had acute renal failure and urate nephrolithiasis. In addition, five nonsynonymous sequence variants and three nonsynonymous sequence variants in *SLC2A9* gene were found in two UK patients suffering from acute renal failure.

DISCUSSION

The molecular basis of urate transport in kidney was uncertain until 2002, when Enomoto et al. discovered hURAT1, which is encoded by *SLC22A12* gene and regulates blood urate levels.[2] Since that time, more than 100 cases with hereditary renal hypouricemia due to mutations in the *SCL22A12* gene have been identified in Japan,[4–6] and this number is unique worldwide. It was found that W258X nonsense mutation is the major cause of renal hypouricemia in Japanese patients.[6]

We have performed mutational analysis of the *SLC22A12* and *SLC2A9* genes in non-Asian patients. Hypouricemia with increased fractional excretion of uric acid is consistent with the findings previously reported[4–6] and this finding confirms the causative role of these genes on primary hereditary renal hypouricemia. There is a growing interest in understanding the genetic determinants of urate homeostasis in view of the fact that recent clinical and epidemiological studies have found that soluble uric acid has an important biological role.[8] Hyperuricemia may be a primary risk factor for several common disorders, including metabolic syndrome, cardiovascular disease, hypertension, and kidney disease.[9,10] Our study suggests that primary hereditary renal hypouricemia is not restricted to East Asian populations, as previously thought. As hypouricemia itself does not induce any symptoms, hereditary renal hypouricemia is sometimes overlooked. Our experience in detection of other inborn errors of metabolism with hypouricemia shows that every finding of persistent hypouricemia needs further detailed purine metabolic investigations in specialized biochemical-genetic laboratories in order to identify different types of hereditary xanthinuria, other causes of primary hypouricemia (such as purine nucleosidase deficiency), and to exclude secondary causes of hypouricemia. These include conditions with increased renal uric acid excretion in conjunction with isolated or generalized tubular defects (Fanconi syndrome, Wilson disease, cystinosis, heavy metal poisoning) or conditions with decreased uric acid synthesis (severe liver disease) or medication with uricosuric agents (salicylates >2 g/day).

In conclusion, our finding of genetic defects in *SCL22A12* and *SLC2A9* provides further evidence of the causative genes of primary renal hypouricemia and supports their important role in the regulation of serum urate levels in humans. Hereditary renal hypouricemia is still an unrecognized condition with a significant incidence in European populations.

REFERENCES

1. Sperling, O. Hereditary renal hypouricemia. *Mol. Genet. Metab.* **2006**, 89, 14–18.
2. Enomoto, A.; Kimura, H.; Chairoungdua, A.; Shigeta, Y.; Jutabha, P.; Cha, S.H.; Hosoyamada, M.; Takeda, M.; Sekine, T.; Igarashi, T.; Matsuo, H.; Kikuchi, Y.; Oda, T.; Ichida, K.; Hosoya, T.; Shimokata, K.; Niwa, T.; Kanai, Y.; Endou, H. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature* **2002**, 417, 447–452.
3. Vitart, V.; Rudan, I.; Hayward, C.; Gray, N.K.; Floyd, J.; Palmer, C.N.; Knott, S.A.; Kolcic, I.; Polasek, O.; Graessler, J.; Wilson, J.F.; Marinaki, A.; Riches, P.L.; Shu, X.; Janicijevic, B.; Smolej-Narancic, N.; Gorgoni, B.; Morgan, J.; Campbell, S.; Biloglav, Z.; Barac-Lauc, L.; Pericic, M.; Klaric, I.M.; Zgaga, L.; Skaric-Juric, T.; Wild, S.H.; Richardson, W.A.; Hohenstein, P.; Kimber, C.H.; Tenesa, A.; Donnelly, L.A.; Fairbanks, L.D.; Aringer, M.; McKeigue, P.M.; Ralson, S.H.; Morris, A.D.; Rudan, P.; Hastie, N. D.; Campbell, H.; Wright, A.F. SLC2A9 is a newly identified urate transporter influencing serum concentration, urate excretion and gout. *Nat. Genet.* **2008**, 40, 437–442.
4. Ichida, K.; Hosoyamada, M.; Hisatome, I.; Enomoto, A.; Hikita, M.; Endou, H.; Hosoya, T. Clinical and molecular analysis of patients with renal hypouricemia in Japan–influence of URAT1 gene on urinary urate excretion. *J. Am. Soc. Nephrol.* **2004**, 15, 164–173.
5. Komatsuda, A.; Iwamoto, K.; Wakui, H.; Sawada, K.; Yamaguchi, A. Analysis of mutation in the urate transporter 1 (URAT1) gene of Japanese patients with hypouricemia in northern Japan and review of the literature. *Ren. Fail.* **2006**, 28, 223–227.
6. Komoda, F.; Sekine, T.; Inatomi, J.; Enomoto, A.; Endou, H.; Ota, T.; Matsuyama, T., Ogata, T.; Ikeda, M.; Awazu, M.; Muroya, K.; Kamimaki, I.; Igarashi, T. The W258X mutation in SLC22A12 is the predominant cause of Japanese renal hypouricemia. *Pediatr. Nephrol.* **2004**, 19, 728–733.
7. Stiburkova, B.; Ichida, K.; Sebesta, I. Novel homozygous insertion in SLC2A9 gene caused renal hypouricemia. *Mol. Genet. Metab.* **2011**, 102, 430–435.
8. Ichida, K. What lies behind serum urate concentration? Insights from genetic and genomic studies. *Genome Med.* **2009**, 1, 118.1–118.10.
9. Johnson, R.J.; Titte, S.; Cade, J.R.; Rideout, B.A.; Oliver, W.J. Uric acid, evolution and primitive cultures. *Semin. Nephrol.* **2005**, 25, 3–8.
10. Bos, M.J.; Koudstaal, P.J.; Hofman, A.; Witteman, J.C.; Breteler, M.M. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke* **2006**, 37, 1503–1507.

