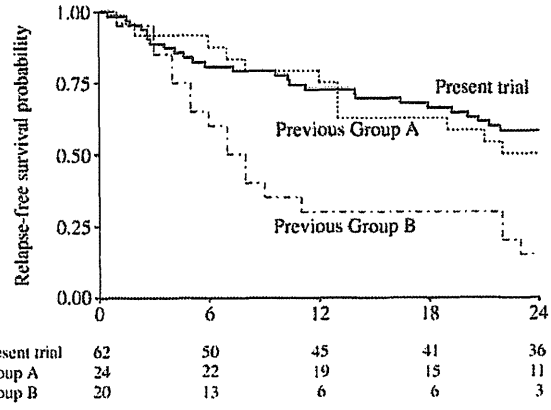

Fig. 3. Comparison with the previous trial [11]. Previous Group A, patients in Group A received conventional cyclosporine according to the same protocol in the previous trial; Previous Group B, patients in Group B received a fixed dose of conventional cyclosporine (2.5 mg/kg) from Month 7 onwards. Most patients in Group B had a trough level <60 ng/mL during this period.

Table 1. Patient's characteristics

Age (years)	Sex (*n*)		Number of relapses before entry (*n*)				Steroid dependence (*n*)		
	Male	Female	NA	≥2–<4/year	≥4–<6/year	≥6/year	NA	(−)	(+)
All ages	48	14	1	17	32	12	1	29	32
0–<3	6	5	0	1	6	4	0	3	8
≥3–<6	18	8	0	7	15	4	0	13	13
≥6–<10	12	0	1	5	5	1	1	4	7
≥10–<15	10	1	0	4	4	3	0	7	4
≥15	2	0	0	0	2	0	0	2	0

NA, not available.

Table 2. Analysis for predictors of relapse

		Univariate			Multivariate		
		Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Sex	Male	2.72	0.82–9.07	0.10	1.77	0.33–9.38	0.49
	Female	1.00			1.00		
Age	≥6 years	2.17	1.01–4.70	0.05	1.95	0.69–5.52	0.19
	<6 years	1.00			1.00		
Steroid dependence	Yes	1.01	0.46–2.20	0.99	1.30	0.52–3.26	0.58
	No	1.00			1.00		
AUC_{0-4h}	Each increment of 100-ng × h/mL	0.95	0.88–1.03	0.19	0.95	0.87–1.04	0.23

CI, confidence interval; AUC_{0-4h}, the area under the concentration-time curve during the first 4 h after treatment.

Table 3. Nephrotoxicity of cyclosporine

Age/sex	Relapse during treatment	Progression to FRNS during treatment	AUC_{0-4h} at 1 month (ng × h/mL)	C2 at 1 month (ng/mL)	Mean trough level (ng/mL)	Hypertension	Renal histology
2.9 male	Yes	No	1063	290	91.1	(+)	Mild arteriolar hyalinosis Mild tubular atrophy Mild interstitial fibrosis
3.9 male	No	No	2160	690	92.4	(−)	Mild arteriolar hyalinosis and vacuolation Mild tubular atrophy Mild interstitial fibrosis
5.4 male	No	No	2251	720	72.2	(−)	Mild arteriolar hyperplasia Mild-to-moderate tubular atrophy No interstitial fibrosis
6.2 male	No	No	975	380	60.0	(−)	Mild arteriolar hyalinosis and vacuolation Mild tubular atrophy Mild interstitial fibrosis
10.8 male	Yes	No	619	160	72.0	(+)	Mild arteriolar hyalinosis Mild tubular atrophy Mild interstitial fibrosis

FRNS, frequently relapsing nephrotic syndrome, AUC_{0-4h}, the area under the concentration-time curve during the first 4 h after treatment; C2, the 2-h post-dose cyclosporine level.

The estimated rate of progression to FRNS was 0.06 (0.02–0.11) per year. The probability of progression (to FRNS)-free survival at Month 24 was 88.5% (95% confidence interval, 80.4–96.5%).

Steroid-resistant nephrotic syndrome did not develop in any patient during the trial.

