TNF-α receptor levels, because his clinical course was quite unexpected. However, there are several reports of increased TNF-α or soluble TNF-α receptor in serum, urine and peripheral blood mononuclear cells at NS relapse or onset [2–5]. To date, there have been two case reports describing the efficacy of TNF-α inhibitors for idiopathic NS. In 2004, Raveh et al. [6] reported the efficacy of infliximab for a boy with steroid-resistant NS. The patient did not respond to treatments with methylprednisolone pulse therapy, cyclosporine, cyclophosphamide, mycophenolate mofetil, Chinese herbs, and pentoxifylline. He finally went into remission with infliximab. In 2009, Leroy et al. [7] reported an interesting case. They encountered a boy with focal segmental glomerular sclerosis who relapsed after renal transplantation. NS continued despite treatments with cyclosporine, cyclophosphamide, mycophenolate mofetil, methylprednisolone pulse therapy, and plasmapheresis. Additional infliximab induced remission at 3 months after the transplantation. PSL was successfully tapered under maintenance with cyclosporine and mycophenolate mofetil. However, a relapse occurred after discontinuation of infliximab at 8 months after the transplantation, and he again suffered from steroid-resistant NS. Surprisingly, combined use of PSL and etanercept induced remission at 10 months. Thereafter, he suffered two relapses whenever etanercept was stopped, but restarting the combined use of PSL and etanercept achieved remission.

Our experience and these previous reports also provide clues for the etiology of NS. TNF-α may be involved in part of its etiology. However, other high TNF-α condition such as sepsis, Kawasaki disease and inflammatory bowel disease are rarely complicated with NS. These observations suggest that TNF-α is not the main inducer of proteinuria, but may affect or enhance the abnormal immune responses in T cells or B cells in NS. As with the first and second cases of rituximab for NS, the incidental use of a drug sometimes creates a new treatment and gives insights into the disease pathogenesis [8, 9]. However, TNF-α inhibitors may induce glomerular diseases including minimal change nephrotic syndrome, systemic lupus erythematosus, ANCA-related glomerulonephritis, and membranous nephropathy based on previous reports [10, 11]. Careful monitoring for new onset of glomerular diseases is necessary when TNF-α inhibitors are used. More clinical experience and investigations of their effects are needed to confirm whether TNF-α inhibitors can be new alternative therapeutic agents for refractory NS.

References

1. Kamei K, Ito S, Nozu K, Fujinaga S, Nakayama M, Sako M, Saito M, Yoneko M, Iijima K (2009) Single dose of rituximab for refractory steroid-dependent nephrotic syndrome in children. Pediatr Nephrol 24:1321–1328
2. Suranyi MG, Guasch A, Hall BM, Myers BD (1993) Elevated levels of tumor necrosis factor-alpha in the nephrotic syndrome in humans. Am J Kidney Dis 21:251–259
3. Bustos C, González E, Muley R, Alonso JL, Egido J (1994) Increase of tumour necrosis factor alpha synthesis and gene expression in peripheral blood mononuclear cells of children with idiopathic nephrotic syndrome. Eur J Clin Invest 24:799–805
4. Lama G, Luongo I, Tirino G, Borriello A, Carangio C, Salsano ME (2002) T-lymphocyte populations and cytokines in childhood nephrotic syndrome. Am J Kidney Dis 39:958–965
5. Tain YL, Liu CA, Yang KD (2002) Implications of blood soluble and cell surface tumor necrosis factor receptors in childhood nephrotic syndrome. Pediatr Nephrol 17:926–932
6. Raveh D, Shemesh O, Ashkenazi YJ, Winkler R, Barak V (2004) Tumor necrosis factor-alpha blocking agent as a treatment for nephrotic syndrome. Pediatr Nephrol 19:1281–1284
7. Leroy S, Guigonis V, Bruckner D, Emal-Aglae V, Deschênes G, Bensman A, Ulinski T (2009) Successful anti-TNFα treatment in a child with posttransplant recurrent focal segmental glomerulosclerosis. Am J Transplant 9:858–861
8. Benz K, Dötsch J, Rascher W, Stachel D (2004) Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. Pediatr Nephrol 19:794–797
9. Nozu K, Iijima K, Fujisawa M, Nakagawa A, Yoshikawa N, Matsuo M (2005) Rituximab treatment for posttransplant lymphoproliferative disorder (PTLD) induces complete remission of recurrent nephrotic syndrome. Pediatr Nephrol 20:1660–1663
10. Chin G, Luxton G, Harvey JM (2005) Infliximab and nephrotic syndrome. Nephrol Dial Transplant 20:2824–2826
11. Stokes MB, Foster K, Markowitz GS, Ebrahimi F, Hines W, Kaufman D, Moore B, Wolde D, D'Agati VD (2005) Development of glomerulonephritis during anti-TNF-alpha therapy for rheumatoid arthritis. Nephrol Dial Transplant 20:1400–1406

Pharmaceuticals Policy and Law 12 (2010) 117–121
DOI 10.3233/PPL-2010-0282
IOS Press

Current situation and critical points for paediatric research in Japan

Hidefumi Nakamura
Division of Clinical Research, National Children's Hospital, National Centre for Child Health and Development, Tokyo, Japan
E-mail: nakamura-hd@ncchd.go.jp

The Ministry of Health, Labour and Welfare (MHLW) and related government agencies have been implementing strategies to facilitate clinical trials in the last few years [1], and the infrastructure for paediatric clinical trials is being strengthened.

The revision of the Pharmaceutical Affairs Law (PAL) in 2002 has enabled physicians to conduct sponsor-investigator trials in Japan. There have been 9 protocols for 6 drugs funded by the MHLW in children completed or ongoing as of August, 2009. The National Centre for Child Health and Development (NCCHD) has been involved in 7 of these protocols. Of these 7 protocols, NCCHD has been involved in project management for 5 protocols.

Keywords: Pharmaceutical Affairs Law, Japan, NCCHD, incentives, paediatrics

1. New Five-year Clinical Trial Promotion Plan (MHLW and MEXT, 2007–2011)

In March 2007, the MHLW and Ministry of Education, Culture, Sports, Science and Technology (MEXT) jointly published the five-year promotion plan. This plan succeeds the previous plan to create networks of clinical institutions. The MHLW chose 10 COEs and 30 affiliated major hospitals, based on therapeutic needs and their performance. For a paediatric clinical trial network, the NCCHD has been chosen as the COE. Among approximately 30 children's hospitals in Japan, the Kanagawa Children's Medical Centre, the Osaka Medical Centre and Research Institute for Maternal and Child Health, and the Tokyo Metropolitan Kiyose Children's Hospital were chosen as affiliated major hospitals. COE receives ∼0.1 billion yen and each affiliated major hospital receives ∼25 million yen annually to build up infrastructure and maintain staff.

2. 5-Year Strategy for the Creation of Innovative Pharmaceuticals and Medical Devices (MHLW, MEXT, METI and Cabinet Office, 2007–2011)

This strategy was made by 3 ministries, the MHLW, the MEXT and the Ministry of Economy, Trade and Industry (METI). The major goals of this strategy is to reinforce clinical research infrastructure to ensure safe and secure patients' access to new drugs and devices [2].

1389-2827/10/$27.50 © 2010 – Network of Centres for Study of Pharmaceutical Law. All rights reserved

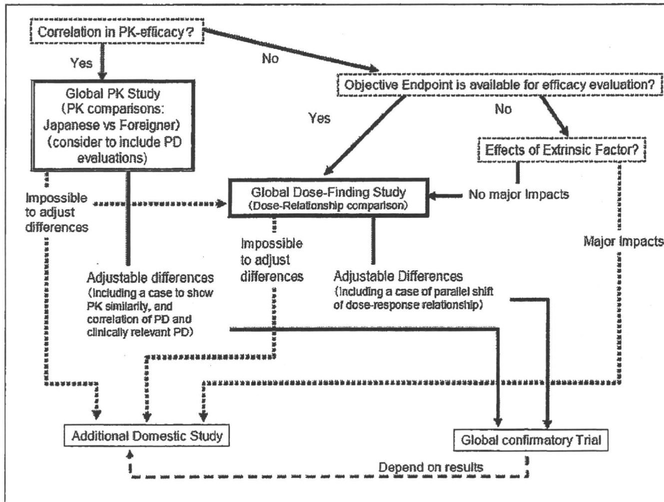


Fig. 1. A reference flow chart for developmental strategies utilizing global trials (Notification #928010).

As a part of the strategy, MHLW decided to spend ~3.5 billion yen to strengthen translational research infrastructure at 4 National Centres to accelerate R & D and promote new partnership with industries. Receiving 0.8 billion yen, NCCHD started to build a new facility devoted for paediatric drug and device developments in August, 2009.

3. Basic principles on global clinical trials

The Pharmaceuticals and Medical Devices Agency (PMDA) and the Evaluation and Licensing Division of the MHLW have issued "basic principles on global clinical trials" in September, 2007 [3] PMDA and the MHLW consider that involvement in global studies is essential to shorten approval lags in Japan.