Nucleosides, Nucleotides and Nucleic Acids, 30:1140–1146, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2011.591746

Taylor & Francis
Taylor & Francis Group

STABLE ISOTOPE DILUTION MASS SPECTROMETRIC ASSAY FOR PRPP USING ENZYMATIC PROCEDURES

Y. Shinohara,[1] Y. Suzuki,[1] H. Hasegawa,[1] M. Nakamura,[1] T. Nishiyama,[2] A. Hiratsuka,[2] and K. Ichida[1]

[1]*Department of Pathophysiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*

[2]*Department of Drug Metabolism and Molecular Toxicology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*

□ *5-Phosphoribosyl-1-pyrophosphate (PRPP) is an important regulator of de novo purine synthesis. A method for the measurement of PRPP in erythrocytes was designed, which is based on the determination of [$^{13}C_5$]glutamate derived from [$^{13}C_5$]glutamine following the utilization of PRPP by the action of amidophosphoribosyltransferase. The present study describes a gas chromatographic-mass spectrometric method for determination of [$^{13}C_5$]glutamate using [$^{13}C_2$]glutamate as an internal standard. The methods involved purification by anion-exchange chromatography using a BondElut SAX and derivatization with isobutyl chlorocarbonate in water-methanol-pyridine. Quantitation was performed by selected ion monitoring of the protonated molecular ions in the chemical ionization mode. The intra-day reproducibility in the amounts of [$^{13}C_5$]glutamate determined was in good agreement with the actual amounts added in erythrocytes. A linear relationship was found between the amount of PRPP added and the amount of [$^{13}C_5$]glutamate formed from [$^{13}C_5$]glutamine using amidophosphoribosyltransferase.*

Keywords PRPP; amidophosphoribosyltransferase; GC-MS; glutamate; stable isotope

INTRODUCTION

5-Phosphoribosyl-1-pyrophosphate (PRPP) is a substrate common to several metabolic pathways including the biosynthesis of purine and pyrimidine nucleotides and the salvage pathway for purines. PRPP also serves as both a substrate and an activator of amidophosphoribosyltransferase (ATase), the first and presumed rate-determining enzyme of de novo purine synthesis. Uric acid is the end product of purine metabolism in humans. Hyperuricemia is a risk factor for gout and results from either overproduction or renal underexcretion of uric acid. Elevated intracellular PRPP is the driving

Received 26 April 2011; accepted 24 May 2011.
Address correspondence to Y. Shinohara, Department of Pathophysiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. E-mail: sinohara@toyaku.ac.jp

force for acceleration of uric acid synthesis in two rare inherited enzyme defects resulting in overproduction hyperuricemia, hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency, and PRPP synthetase (PRS) overactivity.[1,2] Elevation of PRPP is a basis for idiopathic overproduction hyperuricemia, but there is no study about the intracellular concentration of PRPP in the patients with idiopathic overproduction hyperuricemia. Erythrocyte PRPP concentrations are markedly increased in two rare inherited enzyme abnormalities, HPRT deficiency[3] and PRS overactivity,[4] resulting in overproduction hyperuricemia. The measurement of erythrocyte PRPP concentrations is, therefore, an important screening procedure in the investigation of patients with idiopathic hyperuricemia.

A number of radioenzymatic assays have been described for the measurement of PRPP in biological samples.[5–8] The basic approach utilizes the conversion of PRPP to a radioactive derivative through its reaction with a ^{3}H or ^{14}C-labeled base under catalysis by the appropriate phosphoribosyltransferase. The methods have involved trapping of evolved $^{14}CO_2$ or separation of radioactive products by various chromatographic techniques. The present study was carried out to develop a gas chromatography-mass spectrometry (GC-MS) method for determination of PRPP in erythrocytes using ATase and stable-isotopically labeled substrate. We used [$^{13}C_5$]glutamine ([$^{13}C_5$]Gln) as the substrate and measured the enzyme product [$^{13}C_5$]glutamate ([$^{13}C_5$]Glu) by GC-MS using [$^{13}C_2$]glutamate ([$^{13}C_2$]Glu) as the internal standard.

$$[^{13}C_5]\text{Gln} + \text{PRPP} \xrightarrow[Mg^{+}]{\text{ATase}} [^{13}C_5]\text{Glu} + \text{phosphoribosylamine} + \text{PPi}$$

MATERIALS AND METHODS

Chemical and Reagent

Glutamine (Gln), glutamate (Glu), and isobutyl chloroformate were purchased from Wako (Osaka, Japan). [$^{13}C_5$]Gln (98 atom%^{13}C) and [$^{13}C_5$]Glu (98 atom%^{13}C) were purchased from Isotec (Miamisburg, OH, USA). [$^{13}C_2$]Glu (99 atom%^{13}C) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). PRPP sodium salt was purchased from Sigma (St. Louis, MO, USA). A strong anion-exchange solid-phase extraction column BondElut SAX (1 mL/100 mg) was purchased from Varian (Harbor City, OH, USA), and 10% hydrogen chloride in methanol (10% HCl/methanol) was purchased from the Tokyo Chemical Industry (Tokyo, Japan). ATase was prepared as described previously.[9] All other chemicals and solvents were of an analytical grade.

Gas Chromatography-Mass Spectrometry With Selected Ion Monitoring (GC-MS-SIM)

GC-MS-SIM measurements were made with a Shimadzu (Kyoto, Japan) QP-2000 quadrupole gas chromatograph-mass spectrometer. A methylsilicone bonded-phase fused-silica capillary column SPB-1 (10 m × 0.25 mm I.D.) with 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) was connected directly to the ion source. Helium was used as the carrier gas. A split-splitless injection system was used with a septum purge flow-rate of 3.0 mL/minute. The initial column temperature was set at 120°C. After the sample injection, it was maintained for 2 minutes and was increased at 20°C/minute to 220°C and maintained there for 1 minute. The mass spectrometer was operated in a chemical ionization (CI) mode with isobutene as the reagent gas. The ion source temperature was 280°C. SIM was performed on the protonated molecular ions at *m/z* 276, 278, 281 for the N(O)-isobutoxycarbonyl methyl ester (iBCME) derivatives of Glu, [$^{13}C_2$]Glu, and [$^{13}C_5$]Glu, respectively.

Sample Preparation for GC-MS-SIM

Venous blood samples were obtained in heparinized tubes from a healthy volunteer. Erythrocytes were isolated by centrifuging at 900 *g* for 15 minutes at 4°C and washed twice with two volumes of ice-cold saline and then packed by centrifuging at 6000 *g* for 10 minutes at 4°C. Lysates were obtained by freezing and thawing the red blood cells twice. The lysates were stored at −80°C until analysis. The frozen lysates were defrosted in a 4°C water bath. To a polypropylene tube (75 × 15 mm ID) were added 0.05 mL of lysate and 0.2 mL of 50 mM Tris-HCl buffer (pH 7.4). After heating for 45 seconds in boiling water, the sample was immediately chilled on ice. Following the addition of [$^{13}C_2$]Glu (5.10 nmol/0.05 mL) as the internal standard, the sample was deproteinized with 2 mL of methanol. After centrifugation at 900 *g* for 5 minutes, the supernatant was applied to a BondElut SAX cartridge, which was prewashed and activated with 1 mL of methanol, 1 mL of water, 2 mL of 1M NaOH, 4 mL of water, 2 mL of acetic acid, and 4 mL of water. The cartridge was washed with 1 mL of 80% methanol/water, and then the glutamate species were eluted with 0.5 mL of 10% HCl/methanol. After removal of the solvent under a stream of nitrogen at 40°C, the residue was dissolved in 0.5 mL of a mixture of water-methanol-pyridine (30:16:4, v/v), and 0.02 mL of isobutyl chloroformate was added. The mixture was shaken for 10 seconds on a vortex mixer. The sample was extracted with 1 mL of chloroform. After evaporating to dryness under a stream of nitrogen, the residue was dissolved in 0.05 mL of ethyl acetate. A volume of 0.2–1.0 μL of the solution was subjected to GC-MS-SIM.