AUC_{0-4h}, C2 and relapse

The mean AUC_{0-4h} at 1 month was 1493.4±681.2 ng × h/mL, and that of C2 was 486.0 ± 203.9 ng/ml. Table 2 shows the results of Poisson regression analysis for AUC_{0-4h}, adjusted for important prognostic factors. None of the four risk factors analysed [AUC_{0-4h} (continuous), sex (male or female), age (≥6 years or <6 years), steroid dependence (yes or no)] were significantly related to relapse. The hazard ratio for AUC_{0-4h} was 0.95 (95% confidence interval, 0.87–1.04; P=0.23) for each 100-ng × h/mL increment.

The results of Poisson regression analysis using C2 in place of AUC_{0-4h} were similar, and the hazard ratio for C2 was 0.86 (95% confidence interval, 0.64–1.15; P=0.30) for each 100-ng/mL increment.

Growth

Before cyclosporine treatment (at study entry), the mean standard deviation (s.d.) score for body height was −0.27 ± 1.01 (n=62); at the end of the trial, the mean s.d. score for body height was 0.33±0.97 (n=58). The s.d. score for height increased significantly from the start to the end of 2-year treatment (paired t-test, P<0.001).

Adverse events

Renal biopsies were performed in 58 patients at the end of 2 years of treatment. The results are shown in Table 3. Mild nephrotoxicity attributed to cyclosporine occurred in 5 (8.6%) of the 58 patients. Other adverse events are shown in Table 4. Hypertension, defined as a requirement for antihypertensive agents during the trial, was detected in 12.9% of the patients. Severe sequelae of hypertension,

Table 4. Adverse events

Adverse events	Number of events (%)
Hypertrichosis	20 (32.3)
Hypertension	8 (12.9)
Gingival hypertrophy	7 (11.3)
Elevation of alkaline phosphatase	5 (8.1)
Herpes zoster	2 (3.2)
Elevation of serum creatinine	1 (1.6)
General fatigue	1 (1.6)

ALP, alkaline phosphatase.

such as encephalopathy, seizures and cardiac dysfunction, were not detected. One patient had a mild elevation of the serum creatinine concentration, which was transient and resolved. No patient had serious adverse events that required discontinuation of the trial.

Discussion

This prospective, open-label, multicentre trial evaluated the safety and efficacy of 2 years of treatment with microemulsified cyclosporine (Neoral) in children with FRNS. Our results showed that microemulsified cyclosporine significantly decreased the frequency of relapse and increased the probability of relapse-free survival, suggesting that treatment with microemulsified cyclosporine is effective for children with FRNS. Renal biopsy was performed after 2 years of treatment and showed that the treatment protocol was safe in terms of nephrotoxicity.

The significant decrease in the frequency of relapse during 2 years of treatment suggested that microemulsified cyclosporine is effective in children with FRNS. The probability of relapse-free survival in the present trial was compared with that in our previous trial, in which conventional cyclosporine was given to children with FRNS [11]. The probability of relapse-free survival in the present trial (58.1% in 2 years) was higher than the lowest target level, which was the upper limit of the 95% confidence interval for the probability of relapse-free survival in Group B [given a fixed dose of 2.5 mg/kg conventional cyclosporine from Month 7 onwards in the previous trial (37.9%), i.e. standard treatment]. On the other hand, better outcomes in terms of probability of relapse-free survival with microemulsified cyclosporine as compared with conventional cyclosporine were not obtained. The dose of cyclosporine did not differ significantly (data not shown). In this regard, microemulsified cyclosporine was not superior to conventional cyclosporine. Further clinical studies are thus needed to confirm the efficacy of microemulsified cyclosporine in children with FRNS.

