

Original Paper

# Variation of Urate Transport in the Nephrons in Subtypes of Hyperuricemia

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## Key Words

Urate transport in nephrons · Urate secretion · Postsecretory urate reabsorption · Urinary urate excretion · Urate underexcretion · Subtypes of hyperuricemia · Benzbromarone · Benzbromarone-loading urate clearance test

## Abstract

**Background:** Hyperuricemia cases (HU) can be classified into four subgroups by combining the two main causes of hyperuricemia, i.e. urate underexcretion and overproduction. These subgroups are as follows: underexcretion-type cases (UE); overproduction-type cases (OP); combined-type cases, and normal-type cases. Since urinary urate excretion (Uua) and urate clearance differ significantly between UE and OP, urate transport in the nephrons and the intratubular urate contents might also differ. Such differences might help clarify the pathophysiology of urate underexcretion in subgroups of hyperuricemia, and thus reveal its underlying mechanisms. **Methods:** Urate transport coefficients in each subtype of HU were determined employing the previously reported benzbromarone-loading urate clearance tests. The subtype cases of HU were plotted on a graph of urate transport coefficients versus Uua as coordinates. The characteristic features in the distribution of subtype cases on graphs were analyzed in relation to Uua. **Results:** The mean ( $\pm$ standard error) tubular secretion rate (TSR) in the UE ( $48.7 \pm 1.7$  ml/min) was significantly lower and the postsecretory urate reabsorption rate ( $R_2$ ) in the UE ( $0.904 \pm 0.004$ ) was significantly higher than those in the normal controls ( $78.0 \pm 2.1$  ml/min and  $0.877 \pm 0.003$ ) or the OP ( $61.1 \pm 3.2$  ml/min and  $0.861 \pm 0.009$ ). Decrements of TSR and increments of  $R_2$  in the UE were largest in the subtypes of the HU, in terms of case numbers and the deviation rate of the group. Conversely, decrements of TSR and increments of  $R_2$  were smallest in the OP. A significant correlation was identified between TSR and Uua ( $r = 0.345$ ,  $p < 0.0001$ ), and a significant negative correlation was also found between  $R_2$  and Uua ( $r = -0.393$ ,  $p < 0.0001$ ). **Conclusion:** In the UE, hyperuricemia is induced mainly

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by urate underexcretion, which results from the combination of two main causes in urate transporters of the nephron: significantly lower TSR and significantly higher  $R_2$ . Neither of these was observed in OP. Differences in urate transporters in subtypes of the HU might be important not only for understanding the pathophysiology and mechanisms of urate underexcretion and hyperuricemia, but also for providing a strategic therapy for hyperuricemia.

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## Introduction

Hyperuricemia is induced by two main abnormalities in urate metabolism, namely urate underexcretion and urate overproduction [1–5]. In hyperuricemia cases (HU), underexcretion-type cases (UE), which are characterized by significantly lower urate clearance ( $C_{ua}$ ) and overproduction-type cases (OP), which are characterized by higher urinary urate excretion ( $U_{ua}$ ), can be classified by the  $C_{ua}$  test [5, 6]. Since urate is mainly excreted from the nephrons, considerable differences might be found in urate transport in the nephrons between UE and OP, reflecting the differences in urate metabolism between these types. To understand the pathophysiology of urate underexcretion in HU in greater detail, we have investigated quantitative estimation of urate transport in the nephrons employing the benzbramarone (BBR)-loading urate clearance test [7, 8]. Significant differences have previously been noted in urate transport between HU and normal controls (NC) [7], but more marked differences in urate transport were found between UE and OP. Based on these findings, the relationship between urate transport and  $U_{ua}$  and the mechanisms underlying urate underexcretion in HU, particularly in UE, were investigated. Differences in the pathophysiology of urate underexcretion between UE and OP imply the possibility for rational treatment of hyperuricemia based on the specific subtype.

## Materials and Methods

This present study was approved by the ethics committee at Hayashi General Hospital (Director, Dr. M. Nojiri). The participants comprised 172 male gouty patients (age range, 22–82 years) with hyperuricemia, with a serum urate concentration ( $S_{ua}$ )  $>7.0$  mg/dl, along with 30 male volunteers (age range, 19–69 years) who had provided informed consent to act as NC. Prior to examination, administration of medicines affecting  $S_{ua}$  or  $U_{ua}$ , such as BBR, allopurinol, febuxostat [9, 10], bucolome, losartan, diuretics, fenofibrate, or nucleoside derivatives, was discontinued unless otherwise indicated. All patients provided written informed consent prior to enrollment.  $C_{ua}$ , creatinine clearance ( $C_{cr}$ ) and  $C_{ua}$  determined after administering 100 mg of BBR [ $C_{ua}BBR(100)$ ] were determined using the BBR-loading urate clearance test as reported previously [7, 8]. Urate transport coefficients such as urate glomerular filtration (UGF), urate reabsorption, urate reabsorption rate ( $R_1$ ), urate tubular secretion (UTS), tubular secretion rate (TSR), urate postsecretory reabsorption ( $UR_2$ ) and urate postsecretory reabsorption rate ( $R_2$ ) as well as calculated urinary urate excretion ( $U_{ex}$ ) were calculated as reported previously [7].