Calibration Curves and Quantitation

To each of a series of standards containing known amounts of Glu (0.51–51.02 nmol) and [$^{13}C_5$]Glu (0.05–5.10 nmol), 5.10 nmol of [$^{13}C_2$]Glu was added as the internal standard. Each sample was prepared in triplicate. The samples were derivatized and analyzed as described above. After correcting the peak-area values using the values of relative contributions by the equations described previously,[10] the peak-area ratios (*m/z* 276 versus *m/z* 278 for Glu and *m/z* 281 versus *m/z* 278 for [$^{13}C_5$]Glu) were determined. The curves were obtained by an unweighted least-squares linear fitting of the peak-area ratios versus the amounts added to each sample. Erythrocyte concentrations were calculated by comparing the peak-area ratios obtained from the unknown samples with those obtained from standard mixtures.

Accuracy and Precision

Accuracy and precision were determined by assaying four preparations of 0.05 mL portions of lysate spiked with [$^{13}C_5$]Glu (0.51, 1.02, 2.55, and 5.10 nmol). Each sample was prepared in triplicate. Following the addition of [$^{13}C_2$]Glu (5.10 nmol) as the internal standard, the samples were derivatized and measured as described above.

Enzymatic Procedure

A reaction mixture consisting of [$^{13}C_5$]Gln (250 nmol), $MgCl_2$ (5 μmol) and standard PRPP (0.49-47.2 nmol) in 0.45 mL of 50 mM Tris-HCl buffer (pH 7.4) was added in a polypropylene tube (75 × 15 mm ID). The reaction was initiated by the addition of 0.05 mL of ATase solution (0.2 mg protein/mL). After incubation at 37°C for 10 minutes, the reaction was stopped by the addition of 2 mL of ice-cold methanol. Following the addition of [$^{13}C_2$]Glu (5.10 nmol/0.05 mL) as the internal standard, the sample was centrifuged at 900 *g* for 5 minutes. The supernatant was purified, derivatized, and analyzed as described above.

RESULTS AND DISCUSSION

Several derivatization methods have been used to measure glutamate by GC-MS.[11–13] Acid-catalyzed esterification of the carboxylic moiety of amino acids is commonly employed, but the derivatization causes deamidation of glutamine to glutamate.[14] We have previously used the N(O,S)-alkoxycarbonyl alkyl ester derivatives for a simultaneous quantitation of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine,

and homocysteine in plasma by GC-MS using [^{13}C]methionine and [$^{13}C_2$]homocystine as analytical standards.[15] A one-pot derivatization of Glu species to the isobutyloxycarbonyl methyl ester (iBCME) was achieved by the reaction with isobutyl chloroformate in a solution of water-methanol-pyridine. The reaction was completed in seconds at room temperature. The iBCME derivative of Glu showed good chromatographic behavior and eluted at 5.1 minutes. Since iBCME derivatives of [$^{13}C_5$]Glu, [$^{13}C_2$]Glu, and Glu gave strong protonated molecular ions $[M+H]^+$ at m/z 281, 278, 276 in the chemical ionization mass spectra, we have chosen these ions for selected ion monitoring. Reference Gln with a certified purity of 99% was derivatized and subjected to GC-MS-SIM. The Glu detected was 0.88 ± 0.09% ($n = 3$), indicating almost no transformation of Gln to Glu.

Ion-exchange chromatography provides a simple method for extracting amino acids from biological fluids. In the present method, we used an anion-exchange cartridge column BondElut SAX to extract Glu from the reaction mixture prior to GC-MS analysis. The recovery of [$^{13}C_5$]Glu was higher than 90%. In contrast, [$^{13}C_5$]Gln was not retained on the column and eluted almost immediately. The use of the iBCME derivatization and the anion-exchange column made it possible to measure Glu in samples containing Gln without degrading the compound of interest.

Calibration curves were prepared from a series of samples containing varying amounts of [$^{13}C_5$]Glu in the range of 0.05–5.10 nmol and Glu in the range of 5.10–51.0 nmol. When the peak-area ratios were plotted against the molar ratios, good correlations were found ($R = 1.000$). The accuracy and precision of the assays were determined by spiking 50 μL of red blood cell lysates with 0.51–5.10 nmol of [$^{13}C_5$]Glu. The boiling step served to inactivate enzymes, which catalyze Glu generation and utilization in erythrocytes. There was no interference in the vicinity of the peaks analyzed in the SIM. The variance of intra-day precisions of the assay of [$^{13}C_5$]Glu was less than 4%, while the accuracy of the relative errors was −0.92% to 1.27%. The erythrocyte concentration of Glu was 254 ± 7 nmol/mL in a healthy male. The data correspond to previous results in healthy subjects.[16]

The GC-MS method was applied for determination of PRPP. [$^{13}C_5$]Gln was incubated with different concentrations of PRPP in the presence of ATase and the [$^{13}C_5$]Glu formed was determined by GC-MS-SIM. There was a linear relationship between the amount of [$^{13}C_5$]Glu formed and the amount of PRPP added from 0.49 to 47.2 nmol (Figure 1).

In conclusion, a novel GC-MS method has been developed for the assay of PRPP by the determination of [$^{13}C_5$]Glu derived from [$^{13}C_5$]Gln catalyzed by ATase.

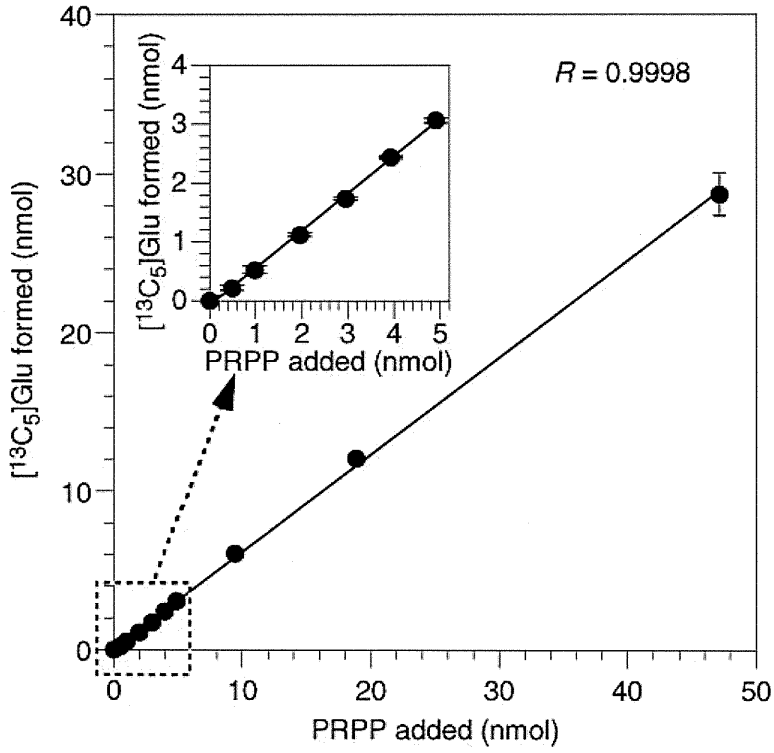

FIGURE 1 Relation between PRPP added and [$^{13}C_5$]Glu formed. All values represent the mean ± SD of three independent experiments.