A reference flow chart for developmental strategies on this Notification is shown in Fig. 1. As there has been limited experience in paediatric global trials in Japan, early consultation with the PMDA and Japanese specialists is strongly recommended before actually plannning/ conducting paediatric global trials in Japan.

4. Incentives for paediatric drug development

Japan does not have regulation equivalent to the EU Paediatric regulation, and there has been a limited success in introducing incentives for companies to develop drugs and devices in children. Recently, discussion between academia, industries and the MHLW/ PMDA has been started for further regulation changes to facilitate paediatric drug development. Revision of the PAL, the primary law governing the drug development in the country is also in scope. Existing incentives are described below. First track review is also considered as a possible incentive, but the PMDA so far has not enough capacity to conduct first track review for all paediatric indications.

5. Extension of the re-examination period

Extension of the reexamination period was introduced in December 2000. The reexamination period is applied to all newly approved indications. Standard reexamination period was 6 years before April 2007 and was then extended to 8 years. Data protection is applied during the reexamination period. When a company conducts clinical trials for a paediatric indication during the reexamination period, the period may be extended up to 10 years. This extension which may be an incentive for companies, has been actually delaying the paediatric drug development process. Based on the examination of recent evaluation reports, many companies wait to start post-marketing trials until the last moment before expiration of the reexamination period.

6. Paediatric premium for comparator pricing method

At present, a higher drug price for paediatric medication is considered as the most feasible incentive for companies. In 2006, paediatric premium was newly introduced for comparator pricing method. The premium was originally 3–10% and only for drugs which have no other pharmacologically comparable drugs in the market. In 2008, the premium was increased to 5–20% and became applicable to all the drugs whose comparators have not received paediatric premium.

7. Activity of the Japan Paediatric Society (JPS) in promoting paediatric drug development

The Committee on Drugs (CoD) of the JPS has been tackling off-label drug problems for more than 20 years. The committee organized a working group to study the approval status of paediatric drugs and made a priority list of off-label drugs in 1998 as part of the MHLW supported research. This list is being revised annually. As

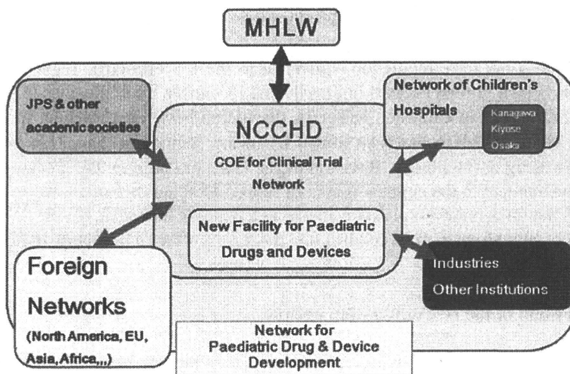


Fig. 2. Schema to show the role of NCCHD in Japanese paediatric drug and device development.

of August, 2009, representatives of 23 associated subspecialty societies have joined this working group. The working group also accumulates information on unlicensed drugs which need to be approved urgently in the country. The list of off-label and unlicensed drugs are utilized for the MHLW projects to solve these problems, and there have been 3 off-label and 5 unlicensed paediatric drugs being newly approved in the last 3 years as results of their effort. This working group is now functioning as a platform to share information and to collaborate for drug and device development in children.

8. Role of the NCCHD as a core for paediatric clinical trials

The NCCHD is playing a central role in setting up a paediatric clinical network in Japan. The NCCHD has project management capability for GCP-compliant clinical trials, has a data center, and offers consultation service both for industries and investigators. The NCCHD has a database for clinical trial capability of 29 children's hospitals and can quickly give names of candidate hospitals and approximate number of subjects if a pharmaceutical industry requests. Through the interaction with the working group of the CoD of JPS, the NCCHD also collaborate with the disease specific networks including nephrology, neurology and neonatology networks. With new facility for paediatric drug and device development being ready by March, 2010, NCCHD will have laboratories including device, formulation and PK labs and will be able to collaborate with industries and other research institutions for technology development. The schema for the role of the NCCHD is shown in Fig. 2.

9. Networking with other Asian countries

There has been a discussion with paediatric pharmacologists and pharmacists in other Asian countries for possible collaboration. As one of the symposiums of the 36th Annual Congress of the Japan Society of Developmental Pharmacology and Therapeutics on November 21, 2009, there will be a discussion on international and Asian networking with Kalle Hoppu from Finland, Min Soo Park from Korea, Li Wang and Zhiping Li from China, and Hidefumi Nakamura as speakers. There will be a continuous discussion, and the next conference hopefully with more asian countries will most likely be held in Korea in 2010.

10. Possible collaboration for international networking

In Japan, industry-sponsored and sponsor-investigator clinical trials are strictly adhered to the ICH GCP, and the network for paediatric clinical trials are being established in a fairly efficient manner. Although there may be some degree of difference in medical care compared to the EU and the US, quality of the trials is comparable. With careful preparation of the protocols and procedures, it is feasible to overcome the difference and include Japan and other Asian countries in multi-national clinical trials. For many orphan drugs and devices, international collaboration is essential to recruit enough number of patients to evaluate efficacy and safety.

References

[1] http://www.mhlw.go.jp/english/wp/wp-hw2/part2/p2c1s5.pdf.
[2] http://www.mhlw.go.jp/bunya/iryou/shinkou/dl/03.pdf.
[3] http://www.pmda.go.jp/english/service/notifications.html.

Am J Physiol Renal Physiol 300: F511–F520, 2011.
First published November 17, 2010; doi:10.1152/ajprenal.00038.2010.

Epithelial-to-mesenchymal transition in cyst lining epithelial cells in an orthologous PCK rat model of autosomal-recessive polycystic kidney disease

Hiroko Togawa,[1] Koichi Nakanishi,[1] Hironobu Mukaiyama,[1] Taketsugu Hama,[1] Yuko Shima,[1] Mayumi Sako,[1] Masayasu Miyajima,[2] Kandai Nozu,[3] Kazuhiro Nishii,[4] Shizuko Nagao,[4] Hisahide Takahashi,[4] Kazumoto Iijima,[3] and Norishige Yoshikawa[1]

[1]*Department of Pediatrics and* [2]*Laboratory Animal Center, Wakayama Medical University, Wakayama;* [3]*Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo; and* [4]*Education and Research Center of Animal Model for Human Disease, Fujita Health University, Toyoake, Aichi, Japan*

Submitted 20 January 2010; accepted in final form 15 November 2010

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* 300: F511–F520, 2011. First published November 17, 2010; doi:10.1152/ajprenal.00038.2010.—In polycystic kidney disease (PKD), cyst lining cells show polarity abnormalities. Recent studies have demonstrated loss of cell contact in cyst cells, suggesting induction of epithelial-to-mesenchymal transition (EMT). Recently, EMT has been implicated in the pathogenesis of PKD. To explore further evidence of EMT in PKD, we examined age- and segment-specific expression of adhesion molecules and mesenchymal markers in PCK rats, an orthologous model of human autosomal-recessive PKD. Kidneys from 5 male PCK and 5 control rats each at 0 days, 1, 3, 10, and 14 wk, and 4 mo of age were serially sectioned and stained with segment-specific markers and antibodies against E-cadherin, Snail1, β-catenin, and N-cadherin. mRNAs for E-cadherin and Snail1 were quantified by real-time PCR. Vimentin, fibronectin, and α-smooth muscle actin (α-SMA) expressions were assessed as mesenchymal markers. E-cadherin expression pattern was correlated with the disease pathology in that tubule segments showing the highest expression in control had much severer cyst formation in PCK rats. In PCK rats, E-cadherin and β-catenin in cystic tubules was attenuated and localized to lateral areas of cell-cell contact, whereas nuclear expression of Snail1 increased in parallel with cyst enlargement. Some epithelial cells in large cysts derived from these segments, especially in adjacent fibrotic areas, showed positive immunoreactivity for vimentin and fibronectin. In conclusion, these findings suggest that epithelial cells in cysts acquire mesenchymal features in response to cyst enlargement and participate in progressive renal fibrosis. Our study clarified the nephron segment-specific cyst profile related to EMT in PCK rats. EMT may play a key role in polycystic kidney disease.

cadherin; Snail1; β-catenin; vimentin; fibronectin

POLYCYSTIC KIDNEY DISEASE (PKD) is characterized by the presence of massively enlarged fluid-filled cysts in the renal tubules and/or collecting ducts. Progressive enlargement of these cysts compromises the normal renal parenchyma, eventually leading to renal failure. Autosomal-dominant polycystic kidney disease (ADPKD; MIM 173900; 173910) is caused by mutation in one of two genes, *PKD1* or *PKD2* (26), and autosomal-recessive polycystic kidney disease (ARPKD; MIM 263200) is caused by mutation in a single gene. The gene causing ARPKD, *PKHD1* (polycystic kidney and hepatic disease 1), is a large gene located on chromosome 6p21.1-p12 (27, 39). It is predicted to yield a novel 4,074-amino acid, multidomain, integral membrane protein called fibrocystin (43), or polyductin (27), of unknown function. Despite impressive advances in the genetic and molecular characterization of PKD gene products, the normal cellular function of these proteins and their role in cyst formation and growth are unknown.