### *Classification of Hyperuricemia Subtypes*

Since the main causes of hyperuricemia are considered to be underexcretion and overproduction in urate metabolism of human subjects, we classified HU into 4 subtypes according to the combination of underexcretion and overproduction, as determined by the urate clearance test [5, 6]. UE were defined as cases in which  $C_{ua}$  was significantly lower than the

**Table 1.** Urate transport in the nephrons and its urinary excretion in each subtype of hyperuricemia

Type	Cases, n (%)	BBR-loading urate clearance tests						Approximate urate transports in nephrons					Uex
		Sua	Uua	Cua	Ccr	R	CuaBBR (100)	UGF	UTS	TSR	UR <sub>2</sub>	R <sub>2</sub>	
NC	30	53.1	7.84	9.76	133	7.34	48.4	7,060	4,130	78.0	3,680	0.877	520
		6.8	1.06	1.28	16	1.23	7.1	1,240	800	11.6	750	0.017	70
		1.2	0.19	0.23	2.9	0.22	1.3	230	150	2.1	140	0.003	13
HU	172 (100)	86.6	7.20	5.73	109	5.24	33.8	9,440	4,480	51.8	4,080	0.892	490
		12.0	2.27	1.88	32	1.57	12.1	2,700	1,500	18.8	1,440	0.044	150
		0.9	0.17	0.14	2.4	0.12	0.9	210	110	1.4	110	0.003	11
UE	117 (68)	86.6	5.98	4.77	100	4.78	31.8	8,650	4,220	48.7	3,890	0.904	415
		11.3	1.27	1.18	30	1.41	11.9	2,570	1,460	18.6	1,430	0.043	90
		1.1	0.12	0.11	2.8	0.14	1.1	240	140	1.7	130	0.004	10
OP	28 (16)	83.3	10.84	8.71	136	6.42	40.0	11,330	5,100	61.1	4,490	0.861	725
		8.6	1.50	0.97	26	1.44	10.8	2,350	1,260	16.8	1,090	0.050	90
		1.6	0.28	0.18	4.9	0.27	2.0	440	240	3.2	210	0.009	40
C	17 (10)	97.1	8.84	6.13	112	5.47	34.1	10,870	5,070	52.2	4,580	0.885	600
		16.7	0.95	1.06	21	1.32	11.3	2,320	1,660	17.8	1,660	0.039	60
		4.1	0.23	0.26	5.0	0.32	2.7	560	400	4.3	400	0.010	15
N	10 (6)	77.3	8.20	7.74	136	5.71	38.3	10,510	4,510	58.4	4,020	0.871	595
		4.6	0.39	0.40	20	0.79	13.8	1,460	1,700	22.3	1,670	0.051	40
		1.5	0.12	0.13	6.3	0.25	4.4	460	540	7.0	540	0.016	10

Upper value = Mean; middle value = standard deviation; lower value = standard error. The following units of measurement apply: Sua, µg/ml; Uua, µg/kg/min; Cua, ml/min/1.73 m<sup>2</sup>; Ccr, ml/min/1.73 m<sup>2</sup>; CuaBBR(100), ml/min/1.73 m<sup>2</sup>; UGF, µg/min; UTS, µg/min; TSR, ml/min; UR<sub>2</sub>, µg/min; Uex, µg/min.

normal range (i.e. <7.3 ml/min) [5, 6]. OP corresponded to those patients in whom Uua was higher than (0.43 Cua + 5.43) µg/min, because OP determined by the labeled urate tracer technique were roughly separated from UE by the line Uua = (0.43 Cua + 5.43) µg/min in the Cua-versus-Uua plot [11, 12], and in whom Cua was higher than 7.3 ml/min. The 4 subtypes of HU were UE, OP, combined-type cases (C) and normal-type cases (N). HU classified as N had both Cua within the normal range and Uua below the criterion line from urate clearance tests. N were characterized by a low-grade elevation of Sua and a short duration of morbidity, representing patients in the early stage of hyperuricemia. Such cases might change to other types of HU during the clinical course.