REFERENCES

1. Becker, M.A. Patterns of phosphoribosylpyrophosphate and ribose-5-phosphate concentration and generation in fibroblasts from patients with gout and purine overproduction. *J. Clin. Invest.* **1976**, 57, 308–318.
2. Becker, M.A. Hyperuricemia and gout, in *The Metabolic & Molecular Bases of Inherited Disease*, 8th ed., ed. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, McGraw-Hill, New York, 2001, pp. 2513–2535.
3. Fox, I.H.; Kelley, W.N. Phosphoribosylpyrophosphate in men: biochemical and clinical significance. *Ann. Int. Med.* **1971**, 74, 424–433.
4. Becker, M.A.; Meyer, L.J.; Wood, A.W.; Seegmiller, J.E. Purine overproduction in man associated with increased phosphoribosylpyrophosphate synthetase activity. *Science* **1973**, 179, 1123–1126.
5. Becker, M.A.; Meyer, L.J.; Seegmiller, J.E. Gout with purine overproduction due to increased phosphoribosylpyrophosphate synthetase activity. *Am. J. Med.* **1973**, 55, 232–242.
6. Tax, W.J.M.; Veerkamp, J.H. A simple and sensitive method for estimating the concentration and synthesis of 5-phosphoribosyl 1-pyrophosphate in red blood cells. *Clin. Chim. Acta* **1977**, 78, 209–216.
7. Ipata, P.L.; Mura, U.; Camici, M.; Giovannitti, M.P. A coupled optical assay for adenine phosphoribosyltransferase and its extension for the spectrometric and radioenzymatic determination of 5-phosphoribosyl-1-pyrophosphate in mixtures and in tissue extracts. *Anal. Biochem.* **1987**, 164, 411–417.
8. King, M.T.; Passonneau, J.V.; Veech, R.L. Radiometric measurement of phosphoribosylpyrophosphate and ribose 5-phosphate by enzymatic procedures. *Anal. Biochem.* **1990**, 187, 179–186.
9. Mantsala, P.; Zalkin, H. Glutamine nucleotide sequence of *Saccharomyces cerevisiae ADE4* encoding phosphoribosylpyrophosphate amidotransferase. *J. Biol. Chem.* **1984**, 259, 8478–8484.
10. Hasegawa, H.; Shinohara, Y.; Hashimoto, T.; Matsuda, R.; Hayashi, Y. Prediction of measurement uncertainty isotope dilution gas chromatography/mass spectrometry. *J. Chromatogr. A* **2006**, 1136, 226–230.
11. Darmaun, D.; Manary, M.J.; Matthews, D.E. A method for measuring both glutamine and glutamate levels and stable isotopic enrichments. *Anal. Biochem.* **1985**, 147, 92–102.

12. Kapetanovic, I.M.; Yonekawa, W.D.; Kupferberg, H.J. Determination of 4-aminobutyric acid, aspartate, glutamate and glutamine and their ^{13}C stable-isotopic enrichment in brain tissue by gas chromatography-mass spectrometry. *J. Chromatogr.* **1987**, 414, 265–274.
13. Anderson, L.W.; Zaharevitz, D.W.; Strong, J.M. Glutamine and glutamate: automated quantification and isotopic enrichments by gas chromatography/mass spectrometry. *Anal. Biochem.* **1987**, 163, 358–368.
14. Montigon, F.; Boza, J.J.; Fay, L.B. Determination of ^{13}C- and ^{15}N-enrichment of glutamine by gas chromatography/mass spectrometry and gas chromatography/combustion/isotope ratio mass spectrometry after N(O,S)-ethoxycarbonyl ethyl ester derivatisation. *Rapid Commun. Mass Spectrom.* **2001**, 15, 116–123.
15. Shinohara, Y.; Hasegawa, H.; Kaneko, T.; Tamura, Y.; Hashimoto, T.; Ichida, K. Analysis of [$^2H_7$]methionine, [$^2H_4$]methionine, methionine, [$^2H_4$]homocysteine and homocysteine in plasma gas chromatography-mass spectrometry to follow the fate of administered [$^2H_7$]methionine. *J. Chromatogr. B* **2010**, 878, 417–422.
16. Hagenfeldt, L.; Arvidsson, A. The distribution of amino acids between plasma and erythrocytes. *Clin. Chim. Acta* **1980**, 100, 133–141.

Nucleosides, Nucleotides and Nucleic Acids, 30:1001–1010, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2011.633955

Taylor & Francis
Taylor & Francis Group

GOUT AND HYPERURICEMIA IN JAPAN: PERSPECTIVES FOR INTERNATIONAL RESEARCH ON PURINES AND PYRIMIDINES IN MAN

Tatsuo Hosoya,[1,2] Iwao Ohno,[2] Kimiyoshi Ichida,[3] and Godefridus J. Peters[4]
[1]*Chairman, Japanese Society of Gout and Nucleic Acid Metabolism*
[2]*Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan*
[3]*Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*
[4]*Department of Medical Oncology, VU University Medical Center, Amsterdam, Netherlands*

□ *One of the best-known disorders in purine metabolism is accumulation of uric acid leading to gout. Gout is a lifestyle disease, which was nicely illustrated in the joint symposium of the Japanese Society of Gout and Nucleic Acid Metabolism and of the Purine and Pyrimidine Society held in February 2011 in Tokyo, Japan. The westernization of the Japanese diet led to an increase in hyperuricemia in Japanese, which subsequently boosted research in this field, as illustrated in this symposium. As a consequence, Japanese nucleotide research also expanded, leading to the development of not only new drugs for treatment of gout, but also for other diseases such as cancer, viral infections, and cardiovascular diseases. The research on inborn errors led to the identification of various genetic polymorphisms affecting drug metabolism, revealing differences between Asians and non-Asians. Such genetic differences may also affect the enzymatic properties of an enzyme or a transporter, necessitating specific inhibitors. This knowledge will help to introduce personalization of treatment. In this symposium, the interaction between various specialties formed an excellent basis for translational research between these specialties but also from the bench to the clinic.*

Keywords Gout; hyperuricemia; febuxostat; transporters; ABCG2/BCRP; antimetabolites; adenosine; deoxycytidine kinase