The results of our study do not allow us to make firm conclusions about whether AUC_{0-4h} and C2 monitoring are clinically useful for titrating the dose of cyclosporine in children with FRNS. AUC_{0-4h} and C2 monitoring have been shown to be a useful method for titrating the dose of cyclosporine, particularly the microemulsified formulation, in adults [22,23] and in children who receive renal transplants [19,20,24]. C2 is the best single time point predictor of AUC_{0-4h}, but the trough level closely correlates with acute rejection [25]. On the other hand, limitations of C2 monitoring in renal transplant recipients have been demonstrated: C2 levels did not predict rejection or toxicity; poor and/or slow absorption were observed in a substantial number of patients; and C2 levels were not dose-proportional [26]. Moreover, in a randomized setting, C2 monitoring was not superior to trough monitoring in terms of graft survival in renal transplant recipients [27]. In the present trial, Poisson regression analysis was used to assess the relations of AUC_{0-4h} and C2 to relapse. The risk of relapse was not dependent on AUC_{0-4h} or C2, probably because the dose of cyclosporine was adjusted on the basis of trough levels, and neither AUC_{0-4h} nor C2 had sufficient variability (or power) to test the relation to relapse. To settle these issues, further studies are required; another new multicentre randomized controlled trial supported by the Ministry of Health, Labour and Welfare, entitled 'Cyclosporine C2 monitoring for frequently relapsing nephrotic syndrome in children: a randomized controlled trial', is now being conducted in Japan to evaluate the safety and efficacy of C2 monitoring for cyclosporine (Neoral).

Improvement in the mean height s.d. score is another encouraging result of our trial. Growth failure is a serious adverse effect of steroids in children. Improvement in the mean height s.d. score is attributed to the steroid-sparing effect of cyclosporine. This effect is an important reason for using immunosuppressants such as cyclosporine in children with FRNS. At the same time, our protocol for the use of prednisolone in this trial appears to be appropriate.

Adverse events associated with cyclosporine were acceptable in our trial. The main adverse events of cyclosporine are nephrotoxicity, neurotoxicity including encephalopathy and seizures, hypertension, gingival hyperplasia, hirsutism, and hypomagnaesemia [10,28–30]. In our trial, five patients (8.6%) had nephrotoxicity, and four (6.9%) had interstitial fibrosis. Fibrosis was mild in all of our patients. However, since irreversibility of interstitial fibrosis has been reported [31] and paediatric patients have a long life expectancy, nephrotoxicity due to cyclosporine should be closely monitored, and renal biopsy should be performed to confirm safety in patients who receive repeated or prolonged treatment with cyclosporine. Hypertension, defined as a requirement for antihypertensive agents, was detected in 12.9% of our patients. Although severe sequelae of hypertension such as seizures did not occur in this study, management of blood pressure is an important concern whenever cyclosporine is administered. No patient had serious adverse events that required the discontinuation of treatment during the trial.

An important limitation of the present trial is the study design: no control group was established. Because of several differences between the present trial and our previous trial, caution should be exercised when comparing the results. The results of the aforementioned randomized controlled trial are awaited to confirm our findings. Our study group was characterized by a significant male preponderance (48 boys and 14 girls), which has also has been reported in children with nephrotic syndrome, including frequently relapsing nephrotic syndrome [7,9]. In our previous study, the male:female ratio was also as high as 35:9.

Another concern is missing data, such as the results of renal biopsy after treatment and measurement of AUC_{0-4h} and C2. Some patients refused repeated renal biopsy because of associated risks. AUC_{0-4h} measurement, which requires multiple blood samples, was inconvenient and was occasionally not performed by the physicians in charge; a single C2 measurement might be more practical. Finally, lack of adequate statistical power, particularly on Poisson regression analysis, was also a weakness of the present study.

A major limitation of cyclosporine treatment for children with FRNS is relapse after drug withdrawal. Several studies have evaluated relapse after cyclosporine treatment, albeit the treatment protocols differed from ours [8,32,33]; most patients had relapse of FRNS after the discontinuation of cyclosporine. Such patients require further treatment with cyclosporine or other immunosuppressants. We are continuing to follow up our patients to better define this critical issue.

In conclusion, treatment with microemulsified cyclosporine (Neoral) for 2 years in a dosage that maintains the trough level between 80 and 100 ng/mL for the first 6 months and 60–80 ng/mL for the next 18 months appears to be safe and effective in children with FRNS. Among several immunosuppressants recommended for children with FRNS, e.g. cyclophosphamide, levamisole, chlorambucil and mycophenolate mofetil, microemulsified cyclosporine is considered an important treatment option. Follow-up studies are being conducted to evaluate the risk of relapse after the withdrawal of cyclosporine.