## Results

### Urate Transport Coefficients by HU Subtype

Significant differences in the urate transport coefficients were noted between the HU and the NC [7], but more marked differences were found between the UE and the OP (table 1). In terms of urate transport, the UE were characterized by the lowest TSR and UTS, by the highest R<sub>2</sub>, and subsequently by the lowest Uex in the subtypes of HU. Conversely, OP were characterized by the highest TSR and UTS, by the lowest R<sub>2</sub>, and subsequently by the highest Uex. Accordingly, the marked difference in Uua between UE and OP was attributed to TSR and R<sub>2</sub> in the subgroups.

**Table 2.** Correlation coefficients of urate transporters and clinical parameters

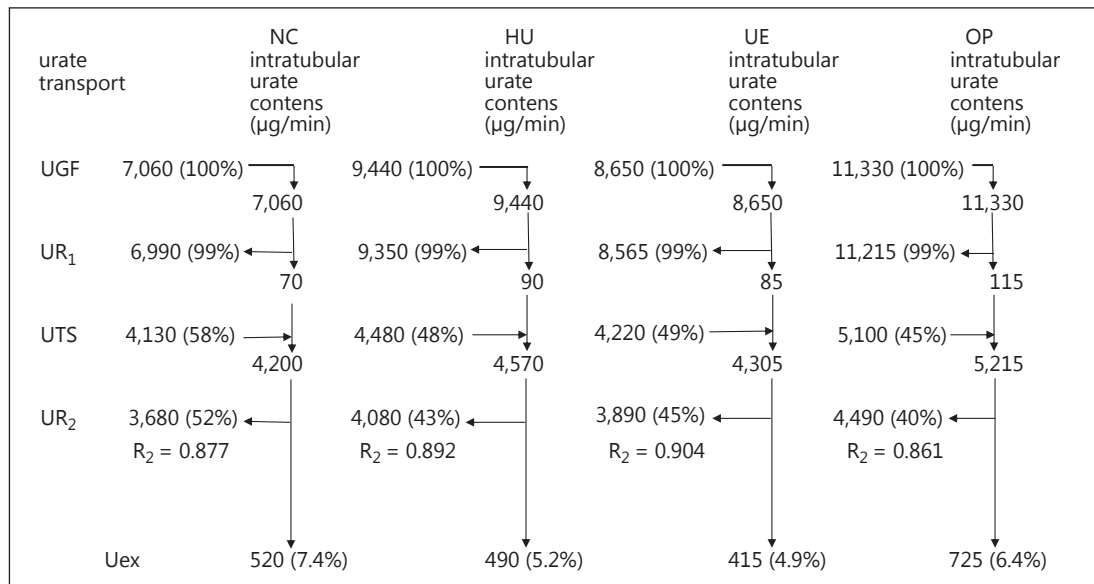
	Sua	Uua	Cua	Ccr	R	UGF	UTS	TSR	UR <sub>2</sub>	R <sub>2</sub>	Uex
Sua		-0.084	-0.633	-0.421	-0.290	+0.314	+0.101	-0.510	+0.116	+0.103	+0.014
Uua			+0.757	+0.506	+0.426	+0.473	+0.344	+0.345	+0.269	-0.393	+0.939
Cua				+0.666	+0.507	+0.196	+0.205	+0.593	+0.135	-0.368	+0.720
Ccr					-0.232	+0.712	+0.280	+0.486	+0.246	-0.151	+0.536
R						-0.480	-0.070	+0.162	-0.120	-0.356	+0.355
UGF							+0.376	+0.102	+0.352	-0.078	+0.584
UTS								+0.773	+0.933	+0.607	+0.388
TSR									+0.756	+0.430	+0.315
UR <sub>2</sub>										+0.655	+0.310
R <sub>2</sub>											-0.380

Regarding urate secretion in the nephrons, UTS in the HU, including in its subtypes, was generally higher than that in NC. The increment of UTS in the UE was minimal in the subtypes of the HU, and UTS in the UE was nearly equal to that in the NC. Although UTS correlated highly with TSR ( $r = 0.733$ ,  $p < 0.0001$ ), as shown in table 2, TSR in all subtypes of the HU was significantly lower than that in the NC and that in the UE was lowest in the subtypes of the HU. Significant correlation was observed between TSR and Uua ( $r = 0.345$ ,  $p < 0.0001$ ) (table 2). Furthermore, the significant correlation between TSR and Cua ( $r = 0.593$ ) was markedly higher than that between UTS and Cua ( $r = 0.205$ ). Since UE were defined as HU with significantly lower Cua than that in NC, urate excretion efficiency in the UE was significantly low, and TSR was considered to be highly correlated with urate excretion and to offer a better parameter than UTS for evaluating Uua. UTS in the OP was markedly higher (+23.5%) than that in the NC and was highest in the subtypes of the HU. These findings suggest that UTS is highly related to urate excretion and possibly related to urate production, because urate metabolism is considered to be in an increased steady state.