INTRODUCTION

The 14th International Symposium on Purine and Pyrimidine Metabolism in Man (PP11) was held from February 18 (Friday) to 21 (Monday), 2011, at Keio Plaza Hotel in Tokyo, Japan. This was the second time that the international symposium took place in Japan, the previous occasion being

Received 6 October 2011; accepted 18 October 2011.
Address correspondence to Godefridus J. Peters, Department of Medical Oncology, VU University Medical Center, De Boelelaan 1117, Amsterdam 1081 HV, Netherlands. E-mail: gj.peters@vumc.nl

the 6th International Symposium on Purine and Pyrimidine Metabolism in Man, in Hakone, in 1988. On the present occasion, the symposium was held jointly with the 44th Annual Meeting of the Japanese Society of Gout and Nucleic Acid Metabolism, which organized a special program on the first day of PP11 (February 18). In relation to the background to PP11, the history of the Japanese Society of Gout and Nucleic Acid Metabolism, and an overview of the 14th International Symposium on Purine and Pyrimidine Metabolism in Man are presented here.

HISTORY OF RESEARCH ON GOUT AND HYPERURICEMIA IN JAPAN

In Japan, gout was a rare disease until 1960. Up to 1959, only 83 cases had been reported since the first case was described by Kondo in 1898. The number of gout patients, however, has rapidly increased since 1960. According to a survey by Nishioka et al.,[1] from 1970 to 1973, among the residents of Toshijima, a Japanese island, the prevalence rate of gout was 0.4%. According to another survey from 1969 to 1974, by Shichikawa,[2] the prevalence rate in Japan was 0.3%. Important factors in the increase in the number of gout patients include changes in Japanese dietary habits due to westernization and increased alcohol consumption.

In addition to gouty arthritis, the prevalence of hyperuricemia, the basic pathological condition in gout, has increased as well. According to the results of two recent large surveys in Japan, the frequency of hyperuricemia in adult males is 21.5% and 26.2%, respectively.[3,4] Comparing the frequencies between age ranges, the highest frequencies were in people in their 30s and 40s, and the frequency in men in their 30s was as high as 30% (Figure 1).[3]

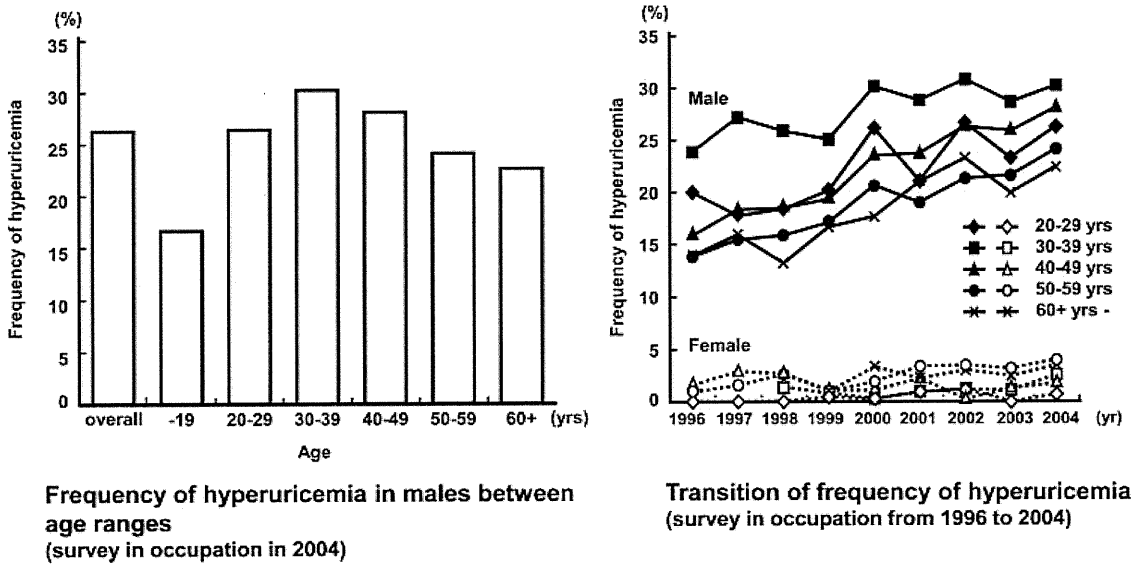

FIGURE 1 Trends in hyperuricemia in Japan (modified from Tomita and Mizuno[3]).

In connection with the above surveys, research in this field increased considerably, leading to the inauguration of the Japanese Society for Uric Acid, Purine and Pyrimidine by Mikanagi et al.[5] in 1977. Initially focusing on uric-acid-related research, the society's research field has gradually expanded to cover purine and pyrimidine metabolism, as well as hyperuricemia and gout, and it was established as the Japanese Association of Purine and Pyrimidine Metabolism in 1989. In 1999, the name was changed to the Japanese Society of Gout and Nucleic Acid Metabolism, with the aim of making the field of research more comprehensive by including a disease name in the title and thus stimulating researchers working in related fields.

In 1988, Dr. Mikanagi organized the 6th International Symposium on Purine and Pyrimidine Metabolism in Man in Japan.[5] Since then, considerable progress has been made with research in this field in Japan, with important studies including the pathophysiology of phosphoribosylpyrophosphate synthetase superactivity, genetic analysis of adenine phosphoribosyl transferase deficiency and hypoxanthine-guanine phosphoribosyl transferase deficiency in Japanese people, reaction mechanism analysis of xanthine oxidoreductase (xanthine dehydrogenase and xanthine oxidase), and identification of the genes responsible for xanthinuria. Furthermore, many Japanese researchers have contributed to research on urate transporters, such as URAT1. In addition, the clinical characteristics of renal hypouricemia that are frequent in Japanese people have been clarified, and the responsible genes, *URAT1* and *GLUT9*, also known as *URATv1*, have been identified. Many important discoveries have been made in Japan, including identifying *ABCG2*, also known as *BCRP* (breast cancer resistance protein) as a causative gene in gout. In addition, new xanthine oxidoreductase inhibitors for urate-lowering therapy have been developed in Japan, 40 years after the development of allopurinol, and a large-scale prospective study on controlling hyperuricemia with respect to renal disorders and cardiovascular accidents is now planned.