PKD cystic epithelia share common phenotypic abnormalities despite the different genetic mutations that underlie the disease (37). Cystic epithelial cells are characterized by altered proliferative activity, a secretory rather than absorptive function, and an abnormal matrix microenvironment (11, 20). The cyst lining epithelial cell pathophysiology in PKD is characterized by dedifferentiation and perturbations of the polarized phenotype. Some of these features resemble an early developmental epithelial cell phenotype. From a developmental perspective, cystogenesis associated with PKD can be considered a state of abnormal tubulogenesis in which proliferative influences predominate over differentiation factors (2, 10).

In relation to an abnormal matrix microenvironment, renal interstitial fibrosis is an important characteristic of PKD (10, 37). One of the key features of fibrosis is epithelial-to-mesenchymal transition (EMT) (17). Epithelial cells undergoing EMT acquire mesenchymal features, which leads to excessive production and deposition of extracellular matrix components (14, 17). Recently, a role of EMT has been implicated in the pathogenesis of PKD. Schieren et al. (34) studied the expression profiles of 12 human ADPKD kidneys using a 7,000 cDNA microarray and concluded that ADPKD was associated with increased EMT markers, suggesting that EMT contributed to the progressive loss of renal function in ADPKD. Song et al. (35) performed global gene profiling on renal cysts from five PKD1 human polycystic kidneys and reported that epithelial dedifferentiation accompanied by EMT may be required for PKD1 cyst growth and disease progression. Chea and Lee (9) reported that the end stages of ADPKD kidneys from five patients were associated with markers of EMT, suggesting that EMT has a role in progression of ADPKD. Okada et al. (25) examined fibroblast-specific protein 1 (FSP1), α-smooth muscle actin (α-SMA), vimentin, and heat shock protein 47 (HSP47) in the recessive *pcy* mouse model of late-onset cystic kidney disease (an ortholog of human adolescent nephronophthisis, NPHP3; MIM 604387) and described that some epithelia among remnant tubules trapped within fibrotic septa around adjacent cysts also acquired the phenotype of FSP1, HSP47 collagen-producing fibroblasts, suggesting a possible role for

Address for reprint requests and other correspondence: K. Nakanishi, Dept. of Pediatrics, Wakayama Medical Univ., 811-1 Kimiidera, Wakayama City, Wakayama 641-8509, Japan (e-mail: knakanis@wakayama-med.ac.jp).

 0363-6127/11 Copyright © 2011 the American Physiological Society

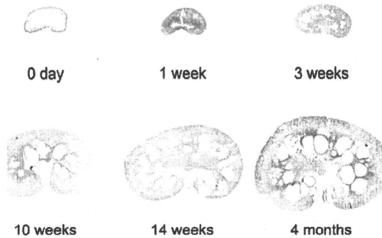


Fig. 1. Cyst formation in PCK rats. Sections with the maximum area for each kidney sample are shown. Renal cysts were evident from 3 wk of age in PCK rats. Multiple pictures obtained using an all-in-one light-field and fluorescence microscope, BIOREVO (BZ-9000, Keyence; original magnification, ×40), were connected using an image-joining software package.

EMT in this model. Many of the proteins implicated in PKD and nephronophthisis are linked to ciliary function and localize to the primary cilia, basal body, and/or centrosome. There are several indications of a relationship between the primary cilium and renal cystic diseases, which belong to a group of disorders with abnormalities in the primary cilium, referred to as ciliopathies (30). The mechanisms by which ciliopathy gene product deficiencies lead to EMT of tubular epithelia are not yet fully understood. It has been shown that EMT is active in a human nephronophthisis (NPHP7; MIM 611498) orthologous mouse model (1). The proposed pathogenesis of EMT in this model is loss of function of downstream effectors of hedgehog signaling, which is active normally in mesenchymal-to-epithelial transition in renal tubulogenesis (1, 45). The similar pathophysiology may also contribute to PKD.

Polarization is essential for epithelial cell function, and apicobasal cell polarity is lost during EMT, a program of events characterized by loss of cell polarity (40). E-cadherin is a calcium-dependent cell adhesion molecule present in most types of renal tubule cells. Assembly of the E-cadherin/β-catenin complex is the first step in formation of a polarized epithelium and plays an important role in maintenance of an epithelial phenotype (29). Recent studies (8, 31) have suggested that aberrant expression of E-cadherin and/or β-catenin may be associated with PKD. Loss of E-cadherin expression is a hallmark of EMT. Recently, it was clarified that Snail1 (known previously as Snail), a transcriptional repressor of E-cadherin expression, plays a role in the process of EMT (4).

In the present study, to explore further evidence of EMT in PKD, we evaluated systematically tubule segment-specific expression of cell adhesion molecules, mesenchymal markers, and Snail1 in the PCK rat, an ARPKD model bearing a mutation in the ortholog of human *PKHD1*.

MATERIALS AND METHODS

PCK model. PCK rats were originally derived from a spontaneous mutation in a strain of Sprague-Dawley rats (15, 16). The animals used in this study were from a colony established at the Education and Research Center of Animal Models for Human Diseases of Fujita Health University. The PCK rats are homozygous for the gene and maintained as such for ease of breeding. In 2002, Ward et al. (43) demonstrated that the mutation in the PCK rat was an exon deletion in the rat homolog of human *PKHD1*. As controls, Sprague-Dawley rats were obtained from Charles River Japan (Kanagawa, Japan). The protocol for the use of rats was approved by the Animal Care and Use Committee at Wakayama Medical University.

Immunohistochemistry. From male PCK (n = 5) and control rats (n = 5) each at *day 0*, 1, 3, 10, and 14 wk, and 4 mo of age, both kidneys were removed rapidly. One kidney was fixed in 6% formaldehyde, and the other was frozen in liquid nitrogen for frozen sections, Western blotting, and real-time PCR. Kidney samples were embedded in paraffin and cut into sections 4 μm thick. Serial sections were incubated for 1 h at room temperature with tubule segment-specific markers (Supplemental Table 1; supplemental data for this article is available online at the *American Journal of Physiology-Renal Physiology* website) (24), and primary antibodies against E-cadherin (1:1,000; BD Transduction Laboratories, San Jose, CA), Snail1 (1:400, ab17732; Abcam, Tokyo, Japan), N-cadherin (1:500; BD Transduction Laboratories), β-catenin (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), Na^+-K^+-ATPase α1-subunit (1:500, clone C464.6; Upstate, Temecula, CA), vimentin (1:200; Dako Cytomation, Glostrup, Denmark), fibronectin (1:400; Dako), α-SMA (1:1,000; Progen Biotecknik, Heidelberg, Germany), and fibrocystin (1:50; Santa Cruz Biotechnology). After washing, they were incubated with the secondary antibody conjugated to a peroxidase-labeled polymer supplied with the Envision plus system (Dako), except for fibronectin. Immunostaining for segment-specific markers, except for *Phaseolus vulgaris* erythroagglutinin (PHA-E), was performed using the labeled streptavidin-biotin complex (LSAB) method employing a LSAB kit (Dako). For PHA-E staining, avidin-binding horseradish peroxidase (HRP) (ExtrAvidin; Sigma, St. Louis, MO) was used. For fibronectin staining, anti-goat HRP-labeled immunoglobulin (Dako) was used as a secondary antibody. Target retrieval solution (Dako) for E-cadherin, Snail1, β-catenin, N-cadherin, vimentin, and fibronectin, and proteinase K (Sigma) for α-SMA and Na^+-K^+-ATPase α1-subunit, were used for antigen retrieval. The samples were visualized with 3,3′-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Masson-trichrome stain was used to assess fibrosis. The samples were photographed using an all-in-one light field and fluorescence microscope, BIOREVO (BZ-9000; Keyence, Osaka, Japan). All stainings had negative controls employing no antibodies or markers.

Segment-specific profile analysis of cyst formation. After immunohistological preparation, segment-specific profile analysis was performed. Cystic tubules were defined as tubules that were ≥50 μm in diameter, as described previously (39). Cyst number was counted in a plane showing the maximum area in each kidney sample.

Western blot analysis. Whole kidney samples were homogenized in lysis buffer (CelLytic MT; Sigma) to extract proteins. The homogenates were centrifuged at 20,000 rpm for 10 min, and the supernatant was collected. The protein concentration was determined using the Bradford method. Ten micrograms of protein lysate were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Immunodetection was performed after the membranes had been blocked in blocking solution

Table 1. *Tubular segment-specific cyst count in PCK rats*

	Age					
Segment	0 days	1 wk	3 wk	10 wk	14 wk	4 mo
PT	+	−	−	+	2+	3+
LH	+	−	2+	3+	5+	5+
DT	−	−	−	−	+	2+
CD	+	−	2+	2+	4+	5+

Cyst numbers were counted in a plane showing the maximum area of each kidney sample. Number of cysts was graded semiquantitatively on a scale from − to 5+: −, 0; +, ≤25; 2+, ≤50; 3+, ≤100; 4+, ≤200; and 5+, >200. PT, proximal tubule; LH, loop of Henle; DT, distal tubule; CD, collecting duct.

