Department of Pediatrics

Hiyoruki Ida, *Professor*
Mitsuyoshi Urashima, *Professor*
Toshio Katsunuma, *Associate Professor*
Yoko Kato, *Associate Professor*
Hiroshi Kobayashi, *Associate Professor*
Masako Fujiwara, *Assistant Professor*
Masaharu Akiyama, *Assistant Professor*
Takashi Urashima, *Assistant Professor*
Tohya Ohashi, *Professor*
Ichiro Miyata, *Associate Professor*
Yasuyuki Wada, *Associate Professor*
Kazue Saito, *Associate Professor*
Yoshihiro Saito, *Assistant Professor*
Hiroshi Tachimoto, *Assistant Professor*
Masahisa Kobayashi, *Assistant Professor*

General Summary

We have 10 subspecialty research groups, which are the Inherited Metabolic Disease group, the Endocrinology group, the Neurology group, the Hematology and Oncology group, the Infectious Diseases and Immunologic Disorders group, the Nephrology group, the Cardiology group, the Allergy group, the Neonatology group, and the Pediatric Psychiatry group. The ultimate aim of each subspecialty group is to supply practical benefits to patients and their families through basic and translational research and clinical study.

Research Activities

Inherited metabolic disease group
The achievements of our group this year are as follows.
1. We developed a novel glycosaminoglycan (GAG) assay method to identify specifically accumulated GAG in a mouse model of mucopoloysaccharidosis type II (MPS II). Using this method, we showed that brain GAG was reduced by lentiviral vector-mediated hematopoietic stem cell gene therapy in MPS II mice.
2. We analyzed the mechanism of cardiomyocyte hypertrophy in Pompe disease using induced pluripotent stem cells. In addition, anti-human CD3 antibody (otelixizumab) induced immune tolerance against infused enzyme in enzyme replacement therapy for Pompe disease in transgenic mice expressing human CD3.
3. Using multiplex ligation-dependent probe amplification and cDNA analysis, we found 4 novel mutations in the α-galactosidase A gene from patients with Fabry disease.
4. We performed genetic diagnoses with a comparative genomic hybridization array and exome analysis in patients with congenital anomalies and intellectual disabilities.

Neurology group
Our research aims to clarify the clinical, genetic, and molecular pathophysiological aspects of childhood genetic epilepsies, especially intractable early-onset epilepsies, and to develop more effective treatments. We are focusing on 2 monogenic epilepsies, Dravet syndrome (mostly caused by defects of sodium channel, voltage-gated, type I, alpha subunit [SCN1A]) and protocadherin 19 (PCDH19)-related female epilepsy. Regarding Dravet syndrome, we have successfully generated a new research platform using patient-

derived induced pluripotent stem cells and have identified a functional vulnerability in the patient-derived neurons.
We investigated visiospatial disturbances in 103 children more than 1 year after onset of acute encephalopathy.

Nephrology group
Yamada and his colleague analyzed the gene expression profiles and differentiation capabilities of bone marrow- and adipose-derived mesenchymal stem cells in a rat model of chronic kidney disease (CKD). The study revealed that uremic toxin in CKD rats had a small effect on the gene expression and differentiation of mesenchymal stem cells. Using data from a nationwide survey, Hirano studied the effect of birth weight on the development of CKD in childhood and showed a strong association between low birth weight and the development of CKD in children.

Infectious diseases and Immunologic Disorders group
We studied the identification of causative pathogens by means of multiplex polymerase chain reaction techniques in pediatric inflammatory diseases and respiratory infectious diseases. We also investigated the pathogenesis of and new treatments for chronic granulomatous disease (CGD) at the Department of Human Genetics of the National Research Institute for Child Health and Development. We found significantly lower incidences of *Bacteroides* and *Clostridium* in patients who had CGD with colitis than in patients who had CGD without colitis. This result suggests that a compositional change of the intestinal microbiota is useful for the early diagnosis of CGD colitis. Furthermore, we reported that thalidomide was effective for treating bowel inflammation in patients with CGD and did not cause progression of fungal or bacterial infections. Thalidomide is an efficacious therapeutic option for patients with CGD. We are planning to attempt gene therapy for certain patients with CGD in the near future.