PRESENTATIONS AT THE JAPANESE SOCIETY OF GOUT AND NUCLEIC ACID METABOLISM

The 44th Annual Meeting of the Japanese Society of Gout and Nucleic Acid Metabolism was held jointly with the 14th International Symposium on Purine and Pyrimidine Metabolism in Man. The Japanese Society of Gout and Nucleic Acid Metabolism had 245 participants and 43 regular submissions over two days, which was approximately the same as the previous year. The conference included several important lectures, such as an educational lecture, "Urate transporters: Recent progress"; a special lecture, "Medical science and treatment in the 21st century"; a congress award lecture, "Research on hyperuricemia based on ATP hypercatabolism"; a symposium, "Progress in gout research"; and a joint symposium with PP11, "Current therapy for

TABLE 1 Sessions of the 44th Annual Meeting of Japanese Society of Gout and Nucleic Acid Metabolism

Session title	Number of presentations
1 Urate-lowering therapy/serum uric acid level	5
2 Urate transporter	7
3 Insulin resistance/food and uric acid	7
4 Purine and pyrimidine metabolism	4
5 Hypertension and hyperuricemia	7
6 Chronic kidney disease/gout	7
7 Genetic investigation of familial juvenile hyperuricemic nephropathy and gout	6

gout and hyperuricemia." The sessions were divided into seven categories by subject area (Table 1), with 14 presentations on basic research, 20 on clinical research, and the other presentations on treating patients using various fundamental medical procedures. The most widely discussed subject area was basic research on urate transporters. Insulin resistance and purine metabolism was the second most covered area. In the clinical research field, reports of drugs lowering serum uric acid and the relationship between serum uric acid and insulin resistance, hypertension, and chronic kidney disease were major topics. In the presentations in the translational research field, genetic analyses of patients with familial juvenile hyperuricemic nephropathy and the relationships between single nucleotide polymorphisms (SNPs) and serum uric acid were reported. The conference can be characterized as covering a variety of themes, from basic to clinical research, and participants had the opportunity to access a wide variety of information in the field of gout and nucleic acid metabolism and to discuss various issues with experts.

In summary, drugs that reduce the uric acid level, the relationship between hyperuricemia and metabolic syndrome and chronic kidney disease, and transporter-related studies were highlighted.

14th INTERNATIONAL SYMPOSIUM ON PURINE AND PYRIMIDINE METABOLISM IN MAN (PP11)

PP11 was held at the Keio Plaza Hotel in Tokyo, Japan. The number of participants was 149 in total, with 53 (one third) from overseas and 96 from Japan. The symposium included researchers from 16 overseas countries, including the United States and Australia, and 10 European countries, two East Asian countries, and two Middle Eastern countries. On February 18, the first day of the Symposium, a joint symposium was co-hosted with the 44th Annual Meeting of the Japanese Society of Gout and Nucleic Acid Metabolism. Following the first day, international oral and poster sessions were held on February 19 and 20.

TABLE 2 Main program of 14th International Symposium on Purine and Pyrimidine Metabolism in Man, PP11

Symposium	Title	Chairs	
1. Joint symposium:	Current therapy for gout and hyperuricemia in the world	N. Kamatani	M.A. Becker
2. Plenary sessions			
Session 1:	Role of uric acid in the pathogenesis of cardiovascular and renal diseases	I. Hisatome	M.A. Lanaspa
Session 2:	Clinical topics of gout and hyperuricemia	H. Yamanaka	S.-Y. Chen
Session 3:	Association between genomic variation and urate metabolism	N. Kamatani	C. Grieger
Session 4:	Recent advances in purine/pyrimidine enzyme regulation, enzyme and regulation of metabolism	T. Morisaki	R.T. Smolenski
Session 5:	Purines/pyrimidines and cancer	T. Ueda	G.J. Peters
Session 6:	Protein structure and catalytic mechanism	L. Okamoto	S. Eriksson
Session 7:	Inborn errors of metabolism/molecular mechanisms of disease	M. Itakura	V. Micheli
Session 8:	Transport of purine and pyrimidine	H. Sakurai	S.K. Nigam
3. Poster session			

The last day of the symposium, February 21, was dedicated to sightseeing, which is one of the traditions of PP symposia, involving a half-day bus tour around Tokyo. The overall number of presentations was 125, consisting of six joint symposia, 42 oral sessions, and 77 poster sessions (Table 2).

Because the symposium was held together with that of the Japanese Society of Gout and Nucleic Acid Metabolism, the first three sessions were focused on novel aspects in gout research and treatment. Various cultural aspects lead to differences in treatment of gout in Asian countries (e.g., China, Taiwan, Korea, Japan) compared with the United States and Europe (with key presentations by Professors Michael Becker and Naoyuki Kamatani). These differences are also partly related to the fact that gouty patients in the United States usually present in a more advanced stage requiring more aggressive treatment. The introduction of febuxostat, which was originally approved in the European Union in 2008, in the United States and Korea in 2009, and in Japan in 2011, has extended the possibilities to treat this disease. In Japan, a lower starting dose (10 mg/day) is underlined with a step-up possibility to 60 mg/day for preventing gouty attack at the medication starting period. Meanwhile, common practice in Europe and the United States is 80–120 mg/day.

Uric acid, which is the endproduct of purine degradation in primates, not only causes gout when precipitated in the joints, but also causes a health risk in cardiovascular disease and chronic kidney disease. Under hyperuricemia conditions, uric acid may impair endothelial-dependent vasodilatation, contributing to cardiovascular risks. Hyperuricemia is a poor prognostic factor of renal function and may result in renal damage, which can be prevented by

allopurinol treatment. Although it was believed that the metabolic syndrome might lead to hyperuricemia, evidence is accumulating that hyperuricemia is important in the development of metabolic syndrome, which is characterized by hyperglycemia, dyslipidemia, and hypertension, accompanied by visceral fat accumulation and insulin resistance. Unfortunately, the prevalence of these symptoms also increases in children with obesity. Obesity may also lead to liver disease due to high fat and fructose intake, whereas alcohol intake is another important risk factor, because this leads to ATP degradation. According to Bruce Cronstein's data, this may lead to accumulation of extracellular adenosine activating adenosine receptors A1 and A2, which regulate many physiological processes, such as ataxia and somnolence. The third session in this area summarized novel mechanisms in the pathogenesis of gout, focusing on transporters and genomic research, the latter leading to identification of polymorphisms in these transporters. The Japanese Center for Genomic Research (RIKEN) has initiated a large GWAS (genome-wide association study) together with other related centers worldwide, which led to the identification of novel risk factors, but most importantly to the identification of patients at risk. GWAS may also help to identify targets for new drugs (e.g., GWAS identified GLUT9/URATv1 and ABCG2/BCRP, and others), or novel targets for existing drugs (genome-wide drug discovery, GWDD). The rest of this session focused on the role of transporters, ABCG2/BCRP, the urate/anion exchanger SLC22A12 (URAT1), and another urate reabsorptive transporter SLC2A9 (GLUT9/URATv1). Novel models were presented on the role of these transporters. Furthermore, a number of relatively frequent polymorphisms such as the Q141K in ABCG2/BCRP as well as some in various SLC transporters are associated with increased risk. The wider importance of these findings is illustrated in another field, cancer treatment, in which Q141K was associated with increased toxicity of treatment with some tyrosine kinase inhibitors. In the poster session, detailed studies on the function of the transporters in the pathogenesis of gout were presented, as well as the use of knockout mice for these and other transporters. One of the posters on ABCG2/BCRP received a poster prize.