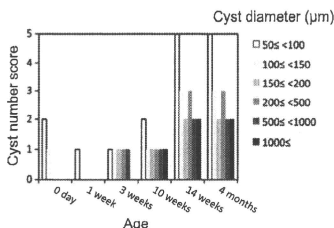


Fig. 2. Cyst number and size in PCK rats. The number and size of the cysts increased markedly from 14 wk of age in PCK rats.

and blotted with an antibody against E-cadherin (1:50,000; BD Transduction Laboratories) and β-catenin (1:5,000; Santa Cruz Biotechnology). An ECL detection system was used for detection (Amersham Biosciences, Little Chalfont, UK). The blots were then reprobed with an antibody against β-actin (1:5,000, Sigma) as a loading control. Images of the blots were captured, and densities of bands were analyzed using a CS Analyzer 3.0 with a charge-coupled device (CCD) camera (ATTO, Tokyo, Japan).

Quantitative real-time PCR. RNA was harvested from kidney lysates using an RNeasy kit (Qiagen, Hilden, Germany). RNA samples were then tested with primers specific for E-cadherin and Snail1 mRNAs, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers used for real-time PCR are shown in Supplemental Table 2. First-strand cDNA was produced from total RNA using a PrimeScript RT reagent kit (Takara Bio, Otsu, Japan). A SYBR Premix Ex Taq kit (Takara Bio) was used to perform quantitative PCR. Data were collected and analyzed with the Thermal Cycler Dice real-time PCR system and software (Takara Bio). The expression of E-cadherin or Snail1 mRNA was calculated relative to that in the control. Each RNA sample was analyzed in triplicate, and each experiment was performed independently at least three times. The $\Delta\Delta C_T$ method was used to analyze the data.

Statistical analysis. Data were analyzed using the Mann-Whitney U-test at a significance level of $P < 0.05$. The results were analyzed with JMP 7.0.1J software (SAS Institute Japan, Tokyo, Japan).

RESULTS

Profile analysis of cyst formation using segment-specific markers. Detection of tubule segments was performed using segment-specific markers (Supplemental Figs. 1 and 2). Renal cysts were evident from 3 wk of age in PCK rats (Fig. 1). Most of these cysts had developed in the loop of Henle (LH) and collecting duct (CD) in the corticomedullary region and outer medulla. There was also cyst formation in distal tubule (DT), in which the number was relatively small but the size was large. However, although small in number, proximal tubule (PT)-derived cysts were present at birth and from 10 wk of age (Table 1). The number and size of the cysts increased markedly from 14 wk of age (Table 1 and Figs. 1 and 2). Interstitial fibrosis and inflammation were also evident from 10 wk of age, in accordance with disease progression (Supplemental Fig. 3).

E-cadherin. Immunohistochemistry showed that E-cadherin was abundant in LH, DT, and CD but was present at only a very low level in PT in control rats at all ages (Supplemental Fig. 1, Fig. 3, and Table 2). This expression pattern was correlated with the pathology of the disease in that the tubule

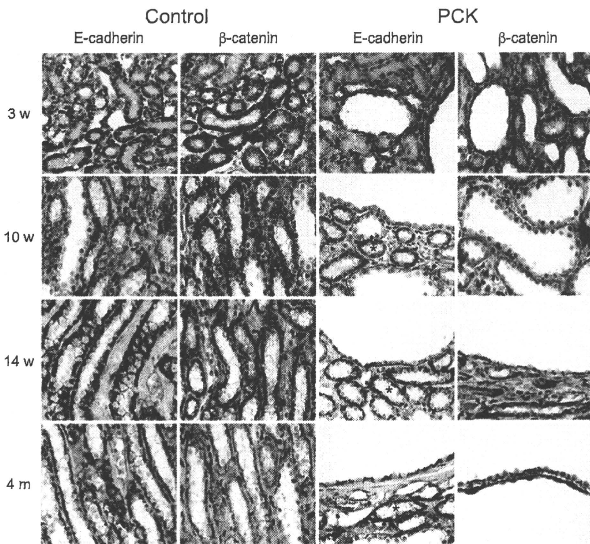


Fig. 3. E-cadherin/β-catenin expression in tubule epithelial cells in control and PCK rats. Immunohistochemistry showed that E-cadherin/β-catenin was abundant in the loop of Henle (LH), distal tubule (DT), and collecting duct (CD) in control rats at all ages. In PCK rats, E-cadherin/β-catenin expression in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with areas showing cyst enlargement, whereas E-cadherin/β-catenin expression in noncystic tubular epithelia remained high (asterisks indicate representative noncystic tubules). Original magnification, ×400; w, week; m, month.

segments showing the highest expression level in control rats had much severer cyst formation in PCK rats. In control rats, E-cadherin was localized primarily in areas of cell-cell contact in a basolateral pattern, and in LH of control rats it was also distributed in a basolateral and cytoplasmic pattern. From 3 wk of age, the level of E-cadherin expression in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement in PCK rats, whereas E-cadherin expression in noncystic tubular epithelia in PCK rats remained at a high level (Table 2 and Fig. 3). Western blot analysis and real-time PCR analysis showed decreased E-cadherin expression in PCK kidneys relative to controls, in accordance with disease progression (Figs. 4 and 5*A*).

Snail1. Snail1 was expressed predominantly in the nuclei of tubule epithelial cells forming large cysts, especially in adjacent fibrotic areas (Fig. 6). Snail1 expression was maximal in enlarging cysts, i.e., the largest cysts showed slightly lower expression of Snail1. There was no significant immunostaining for Snail1 in the controls. Expression of Snail1 mRNA was also upregulated in an age-dependent manner, although it was slightly late for the start of E-cadherin mRNA decrease (Fig. 5*B*).

β-Catenin. Like E-cadherin, β-catenin was strongly expressed in LH, DT, and CD in control rats. β-Catenin was localized primarily in areas of cell-cell contact and was also distributed in the cytoplasm in control rats (Fig. 3). In PCK rats, the level of β-catenin expression demonstrated by immunohistochemistry in cystic tubules was attenuated and localized to areas of lateral cell-cell contact, in accordance with cyst enlargement, whereas β-catenin expression in noncystic tubule epithelia in PCK rats remained high (Table 2 and Fig. 3). These expression patterns were similar to those of E-cadherin. Western blot analysis showed decreased β-catenin expression in PCK kidneys relative to controls, in accordance with disease progression (Fig. 7).

Na^+-K^+-ATPase α1-subunit. To examine the polarization of epithelial cells in PCK rats, we examined the subcellular patterns of Na^+-K^+-ATPase α1-subunit distribution during cyst formation. In control rats, basolateral expression of Na^+-K^+-ATPase α1-subunit was evident in LH and DT, where cyst formation was severe in PCK rats (Fig. 8). In PCK rats, expression of Na^+-K^+-ATPase α1-subunit in cystic tubule epithelia was decreased in accordance with disease progression, whereas basolateral expression of Na^+-K^+-ATPase α1-subunit in noncystic tubule epithelia remained high (Fig. 8).

N-cadherin and mesenchymal markers. In control rats, N-cadherin expression was detected only in PT and was absent in other segments (Table 3). The subcellular distribution of N-cadherin differed between segments S1/S2 and segment S3 of PT. Segments S1/S2 showed mainly apical expression, whereas segment S3 showed mainly basolateral expression. N-cadherin expression began to increase from 10 wk of age in

Table 2. *Epithelial expression of E-cadherin and β-catenin in control and PCK rats*

	Control			PCK (3 wk–4 mo)		
	0 days	1 wk	3 wk–4 mo	Noncystic	Small cyst	Large cyst
			E-cadherin			
Cortex						
PT	± b	± b	± b	± b	± b	No cyst
DT-CD	+ b/c	+ to 2+ b/c	2+ b/c	2+ b/c	+ b/c	± b or l
Outer medulla						
LH	+ b/c	+ b/c	3+ b/c	2+ to 3+ b/c	+ b/c	± b or l
CD	+ b	+ b	+ b	+ b	+ b	± b or l
Inner medulla						
CD	+ b	+ b	+ b	+ b	+ b	± b or l
			β-Catenin			
Cortex						
PT	± b	± b	± b	± b	± b	No cyst
DT-CD	+ b/c	+ b/c	2+ b/c	2+ b/c	+ b/c	+ b or l
Outer medulla						
LH	+ b/c	+ b/c	2+ b/c	2+ b/c	+ b/c	+ b or l
CD	+ b	+ b	+ b	+ b	+ b	+ b or l
Inner medulla						
CD	+ b	+ b	+ b	+ b	+ b	+ b or l

The intensity of staining was graded semiquantitatively on a scale from − to 3+: −, none; ±, trace; +, slight; 2+, moderate; and 3+, intense. A large cyst was defined as a tubule >200 μm in diameter. Dominant pattern of staining: b, basolateral only; b/c, basolateral and cytoplasmic; l, lateral only. No cyst, no cyst formation.