Regarding postsecretory reabsorption of urate, R<sub>2</sub> was significantly higher in the UE than in the NC. Increment of R<sub>2</sub> compared to the NC was highest in the UE (+3.2%) and lowest in the OP (-1.7%) in the subtypes of the HU. A significant negative correlation was obtained between R<sub>2</sub> and Uua ( $r = -0.393$ ,  $p < 0.0001$ ) and also between R<sub>2</sub> and Cua ( $r = -0.368$ ,  $p < 0.0001$ ) (table 2), suggesting that increases in R<sub>2</sub> correlate with decreased urate excretion. R<sub>2</sub> was highly correlated with UR<sub>2</sub> ( $r = 0.655$ ,  $p < 0.0001$ ), as shown in table 2, and UR<sub>2</sub> in all subtypes of the HU was also generally higher than that in NC. Compared to the NC, UR<sub>2</sub> was +6.0% higher in the UE and +22.0% higher in the OP. Although UR<sub>2</sub> was higher in the OP than in the UE, Uua was significantly higher in the OP than in the UE. This is probably because UTS in the OP was very much higher than postsecretory reabsorption. The ratio of UR<sub>2</sub>/UTS was lower in the OP than in the UE. TSR and R<sub>2</sub> in the HU with the combination of underexcretion and overproduction (C) were midway between those for the UE and the OP. Subsequently, Uex in the C was also midway between values in the UE and the OP.

*Estimation of Intratubular Urate Contents in the Nephrons of the Subtypes of HU (fig. 1)*

UGF in all subtypes of the HU was generally higher than that in the NC, and UGF in the OP was markedly higher than that in the UE, but almost all the filtrated urate was thought to be reabsorbed at the urate reabsorption stage. Therefore, intratubular urate contents in all subtypes might be minimal and might not differ significantly. TSR in all subtypes was generally lower than that in the NC, but UTS in all subtypes returned back to NC levels or higher. Intratubular urate contents in all subtypes were thus considered to be higher than those of the NC until the stage

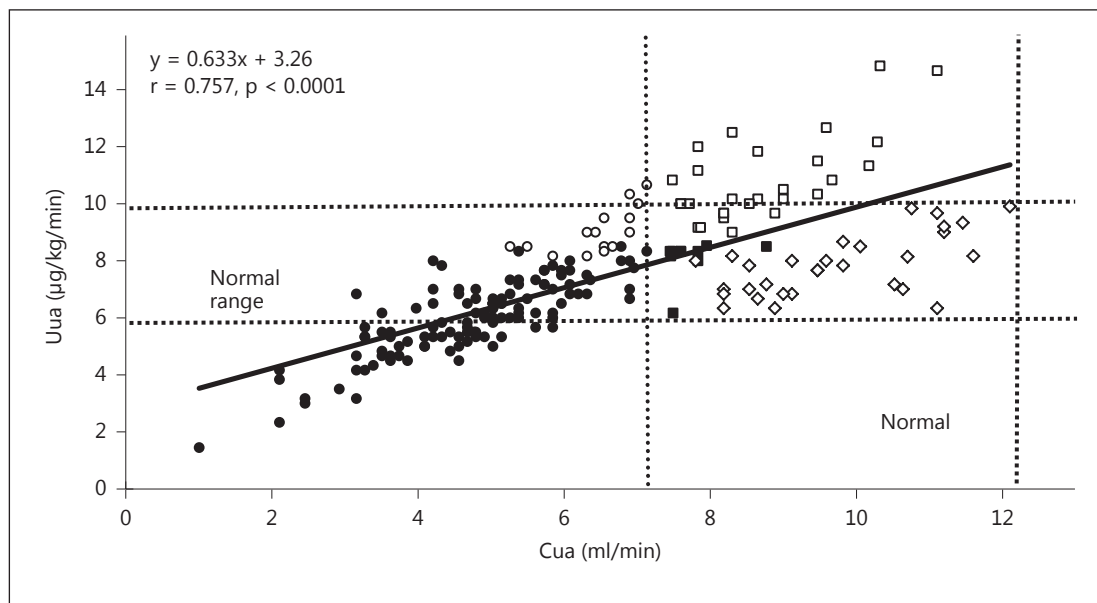


**Fig. 1.** Comparison of intratubular urate contents between UE and OP. UR<sub>1</sub> = Urate reabsorption.

of UTS, but were reduced by reabsorption with significantly higher  $R_2$  in HU subtypes at the stage of UR<sub>2</sub>, especially in the UE with markedly higher  $R_2$ , resulting in significantly lower U<sub>ex</sub>. In the OP, intratubular urate contents were markedly higher than those in the NC at the stage of UTS. Furthermore,  $R_2$  in the OP was significantly lower than that in the NC, and UR<sub>2</sub> in the OP was relatively lower. Subsequently, U<sub>ex</sub> in the OP was markedly higher than that in the NC. Accordingly, a marked difference in U<sub>ua</sub> was evident between the UE and the OP. These findings indicate that both TSR and  $R_2$  are important factors for determining urate excretion efficacy.