In the fourth session, novel aspects on enzyme regulation and metabolism were discussed, with a focus on AMP deaminase (AMPD), which is believed to play a role in cardiovascular disease. A mouse deficient in the isozyme AMPD3 showed aberrant ATP concentrations and may be a good model for human AMPD3 deficiency. The latter may be beneficial under ischemic conditions due to an increase in ATP. Also, other enzymes in purine metabolism can affect ATP levels, such as phosphoribosylpyrophosphate synthetase (PRPP synthetase). Superactivity of this enzyme is associated with gout, whereas a deficiency causes several neurological effects and leads to early death. Degradation enzymes may also play a role in nucleotide homeostasis, as has been found for 5′-nucleotidases and the pyrimidine degradation enzyme, dihydropyrimidine dehydrogenase.

The second half of the symposium was dedicated to metabolism and transport of purines and pyrimidines and their analogs in various diseases, and how inborn errors may affect life and the efficacy of various widely used drugs. The fifth session focused on treatment of cancer, in which various novel treatment modalities were discussed, such as optimalization of drug uptake and metabolism by using various prodrug approaches (liposomes, nanoparticles, lipophilic prodrugs, gene therapy), pharmacological optimalization of nucleoside activation or novel modalities such as miRNA (miR-21) inhibition, leading to increased cell death by gemcitabine. The effect of antimetabolite treatment on the proteome was discussed, as well as the role of hnRNP-impaired splicing of thymidine phosphorylase on drug resistance. Related to this was the sixth session, which focused on protein structure in relation to catalytic mechanisms, which obviously plays a role in drug metabolism and targeting. Several classical enzymes were discussed in this respect: ribonucleotide reductase, thymidylate synthase, xanthine oxidoreductase, and thymidine kinase 2. Novel screening approaches and site-directed mutations were used to identify active sites and develop new drugs.

The study of inborn errors was the impetus for the initiation of the Purine and Pyrimidine symposia. Because Dr. Anne Simmonds played a key role in this research, the seventh session was held in her honor and various inborn errors were discussed by previous collaborators, with emphasis on the impact of Dr. Simmonds on the research field and the careers of her collaborators. Subjects being discussed included the prototype of an important enzyme in inborn errors research, hypoxanthine guanine phosphoribosyltransferase (HGPRT), as well as 5′-nucleotidases, uromodulin, and dihydropyrimidine dehydrogenase. Often neglected but not less important is the nicotinamide metabolism. In the meeting, an important lecture focused on the costs of novel molecular testing. Costs are dependent on how screening is driven, e.g., the phenotype, genotype, metabolites, or mutations. The next step would be how to use these diagnostic tests for intervention with the disease.

In the last session, one of the most important aspects in pathophysiology was discussed, uptake and efflux of purines and pyrimidines and their analogs. Recently, the importance of transport in hyperuricemia was underlined by the characterization of novel transporters and novel aspects of well-known transporters in relation to uric acid accumulation. Urate transporters on the membrane of that renal proximal tubular cell that have been identified are shown in Figure 2.[6] In addition to the transporters described in this figure, the role of the urate efflux transporter NPT4 recently identified in the renal proximal tubule was also discussed. The ATP binding cassette (ABC) transporter ABCG2/BCRP does not only mediate renal secretion of urate, but has also been characterized extensively for its role in cancer drug resistance for a number of unrelated drugs, which include antifolates and some nucleoside analogs.[7] ABCG2/BCRP also plays a role

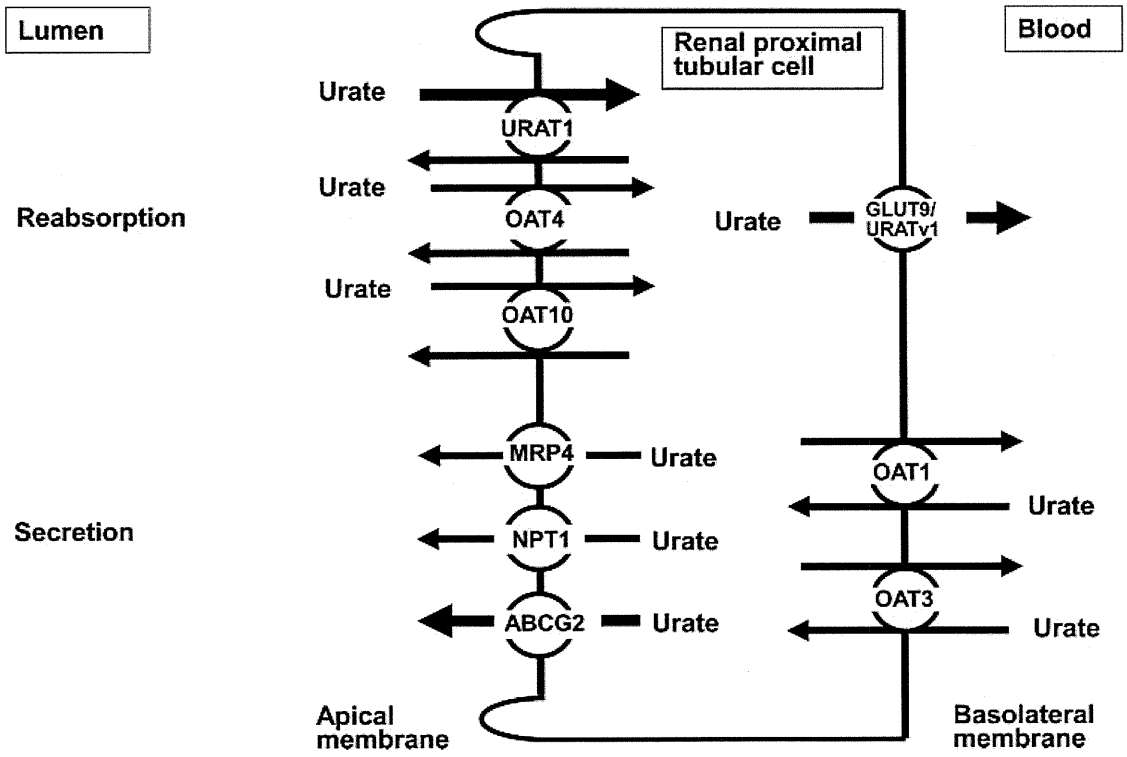

FIGURE 2 Urate transporters at the proximal tubule (modified from Ichida[6]); Abbreviations: ABCG2, ATP-binding cassette, subfamily G, member 2; GLUT, glucose transporter; MRP4, multidrug resistance protein 4; NPT1, sodium-dependent phosphate transport protein 1; OAT, organic anion transporter; URAT1: urate transporter 1; URATv1: voltage-driven urate transporter 1; Note: Meta-analysis of genome-wide association studies showed a relationship between SLC16A9, encoding monocarboxylate transporter 9 (MCT9), and serum urate concentrations, but the localization and function of MCT9 remain unknown. Thus, MCT9 is not depicted in this figure.

in gut epithelium transport of many drugs as well as in the blood–brain barrier.