Hematology and Oncology group
We investigated mutations of exons of 409 tumor-suppressor genes and oncogenes most frequently cited and most frequently mutated in the malignancies associated with congenital anomalies by using the Ion AmpliSeq^{TM} Comprehensive Cancer Panel (Life Technologies, Carlsbad, CA, USA). Moreover, we have a started a project to use whole-exome analysis to identify the genes causing undiagnosed congenital anomaly syndrome.
We performed a cross-sectional survey of pain management during bone marrow aspiration and biopsy and presented the results to institutions belonging to the Tokyo Children's Cancer Study Group. Seeking to acquire relevant pharmacological knowledge and share the information with palliative care teams, we established *Guide to the Pharmacological Management of Symptoms in Children with Cancer*.

Cardiology group
The pediatric cardiology group is interested in both basic and clinical cardiology research to improve outcomes for children with congenital or acquired heart disease.
Ongoing projects are as follows

1. Right heart failure and peroxisomal proliferator-activated receptor gamma
2. The effects of telmisaltan in heart failure
3. The effects of bisoprolol in right heart failure
4. The effects of carperitide in monocrotaline-induced pulmonary hypertension
5. Assessment of cardiopulmonary function in metabolic heart disease
6. Urocoltin and angiopoetin evaluation in congenital heart disease
7. Early diagnosis of renal dysfunction in patients with congenital heart disease
8. Problems of the Fontan operation, with a focus on operative methods
9. Our research on right heart failure and peroxisomal proliferator-activated receptor gamma recieved the Young Investigator Award at the annual meeting of the Association for European Paediatric Cardiology in 2014.

Allergy group

The main subjects of our research are as follows: 1) the role of eosinophils, mast cells, and epithelial cells in the pathology of allergic diseases; 2) pediatric asthma; 3) food allergy; 4) atopic dermatitis; and 5) treatments for allergic diseases. We have organized and performed the following multicenter clinical studies: the PET study (The Preventive Effect of Tulobuterol Patch for the Long-Term Management of Infantile Asthma study), the PARG study (Pediatric Asthma Research for Guideline Update: Add-on use of tulobuterol patch on unstable asthma treated with leukotriene receptor antagonist), the CIT study (A Comparison of Continuous Inhalation Treatment with Salbutamol and Isoproterenol for Severe Pediatric Bronchial Asthma: A multicenter, double-blind, randomized study), the OSCAR study (Optimal Stepdown Therapy for Controlled Pediatric Asthma Responded to SFC), and the ORIMA study (Effect of Oral Immunotherapy in Preschool Children with Milk Allergy study). We have just started the DIFTO study (Daily Versus Intermittent Inhaled Fluticasone in Toddlers with Recurrent Wheezing), a multicenter, double-blind, randomized controlled study, to investigate the effect of intermittent inhaled fluticasone in treating patients with infantile asthma.

Endocrinology group

We used immunohistochemical methods to analyze the expression of urocortin 2, urocortin 3, and inflammatory cytokines in the brain in 2 rat models of surgical left ventricular heart strain (high and low left ventricular pressure). Furthermore, we performed tail-suspension tests for these rats and analyzed behavior patterns.

We identified 3 novel mutations of the *SLC16A2* gene in 3 patients with suspected deficiency of monocarboxylate transporter 8 and performed clinical investigations. We found endocrinological abnormalities in all 3 patients.

We studied the efficacy of growth hormone treatment in children with small for gestational age short stature born with extremely low birth weight. The method of individually adjusting doses of growth hormone was considered the best way of using growth hormone to treat these patients.