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References

1. Hodson EM, Willis NS, Craig JC. Non-corticosteroid treatment for nephrotic syndrome in children. *Cochrane Database Syst Rev* 2008; 23: CD002290
2. Tune BM, Mendoza SA. Treatment of the idiopathic nephrotic syndrome: regimens and outcomes in children and adults. *J Am Soc Nephrol* 1997; 8: 824–832
3. Latta K, von Schnakenburg C, Ehrich JH. A meta-analysis of cytotoxic treatment for frequently relapsing nephrotic syndrome in children. *Pediatr Nephrol* 2001; 16: 271–282
4. Robinson RF, Nahata MC, Mahan JD *et al.* Management of nephrotic syndrome in children. *Pharmacotherapy* 2003; 23: 1021–1036
5. Eddy AA, Symons JM. Nephrotic syndrome in childhood. *Lancet* 2003; 362: 629–639
6. Inoue Y, Iijima K, Nakamura H *et al.* Two-year cyclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 1999; 13: 33–38
7. Tanaka R, Yoshikawa N, Kitano Y *et al.* Long-term ciclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 1993; 7: 249–252
8. Kitano Y, Yoshikawa N, Tanaka R *et al.* Ciclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 1990; 4: 474–477
9. El-Husseini A, El-Basuony F, Mahmoud I *et al.* Long-term effects of cyclosporine in children with idiopathic nephrotic syndrome: a single-centre experience. *Nephrol Dial Transplant* 2005; 20: 2433–2438
10. Cattran DC, Alexopoulos E, Heering P *et al.* Cyclosporin in idiopathic glomerular disease associated with the nephrotic syndrome: workshop recommendations. *Kidney Int* 2007; 72: 1429–1447
11. Ishikura K, Ikeda M, Hattori S *et al.* Effective and safe treatment with cyclosporine in nephrotic children: a prospective, randomized multicenter trial. *Kidney Int* 2008; 73: 1167–1173
12. Kahan BD. Therapeutic drug monitoring of cyclosporine: 20 years of progress. *Transplant Proc* 2004; 36: 378–391
13. Ritschel WA. Microemulsion technology in the reformulation of cyclosporine: the reason behind the pharmacokinetic properties of Neoral. *Clin Transpl* 1996; 10: 364–373
14. Friman S, Backman L. A new microemulsion formulation of cyclosporin: pharmacokinetic and clinical features. *Clin Pharmacokinet* 1996; 30: 181–193
15. Noble S, Markham A. Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral). *Drugs* 1995; 50: 924–941
16. Niaudet P, Reigneau O, Humbert H. A pharmacokinetic study of Neoral in childhood steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 2001; 16: 154–155
17. Pollard SG, Lear PA, Ready AR *et al.* Comparison of microemulsion and conventional formulations of cyclosporine A in preventing acute rejection in de novo kidney transplant patients. The U.K. Neoral Renal Study Group. *Transplantation* 1999; 68: 1325–1331
18. Keown P, Niese D. Cyclosporine microemulsion increases drug exposure and reduces acute rejection without incremental toxicity in de novo renal transplantation. International Sandimmune Neoral Study Group. *Kidney Int* 1998; 54: 938–944
19. Weber LT, Armstrong VW, Shipkova M *et al.* Cyclosporin A absorption profiles in pediatric renal transplant recipients predict the risk of acute rejection. *Ther Drug Monit* 2004; 26: 415–424
20. Ferraresso M, Ghio L, Zacchello G *et al.* Pharmacokinetic of cyclosporine microemulsion in pediatric kidney recipients receiving A quadruple immunosuppressive regimen: the value of C2 blood levels. *Transplantation* 2005; 79: 1164–1168
21. No author listed. Early identification of frequent relapsers among children with minimal change nephrotic syndrome. A report of the International Study of Kidney Disease in Children. *J Pediatr* 1982; 101: 514–518
22. Pescovitz MD, Barbeito R. Two-hour post-dose cyclosporine level is a better predictor than trough level of acute rejection of renal allografts. *Clin Transpl* 2002; 16: 378–382
23. Cole E, Maham N, Cardella C *et al.* Clinical benefits of neoral C2 monitoring in the long-term management of renal transplant recipients. *Transplantation* 2003; 75: 2086–2090
24. Trompeter R, Fitzpatrick M, Hutchinson C *et al.* Longitudinal evaluation of the pharmacokinetics of cyclosporin microemulsion (Neoral) in pediatric renal transplant recipients and assessment of C2 level as a marker for absorption. *Pediatr Transplant* 2003; 7: 282–288
25. Levy G, Thervet E, Lake J *et al.* Patient management by Neoral C(2) monitoring: an international consensus statement. *Transplantation* 2002; 73: 12–18
26. Einecke G, Schutz M, Mai I *et al.* Limitations of C2 monitoring in renal transplant recipients. *Nephrol Dial Transplant* 2005; 20: 1463–1470
27. Kyllonen LE, Salmela KT. Early cyclosporine C0 and C2 monitoring in de novo kidney transplant patients: a prospective randomized single-center pilot study. *Transplantation* 2006; 81: 1010–1015
28. Radhakrishnan J, Halevy D. Cyclosporin treatment of glomerular diseases. *Expert Opin Investig Drugs* 2000; 9: 1053–1063
29. Ishikura K, Ikeda M, Hamasaki Y *et al.* Nephrotic state as a risk factor for developing posterior reversible encephalopathy syndrome in