Although transport of nucleosides and nucleoside analogs has been characterized extensively, the uptake of nucleobases is not well understood. Next to passive diffusion, other transporters were described, which share characteristics with transporters in other organisms, such as bacteria, or have completely different functions, such as vitamin transport. Although the action of a drug only starts after being taken up, this aspect was only discussed in the last presentation, focusing on the differences between equilibrative and concentrative transporters (ENT and CNT), their localization, and their activation.

In the 77 poster presentations, many subjects discussed in the oral presentations were presented in more detail, including the description of ethnic variants of HGPRT, the role of various urate transporters, especially BCRP, and the efficacy of several urate-lowering drugs, such as febuxostat, topiroxostat, and other novel compounds, as well as the role of uric acid in cardiovascular disease. Various presentations focused on the role of purine and pyrimidine metabolism on immune functions and on drugs used to

modulate immune response. An important current diagnostic tool is the identification of relevant genetic polymorphisms, which are not only associated with certain inborn errors but can have profound effects on drug metabolism. This type of research also elucidated the association of certain SNPs with enzyme function and drugs targeted to this specific aberration. Two of these posters were honored with a poster prize. The poster presentations that were selected for the poster prize are:

1. Arenas Hernandez, Monica (Purine Research Laboratory, GSTS Pathology, Guy's and St Thomas' Hospitals, London, United Kingdom): The use of thioguanine nucleotide monitoring to optimize therapy in inflammatory bowel disease.
2. Nakayama, Akiyoshi (Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, Saitama, Japan): ABCG2 is a high-capacity urate transporter and its genetic impairment increases serum uric acid levels in humans.

CONCLUSION

The joint meeting of the Japanese and international societies enabled participants to discuss in detail various novel aspects on gout, with the identification of ABCG2/BCRP as an intriguing transporter in urate homeostasis and the beneficial aspects of novel xanthine oxidase inhibitors, especially febuxostat. The strength of this meeting is also illustrated by its excellent translational environment for scientists from different clinical fields, such as rheumatologists, immunologists, virologists, oncologists, and cardiologists, as well as more basically oriented scientists in biochemistry, molecular biology, genetics, and cell biology. This is a unique property of this meeting, because most current meetings are very specialized in specific diseases or technology. This mix of specialists also recognized the importance of purines and pyrimidines in many diseases, wherein purine and pyrimidine analogs belong to the most successful class of drugs used for treatment of various malignancies and cardiovascular and viral diseases. Furthermore, pyrimidines often form the backbone of various classes of novel types of drugs, such as various tyrosine kinase inhibitors.

REFERENCES

1. Nishioka, K.; Mikanagi, K.; Hirose, K. Clinical study of gout and hyperuricemia. (1) Epidemiological study on the development of gout [in Japanese]. *Ryumachi.* **1974**, 14, 95–105.
2. Shichikawa, K. Epidemiology of gout [in Japanese]. *J. Adult Dis.* **1975**, 5, 331–337.
3. Tomita, M.; Mizuno, S. Does hyperuricemia increase? [in Japanese]. *Gout Nucleic Acid Metab.* **2006**, 30, 1–5.
4. Fujimori, S.; Itoh, H.; Kato, K.; Watanabe, H.; Matsuura, H.; Ogata, N.; Yamanouchi, T. The frequency of hyperuricemia and gout may not increase in Japan [in Japanese]. *Gout Nucleic Acid Metab.* **2006**, 30, 13–19.

5. Mikanagi, K.; Nishioka, K.; Kelley, W.N. (eds). Purine and Pyrimidine Metabolism in Man VI, Parts A and B, *Adv. Exp. Med. Biol.*, **1989**, 253A/B, Plenum Publishing, New York.
6. Ichida, K. What lies behind serum urate concentration? Insights from genetic and genomic studies. *Genome Med.* **2009**, 1, 118.
7. Lemos, C.; Jansen, G.; Peters, G.J. Drug transporters: recent advances concerning BCRP and tyrosine kinase inhibitors. *Brit. J. Cancer* **2008**, 98, 857–862.

Nucleosides, Nucleotides and Nucleic Acids, 30:1091–1097, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2011.633953

Taylor & Francis
Taylor & Francis Group

ABCG2 IS A HIGH-CAPACITY URATE TRANSPORTER AND ITS GENETIC IMPAIRMENT INCREASES SERUM URIC ACID LEVELS IN HUMANS

Akiyoshi Nakayama,[1] Hirotaka Matsuo,[1] Tappei Takada,[2] Kimiyoshi Ichida,[3,4] Takahiro Nakamura,[5,6] Yuki Ikebuchi,[2] Kousei Ito,[2] Tatsuo Hosoya,[4] Yoshikatsu Kanai,[7] Hiroshi Suzuki,[2] and Nariyoshi Shinomiya[1]

[1]*Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan*
[2]*Department of Pharmacy, University of Tokyo Hospital, Faculty of Medicine, University of Tokyo, Tokyo, Japan*
[3]*Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*
[4]*Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan*
[5]*Laboratory for Mathematics, Premedical Course, National Defense Medical College, Tokorozawa, Saitama, Japan*
[6]*Laboratory for Statistical Analysis, Center for Genomic Medicine, Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan*
[7]*Department of Pharmacology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan*

□ *The ATP-binding cassette, subfamily G, member 2 (ABCG2/BCRP) gene encodes a well-known transporter, which exports various substrates including nucleotide analogs such as 3′-azido-3′-deoxythymidine (AZT). ABCG2 is also located in a gout-susceptibility locus (MIM 138900) on chromosome 4q, and has recently been identified by genome-wide association studies to relate to serum uric acid (SUA) and gout. Becuase urate is structurally similar to nucleotide analogs, we hypothesized that ABCG2 might be a urate exporter. To demonstrate our hypothesis, transport assays were performed with membrane vesicles prepared from ABCG2-overexpressing cells. Transport of*

Received 29 May 2011; accepted 17 October 2011.
We would like to thank all the patients and healthy volunteers involved in this study.
The authors are also grateful to C. Okada, Y. Kawamura, and Y. Utsumi for technical support.
This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Defense of Japan, the Ministry of Health, Labour and Welfare of Japan, the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, the Uehara Memorial Foundation, the Takeda Science Foundation, and the Gout Research Foundation of Japan.
This is a proceeding of the poster presentation made at the 14th International Symposium on Purine and Pyrimidine Metabolism in Man, PP11, in Tokyo, Japan (February 18–21, 2011).
Address correspondence to Hirotaka Matsuo, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. E-mail: hmatsuo@ndmc.ac.jp