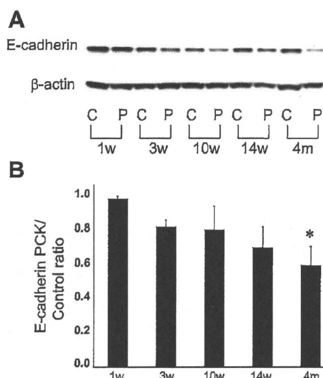


Fig. 4. E-cadherin expression during the disease course. *A*: E-cadherin expression assessed by Western blot analysis of kidney lysates. *B*: protein level of E-cadherin normalized by the expression of β-actin. Bar graph shows means ± SD of the E-cadherin level expressed relative to the control. $*P < 0.05$ vs. 1 wk. C, control; P, PCK rat.

the controls (Table 3). There was no remarkable difference in N-cadherin expression between control and PCK rats until 3 wk of age. N-cadherin expression was evident in cyst epithelial cells in PCK rats from 10 wk of age as cyst enlargement progressed. In PCK rats, all cysts derived from PT showed mainly basolateral expression of N-cadherin, whereas noncystic PT epithelial cells showed apical or basolateral expression of N-cadherin, in accordance with the segmental distribution in control rats (Table 3 and Fig. 9*A*). Cytoplasmic expression of N-cadherin was observed in cyst lining cells derived from LH and CD (Fig. 9, *B* and *C*), indicating de novo synthesis of N-cadherin.

Some tubule epithelial cells forming large cysts, especially in adjacent fibrotic areas, showed positive immunoreactivity for vimentin and fibronectin in PCK rats from 10 wk of age, suggestive of EMT (Fig. 10). Cells with positivity for vimentin and fibronectin were also increased in the interstitium in fibrotic areas around large cysts. Weak α-SMA expression in cyst lining cells was also observed in PCK rats (Fig. 10). Cells with α-SMA positivity were also distributed in the interstitium. There was no significant staining for mesenchymal markers in the controls.

Fibrocystin/polyductin. Although fibrocystin/polyductin was strongly expressed in CD in normal adult kidney, it was widely expressed in epithelial cells in other segments in control rats. In PCK rats, fibrocystin/polyductin expression was reduced in large cysts but not completely absent.

DISCUSSION

The current study provided further evidence of EMT in PKD. The novelty of our study is systematic evaluation of EMT markers in a nephron segment-specific and time-course manner in an orthologous ARPKD model. Clarification of the segment-specific cyst formation profile related to EMT in the PCK rat allows us new insight into PKD pathophysiology as discussed in detail below.

Our studies demonstrate that EMT may play a role in PCK rats, although investigation of mechanistic insights into pathological processes of EMT is needed for further confirmation of EMT in PKD. Recent evidence suggests that fibrocystin/polyductin, polycystin (PC)-1, and PC-2 are all localized at plasma membrane and primary cilium (42). Therefore, analysis of cyst formation mechanism in PCK rats would likely provide valuable information about not only ARPKD but also ADPKD in humans. Because our study was performed by focusing on specific segments in rats at different ages, it clarified the process of disease progression in vivo. Sato et al. (33) reported that cholangiocytes of the PCK rat may acquire mesenchymal features and participate in progressive hepatic fibrosis by producing extracellular matrix molecules, which seems to be a different event from EMT. The reason of this difference is unknown. There is the possibility of organ-specific features in the model.

The data presented in the present study reveal that tubule segments with the highest level of E-cadherin expression in control rats showed much severer cyst formation in PCK rats. The expression level and pattern of cadherins differed among the tubule segments. In control rats, N-cadherin was predominant in PT and absent in other segments, whereas in control rats E-cadherin was highly expressed in LH, DT, and CD but

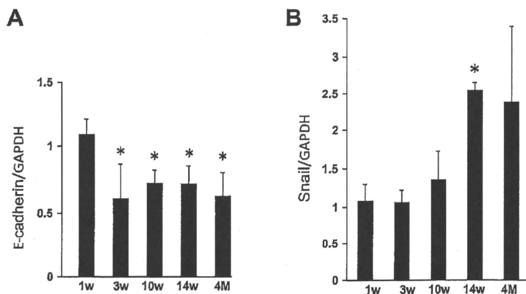


Fig. 5. E-cadherin and Snail1 mRNAs assessed by real-time quantitative RT-PCR in PCK rats. Bar graphs show means ± SD of mRNA level expressed relative to the control. *A*: the ratio of the E-cadherin mRNA level relative to GAPDH was decreased after 3 wk. $*P < 0.05$ vs. 1 wk. *B*: the ratio of Snail1 mRNA level was upregulated after 10 wk. $*P < 0.05$ vs. 1 and 3 wk.

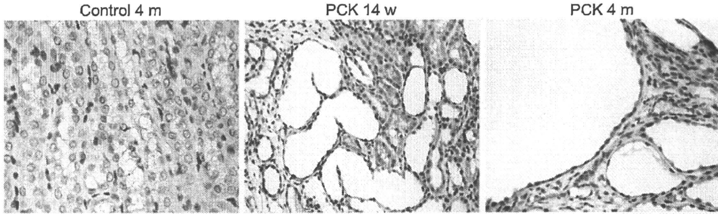


Fig. 6. Nuclear Snail1 expression in PCK rats. Representative images from PCK rats at 14 wk and 4 mo and from a control rat at 4 mo are shown (original magnification, ×400; m, months). Snail1 was expressed predominantly in the nuclei of tubule epithelial cells forming large cysts. On the other hand, there was no significant immunostaining of Snail1 in control rats.

present at a very low level in PT. Moreover, E-cadherin expression was strongest in the thick ascending limb (THAL) of LH in the outer medullary segment. These localization patterns of cadherins were in accord with results reported previously (29). On the other hand, Lager et al. (16) described that PCK rats develop renal cysts in LH, DT, and CD. In our study, the segments most affected in PCK rats were the medullary THAL and CD in the outer medulla, where the level of E-cadherin expression is highest in control rats. In addition, the expression of E-cadherin decreased gradually in accordance with cyst enlargement in PCK rats. These findings raise the possibility that, in the PCK model, differences in cadherin expression may be a marker of susceptibility to cystogenesis. Nephron segment-specific cyst formation is a key feature of PKD. Our observations may therefore provide an important clue to the mechanism of segment-specific cyst formation.

In the present study, E-cadherin expression decreased gradually and was limited to the lateral-lateral cell membrane, in accordance with cyst enlargement. Loss of E-cadherin expression is closely linked to plasma membrane destabilization, which is also observed in fetal development and certain pathological processes including carcinogenesis, metastasis of cancer cells, and EMT. Aberrant E-cadherin expression has been reported in PKD (7, 28). Huan and van Adelsberg (13) reported that the PKD1 gene product PC-1 formed a complex containing E-cadherin and β-catenin. Roitbak et al. (32) reported that the PC-1/PC-2/E-cadherin/β-catenin complex was disrupted in cells from ADPKD patients and that loss of E-cadherin expression was compensated by N-cadherin expression, which apparently stabilized β-catenin in place of E-cadherin. In our present study, segments of epithelial cells in areas of severe cyst formation showed de novo expression of N-cadherin, whereas it was negative in control rats. Expression of N-cadherin might therefore represent transdifferentiation from an epithelial to a mesenchymal phenotype, as well as loss of E-cadherin. These findings suggest that cadherin-related functions may play a key role in PKD.

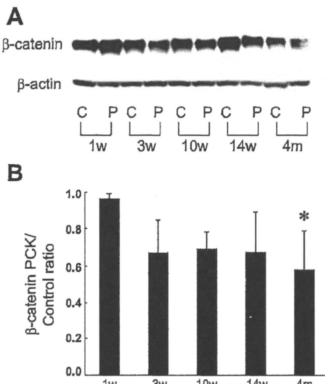


Fig. 7. β-Catenin expression during the disease course. *A*: β-catenin expression assessed by Western blot analysis of kidney lysates. *B*: protein level of β-catenin normalized by the expression of β-actin. Bar graph shows means ± SD of the β-catenin level expressed relative to the control. **P* <0.05 vs. 1 wk.

In the present study, expression of the Na^+-K^+-ATPase α1-subunit in cystic tubular epithelia decreased in parallel with disease progression in PCK rats, suggesting a loss of apicobasal cell polarity. Avner et al. (3) reported that the apical membrane Na^+-K^+-ATPase α1-subunit persisted in cystic kidneys of the PCK mouse, suggesting that its expression might be a manifestation of the relatively undifferentiated phenotype of the epithelial cells lining CD cysts. Although there is a difference in the abnormal expression pattern of the Na^+-K^+-ATPase α1-subunit in cystic epithelium between the PCK rat and CPK mouse models, these findings may suggest diversity of the peripheral phenotype of epithelial cells from the common pathophysiology of PKD.