#### Correlation between Urate Transport Coefficients (table 2)

S<sub>ua</sub> has been used as a primary parameter for differential diagnosis and understanding the urate metabolism underlying hyperuricemia in clinical practice for gouty patients; however, correlation coefficients between U<sub>ua</sub> and urate transport coefficients were considered to be more valuable than those between S<sub>ua</sub> and urate transport coefficients for investigating the pathophysiology of intratubular urate contents and mechanisms of urate underexcretion in hyperuricemia, because the former were generally higher than the latter. Among the correlation coefficients between C<sub>ua</sub> and urate transport coefficients, TSR and  $R_2$  (negative) were found to show higher correlations than the others, both of which might be valuable transporters for determining U<sub>ua</sub>, but might not be related to UGF, because correlation coefficients of UGF and TSR as well as those of UGF and  $R_2$  were low. C<sub>cr</sub> showed a significantly higher correlation with TSR, but a lower one with  $R_2$ . High-grade correlation coefficients were obtained between UTS and TSR ( $r = 0.773$ ,  $p < 0.0001$ ), both of which are closely related to urate secretion, and UTS was calculated as a product of TSR and S<sub>ua</sub> [7]. A relationship similar to that between UTS and TSR was also seen for UR<sub>2</sub> and  $R_2$  ( $r = 0.655$ ,  $p < 0.0001$ ), both of which are closely related to urate postsecretory reabsorption, and UR<sub>2</sub> is a product of  $(0.01 \text{ UGF} + \text{UTS})$  and  $R_2$  [7]. High-grade correlation coefficients were also obtained between UTS and UR<sub>2</sub> ( $r = 0.993$ ,  $p < 0.0001$ ), which was higher than the correlation coefficient between  $R_2$  and UR<sub>2</sub>, suggesting that UR<sub>2</sub> was much more influenced by UST than by  $R_2$ . This is why correlation coefficients between UR<sub>2</sub> and U<sub>ua</sub> ( $r = 0.269$ ,  $p < 0.0001$ ) were not negative, in contrast to the common concept that larger UR<sub>2</sub> would result in smaller U<sub>ua</sub>.



**Fig. 2.** Distribution of each type of HU on a plot evaluating Uua as a function of Cua. ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.

*Distribution of HU Subtypes on Graphs of Uua versus Cua as Coordinates (fig. 2)*

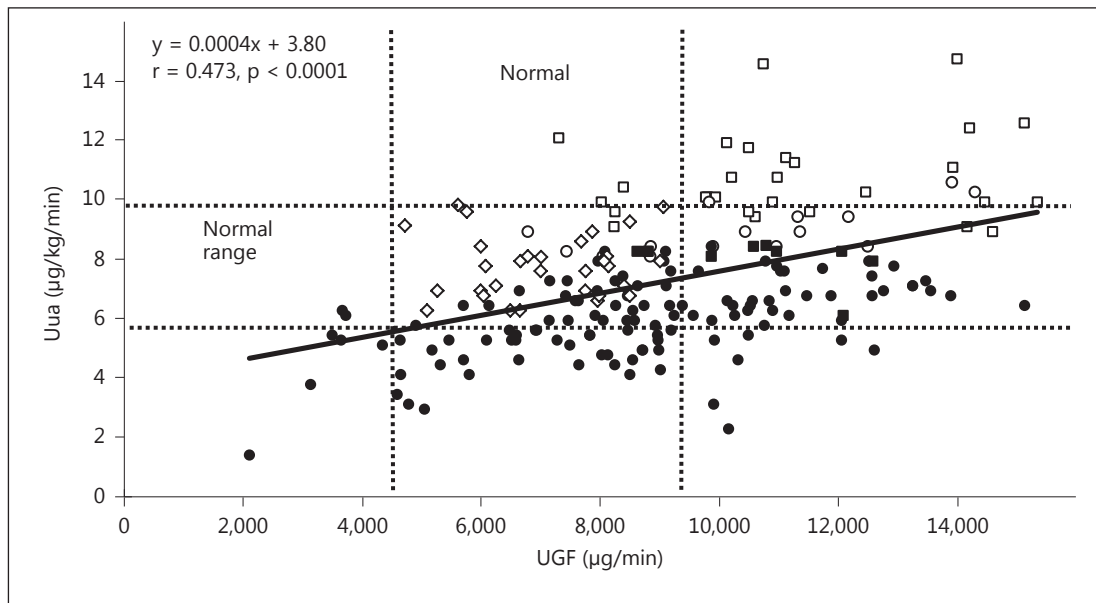
The OP were mainly located in the area of significantly higher Uua and the normal Cua. Conversely, UE were located in the significantly lower Cua range, but not all UE were located in the significantly lower Uua range area. Only approximately 1/3 of the UE were located in the significantly lower Uua range, with 2/3 in the normal Uua range, although UE were defined as cases in which Cua was significantly lower than that in NC, suggesting that the expected decrease in Uua with decreased Cua was restored by increased Sua in the UE. The C and the N were located in border areas between the distribution of UE and OP. Since the UE and the OP showed great differences in the distribution on the graph of Uua versus Cua as coordinates, together with differences in urate transport coefficients, the distribution of the UE and the OP on graphs of urate transport coefficients versus Uua as coordinates was investigated to provide a better understanding of the pathophysiology and mechanisms of urate underexcretion in hyperuricemia.