paediatric patients with nephrotic syndrome. *Nephrol Dial Transplant* 2008; 23: 2531–2536
30. Ishikura K, Ikeda M, Hamasaki Y *et al.* Posterior reversible encephalopathy syndrome in children: its high prevalence and more extensive imaging findings. *Am J Kidney Dis* 2006; 48: 231–238
31. Hamahira K, Iijima K, Tanaka R *et al.* Recovery from cyclosporine-associated arteriolopathy in childhood nephrotic syndrome. *Pediatr Nephrol* 2001; 16: 723–727
32. Hino S, Takemura T, Okada M *et al.* Follow-up study of children with nephrotic syndrome treated with a long-term moderate dose of cyclosporine. *Am J Kidney Dis* 1998; 31: 932–939
33. Hulton SA, Neuhaus TJ, Dillon MJ *et al.* Long-term cyclosporin A treatment of minimal-change nephrotic syndrome of childhood. *Pediatr Nephrol* 1994; 8: 401–403

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Tyrosine phosphorylation–dependent activation of TRPC6 regulated by PLC-γ1 and nephrin: effect of mutations associated with focal segmental glomerulosclerosis

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ABSTRACT Transient receptor potential canonicals (TRPCs) play important roles in the regulation of intracellular calcium concentration. Mutations in the *TRPC6* gene are found in patients with focal segmental glomerulosclerosis (FSGS), a proteinuric disease characterized by dysregulated function of renal glomerular epithelial cells (podocytes). There is as yet no clear picture for the activation mechanism of TRPC6 at the molecular basis, however, and the association between its channel activity and pathogenesis remains unclear. We demonstrate here that tyrosine phosphorylation of TRPC6 induces a complex formation with phospholipase C (PLC)-γ1, which is prerequisite for TRPC6 surface expression. Furthermore, nephrin, an adhesion protein between the foot processes of podocytes, binds to phosphorylated TRPC6 via its cytoplasmic domain, competitively inhibiting TRPC6–PLC-γ1 complex formation, TRPC6 surface localization, and TRPC6 activation. Importantly, FSGS-associated mutations render the mutated TRPC6s insensitive to nephrin suppression, thereby promoting their surface expression and channel activation. These results delineate the mechanism of TRPC6 activation regulated by tyrosine phosphorylation, and imply the cell type–specific regulation, which correlates the FSGS mutations with deregulated TRPC6 channel activity.

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Abbreviations used: CD, cytoplasmic domain; EGF, epidermal growth factor; FITC, fluorescein isothiocyanate; FSGS, focal segmental glomerulosclerosis; GST, glutathione *S*-transferase; HA, hemagglutinin; HBS, HEPES-buffered saline; LC-MS/MS, liquid chromatograph-mass/mass spectrometry; NP40, Nonidet P-40; PH, pleckstrin homology; PLC, phospholipase C; PMSF, phenylmethylsulfonyl fluoride; PTK, protein tyrosine kinase; SH2, Src homology 2; TRPC, transient receptor potential canonical.