Renal fibrosis, like that in PKD, is considered to be a common key pathway leading to renal failure, irrespective of the underlying disease (17). Although EMT is known to play a significant role in renal fibrosis, the exact mechanism involved is unclear. In the present study, some tubule epithelial cells in large cysts in PCK rats showed phenotypic and morphological change, including loss of E-cadherin expression, and de novo expression of N-cadherin, vimentin, and fibronectin. Loss of E-cadherin expression is thought to be an early event that precedes EMT (17). In PCK rats, even at 3 wk of age, E-cadherin expression was evidently decreased in cystic epithelial cells. On the other hand, mesenchymal markers were expressed after 10 wk of age. At the same time, in interstitial fibrotic areas adjacent to large cysts, expression of vimentin

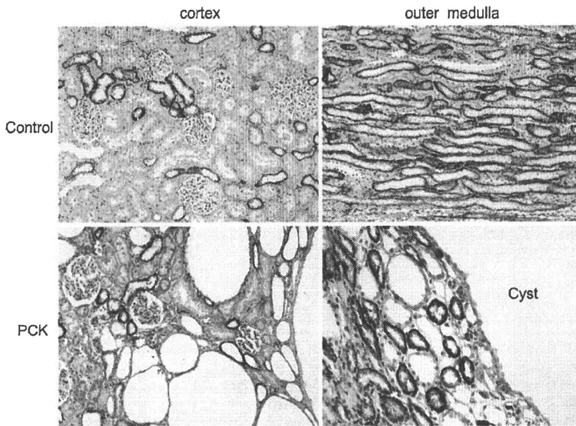


Fig. 8. Expression of Na^+-K^+-ATPase α1-subunit in PCK and control rats. Representative images from PCK and control rats at 14 wk are shown (original magnification, ×200). In control rats, basolateral expression of Na^+-K^+-ATPase α1-subunit was evident in the LH and DT. In PCK rats, expression of Na^+-K^+-ATPase α1-subunit in cystic epithelia decreased in parallel with disease progression, whereas its basolateral expression in noncystic epithelia remained high.

and fibronectin was upregulated, suggesting an accumulation of fibroblasts. α-SMA is a molecular marker of myofibroblasts. The presence of activated myofibroblasts is thought to be one of the most distinctive features of interstitial fibrosis. It has been reported that the expression of α-SMA is increased in rat models of unilateral ureter obstruction (UUO) (28). In our present study of PCK rats, weak α-SMA expression in cyst lining cells was also observed, in addition to a few α-SMA-positive cells scattered in the fibrotic area. As discussed in previous reports, such fibroblasts may have a different origin. Iwano et al. (14) showed that 36% of new fibroblasts originated from local EMT and that ~15% were from bone marrow, the rest being local proliferation. Together, the data suggest that EMT of tubule epithelial cells may contribute to renal fibrosis in the PCK rat. These observations raise the question of whether EMT is a PKD-specific pathophysiology or merely a common key pathway leading to renal failure, regardless of the underlying disease. However, the early change of E-cadherin expression before evident fibrosis suggests the former possibility. Further investigations are needed to clarify this issue.

Although the mechanism of EMT appears complex, its consequences are likely mediated in part through the effects of β-catenin. Disruption of the E-cadherin/β-catenin complex induces loss of β-catenin in the cell membrane. Cytoplasmic free β-catenin works as an essential signal mediator in the Wnt signal pathway. The Wnt/β-catenin signal pathway mediates many inductive events such as kidney development and cancer. Cytoplasmic β-catenin translocates to the nucleus and binds

Table 3. *Epithelial expression of N-cadherin in control and PCK rats*

	Control		PCK Rat (≥10 wk)		
	≤3 wk	≥10 wk	Noncystic	Small cyst	Large cyst
Cortex					
PT					
S1, S2	± a	2+ a	2+ a	2+ b	No cyst
S3	± b	+ b	+ b	2+ b	No cyst
DT-CD	−	−	−	−	± to + c
Outer medulla					
LH	−	−	−	−	± to + c
CD	−	−	−	−	± to + c
Inner medulla					
CD	−	−	−	−	± to + c

The intensity of staining was graded semiquantitatively on a scale from − to 3+: −, none; ±, trace; +, slight; 2+, moderate; and 3+, intense. A large cyst was defined as a tubule >200 μm in diameter. Dominant pattern of staining: a, apical; b, basolateral; c, cytoplasmic. S1–S3, PT segments.

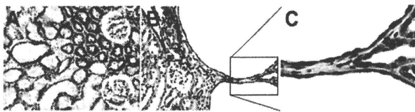


Fig. 9. N-cadherin expression in tubule epithelial cells in PCK rats. Representative images from PCK rats at 4 mo are shown (original magnification, ×400). *A*: all cysts derived from the proximal tubule showed mainly basolateral expression of N-cadherin, whereas noncystic proximal tubule epithelia showed apical or basolateral expression of N-cadherin. *B* and *C*: cytoplasmic expression of N-cadherin was observed in cyst lining cells derived from the LH and CT, indicating de novo synthesis of N-cadherin.

with T-cell factor/lymphoid enhancing factor (TCF/LEF) in the nucleus, thus activating various target genes (5). Aberrant β-catenin signaling is reported to play a role in cyst formation. Nuclear catenin staining has been found in the bcl-2 null mouse model of PKD (36). Muto et al. (21) showed that the expression of E-cadherin and β-catenin was attenuated in transgenic mice with targeted deletion of exon 2–6 of *Pkd1*. We speculate the alterations of β-catenin expression are associated with cyst enlargement.

We observed a clear increase in the nuclear expression of Snail1 in PCK rats, and Snail1 mRNA was also upregulated. These findings may support a role of EMT in the PCK rat model. One possible mechanism to account for the loss of E-cadherin expression is a reduction of gene expression. The transcription factor Snail1 is activated and binds to specific DNA sequences known as E-boxes in the promoter of the E-cadherin gene. Subsequently, transcriptional repression of E-cadherin occurs during EMT and cancer (6, 38). It has been reported that Madin-Darby canine kidney (MDCK) cells transfected with Snail1 show downregulation of E-cadherin expression and increased expression of mesenchymal markers (7). In our study, Snail1 mRNA upregulation was slightly late for the start of E-cadherin mRNA decrease. The reason of this gap between the E-cadherin expression and its inhibitor Snail1 level is unknown. Several possibilities are conceivable. Because of the nature of our study, the change of expression levels on time course in an individual rat is not available. Therefore, if expression levels vary widely among individuals, the mean of the data may also vary. Another possibility is that the initiation of decrease in E-cadherin may be caused by other factor(s) than Snail1. Snail1 may work for the continuity of suppression of E-cadherin.

The *PKHD1* gene product, fibrocystin/polyductin, is a membrane-bound protein widely expressed in epithelial cells (46). The cellular functions of fibrocystin/polyductin are not fully understood but likely include roles in epithelial cell proliferation. Fibrocystin/polyductin seems to participate in tubule morphogenesis. Ward et al. (43) showed that fibrocystin/polyductin was strongly expressed in CD of fetal and adult human kidney. Fibrocystin/polyductin also has been detected in LH in fetal kidney (19, 43). The PCK rat has a mutation in the ortholog of human *PKHD1*. Immunohistochemical analysis has shown that fibrocystin/polyductin expression is reduced in the kidney of the PCK rat but not completely absent (46). Similar findings also have been obtained by Western blot analysis (18). Our observations in fibrocystin/polyductin in the PCK rat are similar to those reported previously. Loss of E-cadherin and β-catenin might disrupt the PC1/E-cadherin/β-catenin complex and induce loss of function of PC1 as a mechanosensor, as has been reported in ADPKD (32). It is known PC-1 and PC-2, which are mutated in ADPKD, interact though their COOH-terminal cytoplasmic tail (41). Moreover, recent evidence suggests that fibrocystin/polyductin and polycystins are colocalized at the primary cilia (23, 42, 44). Fibrocystin/polyductin is reportedly related to the flow-stimulated intracellular calcium signaling pathway (12, 22). These findings suggest that the pathophysiology of the PCK rat model may involve a common pathway that alters ciliary functions, thus leading to cyst formation.

In conclusion, we have shown that nephron segments exhibiting severe cyst formation correspond to the segments with high expression of E-cadherin and fibrocystin/polyductin in PCK rats. These distribution patterns are probably associated with cyst formation in PKD. E-cadherin expression was shown to decrease in parallel with cyst enlargement, suggesting that adhesion molecules play a key role in cyst formation and enlargement in PCK rats. In addition, epithelial cells in cysts were shown to acquire mesenchymal features in response to cyst enlargement and probably participate in the progression of renal fibrosis. EMT may play an important role in the pathophysiology of PKD, and we speculate that it could become a potential therapeutic target for human PKD.

ACKNOWLEDGMENTS

Parts of this study were presented at the 40th, 41st, and 42nd annual meetings of the American Society of Nephrology in San Francisco, CA (2007), Philadelphia, PA (2008), and San Diego, CA (2009) and have been published in abstract form (*J Am Soc Nephrol* 18: 361A, 2007; *J Am Soc Nephrol* 19: 362A, 2008; and *J Am Soc Nephrol* 20: 274A, 2009).