*Distribution of HU Subtypes on Graphs of Urate Transport Coefficients versus Uua as Coordinates*

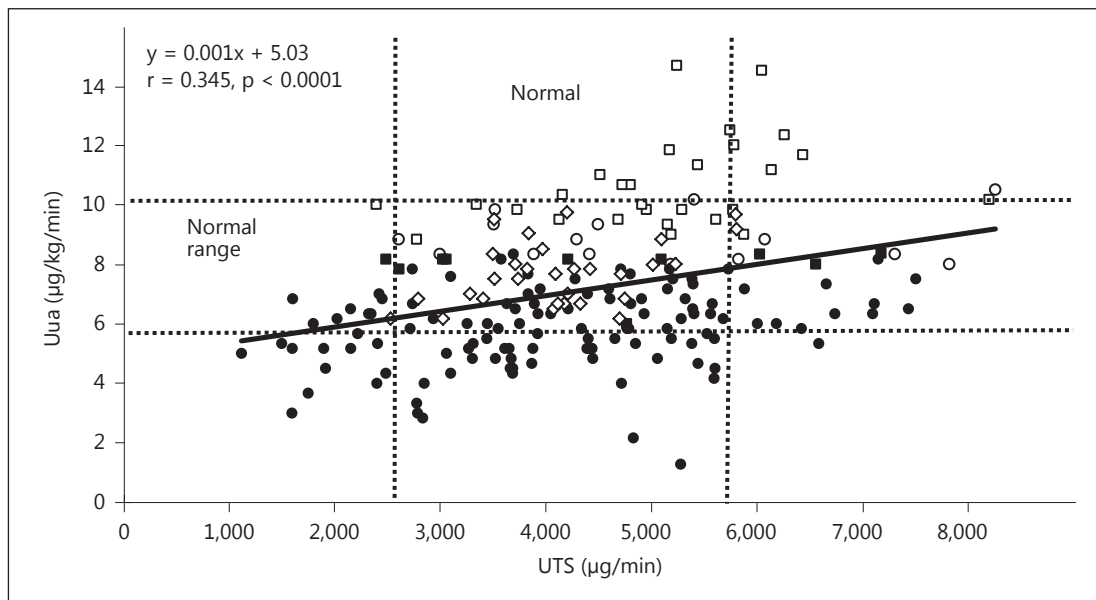
*UGF-versus-Uua Graph (fig. 3)*

As a whole, a significant correlation was obtained between UGF and Uua ( $r = 0.473$ ,  $p < 0.0001$ ). The distribution of UE was spread widely in the horizontal (UGF) direction and mainly located in the normal to the higher UGF range, but was limited in a relatively narrow area in the vertical (Uua) direction, that is the lower half of the normal Uua range and the upper part of the lower Uua range. Approximately 40% of the UE were located in the significantly higher UGF range, but the majority of the UE in the high UGF was located within the normal range of Uua. The Uua/UGF ratio in UE was lower than that in NC, suggesting that urate underexcretion in UE might be functioning in the lower areas of UGF in the intratubular urate flow. The OP were located mainly in the higher UGF and the higher Uua area. The Uua/UGF ratio was not decreased in the OP. There were only 7 cases (6.0%) of all 117 UE in the significantly lower UGF and lower Uua range, in which low UGF could be explained as a direct cause of urate underexcretion.