INTRODUCTION

Dynamic changes in calcium concentration trigger a plethora of cellular responses, including secretion, contraction, cell growth, survival, and differentiation by versatile regulatory mechanisms (Berridge *et al.*, 2000). The increase in Ca^{2+} concentration is initiated by the opening of Ca^{2+}-permeable channels on the plasma membrane or on the endoplasmic reticulum resulting from direct receptor activation by ligands or indirect activation through the intracellular signaling pathways (Putney, 1986, 2009). The mammalian homologues of *Drosophila* transient receptor potential canonical, the TRPCs, are potent plasma membrane channels that contribute to changes in the cytosolic free Ca^{2+} concentration (Birnbaumer *et al.*, 1996), either by acting as Ca^{2+} entry pathways on the plasma

membrane or by modulating the membrane-driving force for Ca^{2+} entry through changing the membrane potential (Nilius *et al.*, 2007; Abramowitz and Birnbaumer, 2009; Kiselyov and Patterson, 2009). Among the seven mammalian TRPC channels, a subfamily of TRPC3, 6, and 7 can be defined by sequence similarity. These proteins form a nonselective cation channel that is activated by receptor stimulation or by the exogenous application of diacylglycerol analogues (Hofmann *et al.*, 1999; Okada *et al.*, 1999; Inoue *et al.*, 2001; Trebak *et al.*, 2003).

One of the mechanisms for the regulation of TRPC channel activity is insertion of channels into the plasma membrane. TRPC3, 4, and 6 are translocated to the plasma membrane upon stimulation of Gq-coupled receptors or receptor tyrosine kinases (Cayouette *et al.*, 2004; Odell *et al.*, 2005; Smyth *et al.*, 2006). Phosphorylation of TRPC channels by protein kinase C, protein kinase G, or Src family tyrosine kinase also causes their membrane insertion, and a number of phosphorylation sites have been documented on these channels (Kiselyov and Patterson, 2009; Nishida *et al.*, 2010). Src family kinase interacts with all TRPC channels (Kawasaki *et al.*, 2006), and TRPC4 and 6 undergo tyrosine phosphorylation by Src family kinase upon epidermal growth factor (EGF) stimulation (Hisatsune *et al.*, 2004; Odell *et al.*, 2005). Fyn phosphorylates TRPC6 and increases its diacylglycerol-stimulated single channel activity (Hisatsune *et al.*, 2004). Phosphorylation-independent interaction with phospholipase C (PLC)-γ also induces membrane insertion of TRPC3 (van Rossum *et al.*, 2005). The precise mechanisms of the activation and regulation of TRPCs remain to be clarified, however.

It is widely recognized that dysregulation of TRPC channels can result in the pathogenesis of various diseases, such as cardiovascular, neurodegenerative, respiratory, and renal diseases (Nilius *et al.*, 2005, 2007; Abramowitz and Birnbaumer, 2009; Woudenberg-Vrenken *et al.*, 2009). TRPC6 is the essential component of receptor-operated cation channels in vascular smooth muscle cells (Inoue *et al.*, 2001), and has been implicated in hypoxia-induced pulmonary hypertension (Lin *et al.*, 2004; Wang *et al.*, 2006). TRPC3 and TRPC6 promote cardiac hypertrophy through activation of calcineurin and its downstream effector, nuclear factors of activated T-cells (Bush *et al.*, 2006; Kuwahara *et al.*, 2006; Nakayama *et al.*, 2006; Onohara *et al.*, 2006). Recently mutations in the *TRPC6* gene have been linked to the human proteinuric kidney disease, focal segmental glomerulosclerosis (FSGS) (Reiser *et al.*, 2005; Winn *et al.*, 2005). In this disease, the glomerular epithelial cells (podocytes) and the specific cellular junctional structure between podocyte foot processes, called the slit diaphragm, lose their integrity, disrupting glomerular filtration barrier (Tryggvason *et al.*, 2006; Patrakka and Tryggvason, 2009). TRPC6 is expressed in podocytes, and binds to nephrin and podocin (Reiser *et al.*, 2005; Huber *et al.*, 2006), which are the critical components of the slit diaphragm, forming an essential part of the glomerular permeability barrier in the kidney (Tryggvason *et al.*, 2006). Although overexpression of TRPC6 in the mouse kidney resulted in the induction of proteinuria (Moller *et al.*, 2007; Krall *et al.*, 2010), how the channel activity of mutated TRPC6 is involved in the pathogenesis remains unclear. Some mutations (P112Q, R895C, E897K) enhanced angiotensin II receptor–mediated activation of TRPC6 when expressed in HEK293 cells, but neither the S270T nor the N143S missense mutations, nor a 57-amino-acid truncation (K874X) mutation, altered the channel activity (Reiser *et al.*, 2005; Winn *et al.*, 2005). These channel activities correlated well with the extent of downstream nuclear factor of activated T-cells activation (Schlondorff *et al.*, 2009). In contrast, the P112Q mutation increased the plasma membrane expression of TRPC6 (Winn *et al.*, 2005), suggesting that changes in surface expression may also contribute to the pathogenesis of the disease.