Publications

Yamaoka M, Akiyama M, Yokokawa Y, Terao Y, Yokoi K, Kato T, Fukushima T, Sakurai H, Ida H. Multidisciplinary therapy including proton beam radiotherapy for an Ewing's sarcoma family tumor of maxillary sinus in a 4-year-old girl. *Head Neck.* 2013; **35:** E386-90.

Akiyama M, Ozaki K, Kawano T, Yamada O, Kawauchi K, Ida H, Yamada H. Telomerase activation as a repair response to radiation-induced DNA damage in Y79 retinoblastoma cells. *Cancer Lett.* 2013; **340:** 82-7.

Katsunuma T, Fujisawa T, Nagao M, Akazawa A, Nomura I, Yamaoka A, Kondo H, Masuda K, Yamaguchi K, Terada A, Ikeda M, Nishioka K, Adachi Y, Kurihara K. Effects of transdermal tulobuterol in pediatric asthma patients on long-term leukotriene receptor antagonist therapy: results of a randomized, open-label, multicenter clinical trial in Japanese children aged 4-12 years. *Allergol Int.* 2013; **62:** 37-43.

To M, Takagi D, Akashi K, Kano I, Haruki K, Barnes PJ, Ito K. Sputum plasminogen activator inhibitor-1 elevation by oxidative stress-dependent nuclear factor-κB activation in COPD. Chest. 2013; **144:** 515-21.

Sato Y, Fujiwara M, Kobayashi H, Ida H. Massive accumulation of glycosaminoglycans in the aortic valve of a patient with Hunter syndrome during enzyme replacement therapy. *Pediatr Cardiol.* 2013; **34:** 2077-9.

Akiyama K, Shimada Y, Higuchi T, Ohtsu M, Nakauchi H, Kobayashi H, Fukuda T, Ida H, Eto Y, Crawford BE, Brown JR, Ohashi T. Enzyme augmentation therapy enhances the therapeutic efficacy of bone marrow transplantation in mucopolysaccharidosis type II mice. *Mol Genet Metab.* 2014; **111:** 139-46.

Ishikawa A, Enomoto K, Tominaga M, Saito T, Nagai J, Furuya N, Ueno K, Ueda H, Masuno M, Kurosawa K. Pure duplication of 19p13.3. Am J Med Genet A. 2013; **161A**: 2300-4.

Hirano D, Fujinaga S, Nishizaki N, Kanai H, Ida H. Role of ultrasound in revealing complications following percutaneous renal biopsy in children. *Clin Nephrol.* 2013; **80:** 426-32.

Higurashi N, Nakamura M, Sugai M, Ohfu M, Sakauchi M, Sugawara Y, Nakamura K, Kato M, Usui D, Mogami Y, Fujiwara Y, Itoh T, Ikeda H, Imai K, Takahashi Y, Nukui M, Inoue T, Okazaki S, Kirino T, Tomonoh Y, Inoue T, Takano K, Shimakawa S, Hirose S. PCDH19-related female-limited epilepsy: Further details regarding early clinical features and therapeutic efficacy. *Epilepsy Res.* 2013; **106:** 191-9.

Higurashi N, Uchida, T, Lossin C, Misumi Y, Okada Y, Akamatsu W, Imaizumi Y, Zhang B, Nabeshima K, Mori MX, Katsurabayashi S, Shirasaka Y, Okano H, Hirose S. A human Dravet syndrome model from patient induced pluripotent stem cells. *Mol Brain.* 2013; **6:** 19.

Higurashi N, Okano H, Hirose S. The effect of SCN1A mutations on patient-derived GABAergic neurons: what are the implications for future Dravet syndrome therapeutics? *Future Neurol.* 2013; **8:** 487-9.

Kawai T, Watanabe N, Yokoyama M, Arai K, Oana S, Harayama S, Yasui K, Ohishi T, Onodera M. Thalidomide attenuates excessive inflammation without interrupting lipopolysaccharide-driven inflammatory cytokine production in chronic granulomatous disease. *Clin Immunol.* 2013; **147:** 122-8.