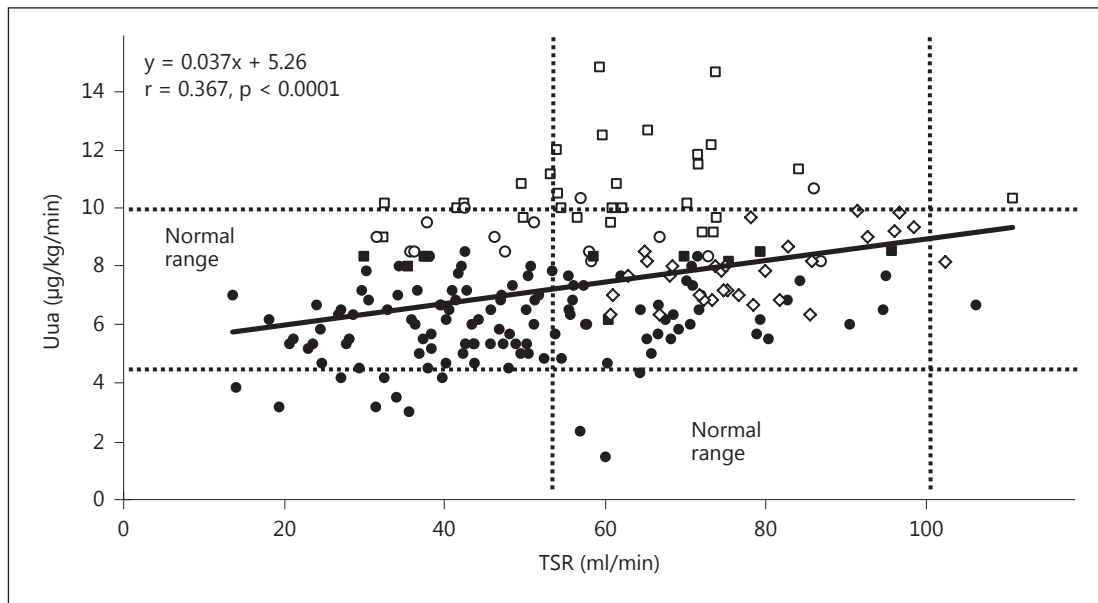
**Fig. 3.** Distribution of each type of HU on a plot evaluating Uua as a function of UGF. ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.



**Fig. 4.** Distribution of each type of HU on a plot evaluating Uua as a function of UTS. ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.

#### UTS-versus-Uua Graph (fig. 4)

A significant correlation was obtained between UTS and Uua ( $r = 0.345, p < 0.0001$ ). The distribution of the UE was spread in the horizontal (UTS) direction and was in the lower to the higher UTS range, but was limited to a narrow area in the vertical (Uua) direction. The majority (73%) of UE was located in the normal UTS range, and the distribution could be



**Fig. 5.** Distribution of each type of HU on a plot evaluating Uua as a function of TSR. ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.

roughly shifted vertically to a lower Uua compared to the NC. That is, approximately half of these UE in the normal UTS range were shown to have significantly lower Uua. Accordingly, the Uua/UTS ratio in these UE was lower than that in the NC, indicating that Uua in the UE was lower than that in the NC even if UTS in the UE was the same as that in the NC, suggesting that factors other than low UTS might be more significant causes of urate underexcretion. The OP were mainly located in the higher part in the normal UTS range area to the lower part in the higher UTS and the higher Uua range, in which Uua/UTS was higher than in the NC even if UTS was the same between the UE and the NC. There were only 11 UE (9.4%) located in the significantly lower UTS and lower Uua range, in which low UTS could be explained as a direct cause of urate underexcretion.

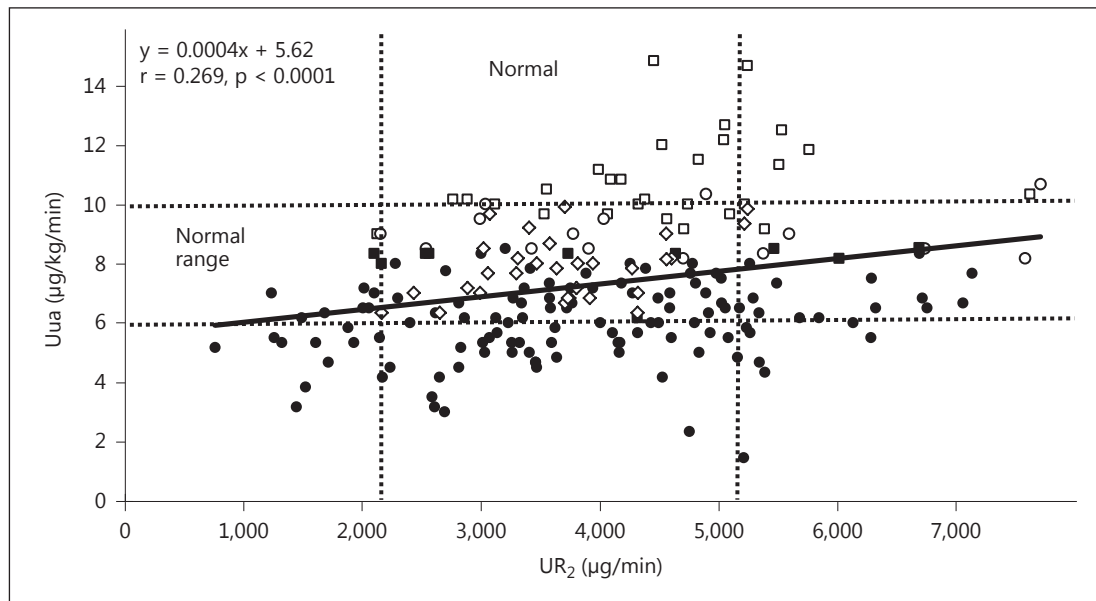
#### TSR-versus-Uua Graph (fig. 5)