In the present study we investigated the molecular basis of the translocation of TRPC6 by using HEK293T cells and cultured podocytes. We present evidence that surface expression of TRPC6 is regulated by its phosphorylation at Y284 by Src family kinase and interaction with PLC-γ1. Notably, a slit diaphragm protein, nephrin, interacts with phosphorylated TRPC6, and suppresses its translocation by interfering with TRPC6–PLC-γ1 binding. Importantly, FSGS-causing mutations dramatically weaken the nephrin–TRPC6 interaction, resulting in an increased membrane expression and Ca^{2+} channel activity of TRPC6 in living cells. Collectively, our results provide a correlation between the FSGS-associated mutations with deregulated TRPC6 activity, which may underlie the pathogenesis of this disease.

RESULTS

Phosphorylation of TRPC6 Tyr-284 is necessary for its trafficking to the plasma membrane

Because regulated translocation of TRPC channels to the plasma membrane has been proposed as a mechanism for their activation, we examined, by a surface biotinylation assay, whether phosphorylation of TRPC6 induces its translocation to the plasma membrane. HEK293T cells expressing hemagglutinin (HA)-tagged TRPC6 were stimulated with EGF for various times before biotinylation. Biotinylated TRPC6 was barely detectable under basal conditions (Figure 1A). TRPC6 appeared at the plasma membrane within 1 min after the stimulation, and its level reached a plateau at 3 min, which lasted for at least 60 min. The phosphorylation of TRPC6 by Src family protein tyrosine kinases (PTKs) has been implicated in physiological stimulation, because PP2, a specific inhibitor of Src family PTK, or a dominant-negative form of Fyn abrogates EGF-induced tyrosine phosphorylation of TRPC6 (Hisatsune *et al.*, 2004). Indeed, tyrosine phosphorylation (Figure 1B) and increased cell surface localization of TRPC6 (Figure 1C) were induced by coexpression of a constitutively active Fyn.

TRPC6 has 23 tyrosine residues in its cytoplasmic regions. To address which tyrosine residue of TRPC6 is critical for its membrane trafficking, we analyzed the effect of a single phenylalanine substitution for each of eight tyrosine residues (Y31, Y50, Y85, Y107, Y206, Y208, Y284, Y895) on the surface localization. Y107, Y206, Y208, and Y284 correspond to the previously reported phosphorylation sites Y49, Y148, Y150, and Y226 in TRPC3, respectively (Kawasaki *et al.*, 2006). Y31, Y50, Y85, and Y895 were predicted to be binding sites for Src homology 2 (SH2)-containing proteins by the motif search program in ScanSite (http://scansite.mit.edu/). All of these eight tyrosine residues are conserved between several animal species (human, rat, mouse, dog). Among them, the Y284F mutation dramatically decreased the Fyn-induced surface expression of TRPC6 in HEK293T cells (Figure 1D) and in cultured podocytes (Figure 1E).

PLC-γ1 binds to phosphorylated TRPC6 and controls its surface expression

We hypothesized that the phosphorylation of TRPC6 Y284 provides a binding site for a protein that is crucial for TRPC6 trafficking. To identify such a protein, we performed in vitro binding assays using phosphorylated or nonphosphorylated TRPC6 peptide around Y284 (the same peptide used as an immunogen for anti–pY284 TRPC6 described in *Materials and Methods*). Both peptides were immobilized to a coupling gel and incubated with HEK293T cell lysates, and the bound proteins were subjected to SDS–PAGE followed by silver