GRANTS

This work was supported by Grants-in-Aid for Scientific Research 20790739 (to H. Togawa), 18790730 (to M. Sako), and 20591285 (to K.

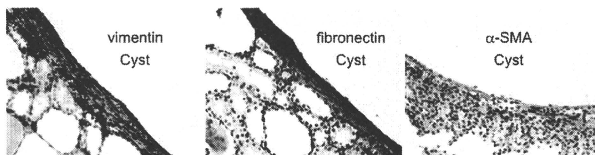


Fig. 10. Mesenchymal markers in tubule epithelial and interstitial cells in PCK rats. Representative images from PCK rats at 4 mo are shown (original magnification, ×400). Mesenchymal markers (vimentin, fibronectin) are expressed de novo in cystic epithelial cells, especially in adjacent fibrotic areas. Cells positive for vimentin and fibronectin are also increased in fibrotic areas around large cysts. Weak expression of α-smooth muscle actin (α-SMA) in cyst lining cells was also observed. α-SMA-positive cells were also distributed in the interstitium.

Nakanishi) from the Japan Society for the Promotion of Science and by a grant from Wakayama Medical University (to H. Togawa).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **Attanasio M, Uhlenhaut NH, Sousa VH, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, Seelow D, Nürnberg G, Becker C, Chudley AE, Nürnberg P, Hildebrandt F, Treier M.** Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat Genet* 39: 1018–1024, 2007.
2. **Avner ED, Woychik RP, Dell KM, Sweeney WE.** Cellular pathophysiology of cystic kidney disease: insight into future therapies. *Int J Dev Biol* 43: 457–461, 1999.
3. **Avner ED, Sweeney WE Jr, Nelson WJ.** Abnormal sodium pump distribution during renal tubulogenesis in congenital murine polycystic kidney disease. *Proc Natl Acad Sci USA* 89: 7447–7451, 1992.
4. **Barrallo-Gimeno A, Nieto MA.** The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* 132: 3151–3161, 2005.
5. **Benzing T, Simons M, Walz G.** Wnt signaling in polycystic kidney disease. *J Am Soc Nephrol* 18: 1389–1398, 2007.
6. **Boutet A, De Frutos CA, Maxwell PH, Mayol MJ, Romero J, Nieto MA.** Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. *EMBO J* 25: 5603–5613, 2006.
7. **Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA.** The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2: 76–83, 2000.
8. **Charron AJ, Nakamura S, Bacallao R, Wandinger-Ness A.** Compromised cytoarchitecture and polarized trafficking in autosomal dominant polycystic kidney disease cells. *J Cell Biol* 149: 111–124, 2000.
9. **Chea SW, Lee KB.** TGF-beta mediated epithelial-mesenchymal transition in autosomal dominant polycystic kidney disease. *Yonsei Med J* 50: 105–111, 2009.
10. **Dell KM, McDonald RA, Watkins SL, Avner ED.** Polycystic kidney disease. In: *Pediatric Nephrology* (5th ed.), edited by Avner ED, Harmon WE, and Niaudet P. Philadelphia, PA: Lippincott Williams & Wilkins, 2004, p. 675–699.
11. **Grantham JJ.** Mechanisms of progression in autosomal dominant polycystic kidney disease. *Kidney Int Suppl* 63: S93–S97, 1997.
12. **Hiesberger T, Gourley E, Erickson A, Koulen P, Ward CJ, Masyuk TV, Larusso NF, Harris PC, Igarashi P.** Proteolytic cleavage and nuclear translocation of fibrocystin is regulated by intracellular Ca^{2+} and activation of protein kinase C. *J Biol Chem* 281: 34357–34364, 2006.
13. **Huan Y, van Adelsberg J.** Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. *J Clin Invest* 10: 1459–1468, 1999.
14. **Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG.** Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 110: 341–350, 2002.
15. **Katsuyama M, Masuyama T, Komura I, Hibino T, Takahashi H.** Characterization of a novel polycystic kidney rat model with accompanying polycystic liver. *Exp Anim* 49: 51–55, 2000.
16. **Lager DJ, Qian Q, Bengal RJ, Ishibashi M, Torres VE.** The pck rat: a new model that resembles human autosomal dominant polycystic kidney and liver disease. *Kidney Int* 59: 126–136, 2001.
17. **Liu Y.** Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* 15: 1–12, 2004.
18. **Masyuk TV, Huang BQ, Ward CJ, Masyuk AI, Yuan D, Splinter PL, Punyashthiti R, Ritman EL, Torres VE, Harris PC, LaRusso NF.** Defects in cholangiocyte fibrocystin expression and ciliary structure in the PCK rat. *Gastroenterology* 125: 1303–1310, 2003.
19. **Menezes LF, Cai Y, Nagasawa Y, Silva AM, Watkins ML, Da Silva AM, Somlo S, Guay-Woodford LM, Germino GG, Onuchic LF.** Polyductin, the PKHD1 gene product, comprises isoforms expressed in plasma membrane, primary cilium, and cytoplasm. *Kidney Int* 66: 1345–1355, 2004.
20. **Murcia NS, Sweeney WE, Avner ED.** New insights into the molecular pathophysiology of polycystic kidney disease. *Kidney Int* 55: 1187–1197, 1999.
21. **Muto S, Aiba A, Saito Y, Nakao K, Nakamura K, Tomita K, Kitamura T, Kurabayashi M, Nagai R, Higashihara E, Harris PC, Katsuki M, Horie S.** Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant. *Hum Mol Genet* 11: 1731–1742, 2002.
22. **Nagano J, Kitamura K, Hujer KM, Ward CJ, Bram RJ, Hopfer U, Tomita K, Huang C, Miller RT.** Fibrocystin interacts with CAML, a protein involved in Ca^{2+} signaling. *Biochem Biophys Res Commun* 338: 880–889, 2005.
23. **Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J.** Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129–137, 2003.
24. **Ohse T, Inagi R, Tanaka T, Ota T, Miyata T, Kojima I, Ingelfinger JR, Ogawa S, Fujita T, Nangaku M.** Albumin induces endoplasmic reticulum stress and apoptosis in renal proximal tubular cells. *Kidney Int* 70: 1447–1455, 2006.
25. **Okada H, Ban S, Nagao S, Takahashi H, Suzuki H, Neilson EG.** Progressive renal fibrosis in murine polycystic kidney disease: an immunohistochemical observation. *Kidney Int* 58: 587–597, 2000.
26. **Ong AC, Harris PC.** Molecular pathogenesis of ADPKD: The polycystin complex gets complex. *Kidney Int* 67: 1234–1247, 2005.
27. **Onuchic LF, Furu L, Nagasawa Y, Hou X, Eggermann T, Ren Z, Bergmann C, Senderek J, Esquivel E, Zeltner R, Rudnik-Schoneborn S, Mrug M, Sweeney W, Avner ED, Zerres K, Guay-Woodford LM, Somlo S, Germino GG.** PKHD1, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am J Hum Genet* 70: 1305–1317, 2002.
28. **Picard N, Baum O, Vogetseder A, Kaissling B, Le Hir M.** Origin of renal myofibroblasts in the model of unilateral ureter obstruction in the rat. *Histochem Cell Biol* 130: 141–155, 2008.
29. **Prozialeck WC, Lamar PC, Appelt DM.** Differential expression of E-cadherin, N-cadherin and beta-catenin in proximal and distal segments of the rat nephron. *BMC Physiol* 4: 10, 2004.
30. **Quinlan RJ, Tobin JL, Beales PL.** Modeling ciliopathies: Primary cilia in development and disease. *Curr Top Dev Biol* 84: 249–310, 2008.
31. **Rocco MV, Neilson EG, Hoyer JR, Ziyadeh FN.** Attenuated expression of epithelial cell adhesion molecules in murine polycystic kidney disease. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F679–F686, 1992.
32. **Roitbak T, Ward CJ, Harris PC, Bacallao R, Ness SA, Wandinger-Ness A.** A polycystin-1 multiprotein complex is disrupted in polycystic kidney disease cells. *Mol Biol Cell* 15: 1334–1346, 2004.
33. **Sato Y, Harada K, Ozaki S, Furubo S, Kizawa K, Sanzen T, Yasoshima M, Ikeda H, Sasaki M, Nakanuma Y.** Cholangiocytes with mesenchymal features contribute to progressive hepatic fibrosis of the polycystic kidney rat. *Am J Pathol* 171: 1859–1871, 2007.
34. **Schieren G, Rumberger B, Klein M, Kreutz C, Wilpert J, Geyer M, Faller D, Timmer J, Quack I, Rump LC, Walz G, Donauer J.** Gene profiling of polycystic kidneys. *Nephrol Dial Transplant* 21: 1816–1824, 2006.
35. **Song X, Di Giovanni V, He N, Wang K, Ingram A, Rosenblum ND, Pei Y.** Systems biology of autosomal dominant polycystic kidney disease (ADPKD): computational identification of gene expression pathways and integrated regulatory networks. *Hum Mol Genet* 18: 2328–2343, 2009.
36. **Sorenson CM.** Nuclear localization of beta-catenin and loss of apical brush border actin in cystic tubules of bcl-2 −/− mice. *Am J Physiol Renal Physiol* 276: F210–F217, 1999.
37. **Sweeney WE Jr, Avner ED.** Molecular and cellular pathophysiology of autosomal recessive polycystic kidney disease (ARPKD). *Cell Tissue Res* 326: 671–685, 2006.
38. **Takkunen M, Grenman R, Hukkanen M, Korhonen M, García de Herreros A, Virtanen I.** Snail-dependent and -independent epithelial-mesenchymal transition in oral squamous carcinoma cells. *J Histochem Cytochem* 54: 1263–1275, 2006.
39. **Tao Y, Kim J, Schrier RW, Edelstein CL.** Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol* 16: 46–51, 2005.
40. **Thiery JP, Sleeman JP.** Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* 7: 131–142, 2006.
41. **Tsiokas L, Kim E, Arnould T, Sukhatme VP, Walz G.** Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci USA* 94: 6965–6970, 1997.
42. **Wang S, Zhang J, Nauli SM, Li X, Starremans PG, Luo Y, Roberts KA, Zhou J.** Fibrocystin/polyductin, found in the same protein complex

with polycystin-2, regulates calcium responses in kidney epithelia. *Mol Cell Biol* 27: 3241–3252, 2007.
43. **Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, Kubly V, Cunningham JM, Bacallao R, Ishibashi M, Milliner DS, Torres VE, Harris PC.** The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* 30: 259–269, 2002.
44. **Yoder BK, Hou X, Guay-Woodford LM.** The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* 13: 2508–25016, 2002.
45. **Yu J, Carroll TJ, McMahon AP.** Sonic hedgehog regulates proliferation and differentiation of mesenchymal cells in the mouse metanephric kidney. *Development* 129: 5301–5312, 2002.
46. **Zhang MZ, Mai W, Li C, Cho SY, Hao C, Moeckel G, Zhao R, Kim I, Wang J, Xiong H, Wang H, Sato Y, Wu Y, Nakanuma Y, Lilova M, Pei Y, Harris RC, Li S, Coffey RJ, Sun L, Wu D, Chen XZ, Breyer MD, Zhao ZJ, McKanna JA, Wu G.** PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc Natl Acad Sci USA* 101: 2311–2316, 2004.

V.　資料

JSKDC Japanese Study Group of Kidney Disease in Children

平成 22 年度厚生労働科学研究費補助金（医療技術実用化総合研究事業）
「小児ネフローゼ症候群における適応外使用免疫抑制薬の有効性・安全性の検証と
治療法の確立を目指した多施設共同臨床研究（H22-臨研推-一般-005）」

初発寛解後早期に再発する小児ステロイド感受性ネフローゼ症候群患者を対象とした標準治療（再発時プレドニゾロン治療）と標準治療＋高用量ミゾリビン併用治療の多施設共同オープンランダム化比較試験（JSKDC05）

研究実施計画書

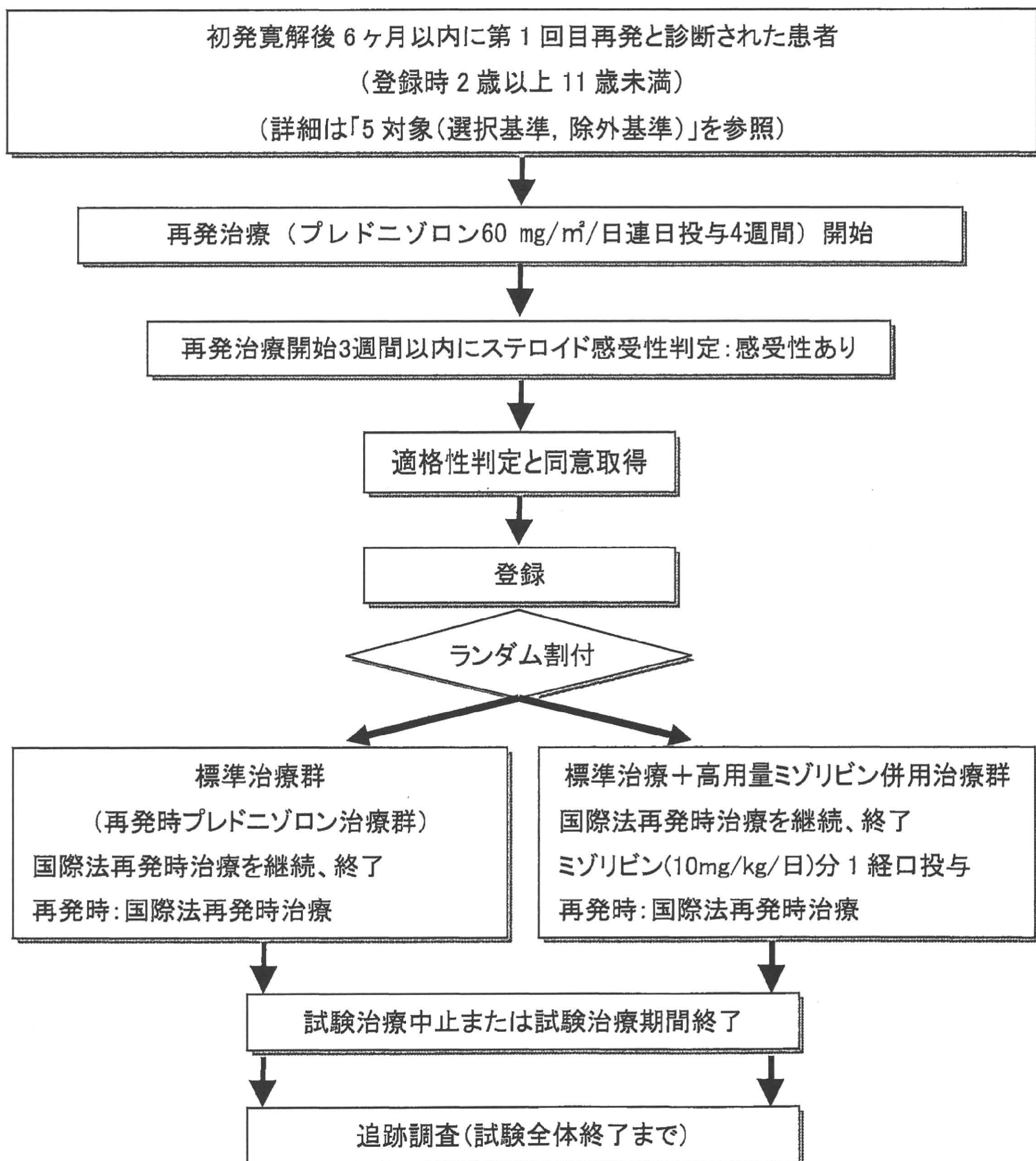
研究代表者:中西　浩一
和歌山県立医科大学小児科
〒641-8509　和歌山市紀三井寺 811-1
TEL：073-441-0632
FAX：073-444-9055

0　概要

0.1　試験名

初発寛解後早期に再発する小児ステロイド感受性ネフローゼ症候群患者を対象とした標準治療（再発時プレドニゾロン治療）と標準治療＋高用量ミゾリビン併用治療の多施設共同オープンランダム化比較試験

0.2　試験の構成



0.3 目的

初発寛解後早期に再発する小児ステロイド感受性ネフローゼ症候群患者を対象としたオープンランダム化並行群間比較試験によって，標準治療（再発時プレドニゾロン治療）＋高用量ミゾリビン併用治療が標準治療に対して，頻回再発抑制効果に優れることを検証する。

主要評価項目

頻回再発までの期間

副次評価項目

無再発期間，無再発割合，再発回数（回/患者），ステロイド依存性までの期間，ステロイド抵抗性までの期間，ステロイド総投与量（mg/m^2/患者）

その他の評価項目

試験薬ミゾリビンの薬物血中濃度（CO，C3），薬物動態

安全性評価項目

有害事象発現割合

0.4 対象（選択基準，除外基準）

小児ステロイド感受性ネフローゼ症候群患者（発症年齢が 2 歳以上 11 歳未満）のうち，初発寛解後 6 ヶ月以内に再発しステロイド感受性*を示す患者で，法的保護者から本試験の参加に対する同意が得られた患者。ただし，二次性ネフローゼ症候群と診断された患者，試験治療により病状を悪化させるおそれのある患者は除外する（5.1，5.2）。

*適格性判断時のみ，再発時治療開始 3 週間でステロイド感受性を判定する。

0.5 試験治療

登録された患者に対し，ランダム化割付結果に従い，標準治療群（再発時プレドニゾロン治療群）または標準治療＋高用量ミゾリビン併用治療群の試験治療（6.4）を行う。再発を認めた場合は再発時治療（6.5）を行う。

0.6 目標症例数と治験実施予定期間

目標症例数：120 例（標準治療群 60 例，標準治療＋高用量ミゾリビン併用治療群 60 例）
登録予定期間：2011 年 2 月～2015 年 1 月（4 年間）
試験実施予定期間：2011 年 2 月～2017 年 1 月（6 年間）