A significant correlation was obtained between TSR and Uua in the HU and the NC ( $r = 0.367, p < 0.0001$ ). Approximately 2/3 of the UE were distributed in the significantly lower TSR range, where the UE were located in the lower to the lower part of the normal Uua range. UE in the normal TSR range were located in the lower to the lower part of the normal Uua range. The ratio of Uua/TSR in the UE was nearly equal to that in the NC. These findings suggest that TSR correlates highly with Uua, and low TSR is one of the main causes of urate underexcretion in UE. The majority of the OP was located in the higher part of the normal TSR and the higher Uua range. Generally, the level of reduction in the TSR in the UE compared to the NC was higher than that in UTS compared to the NC, and suggested that TSR is a better parameter for estimating underexcretion in HU. Decreased TSR was compensated for by increased Sua at the stage of urate secretion in HU.

#### UR<sub>2</sub>-versus-Uua Graph (fig. 6)

The distribution of the UE was spread in the horizontal (UR<sub>2</sub>) direction from the lower to the higher UR<sub>2</sub> range. Approximately 2/3 of the UE were located in the normal UR<sub>2</sub> range,





**Fig. 6.** Distribution of each type of HU on a plot evaluating Uex as a function of  $R_2$ . ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.

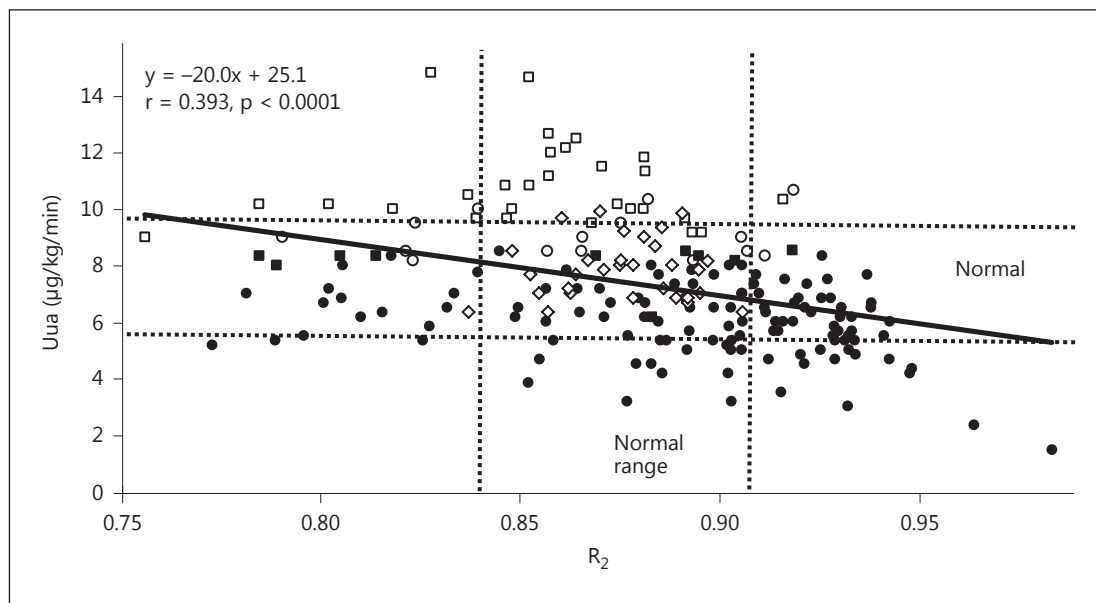
where these UE were located in the significantly lower to the lower normal Uua range. That is, half of these UE were located in the significantly lower Uua range. The Uua/ $UR_2$  ratio in the UE was lower than that in the NC, suggesting that other factors except  $UR_2$  could be the main cause of urate underexcretion. A significant correlation between  $UR_2$  and Uua was noted ( $r = 0.269$ ,  $p < 0.00001$ ) although the coefficient value was not high.

#### $R_2$ -versus-Uua Graph (fig. 7)

A significant negative correlation was obtained between  $R_2$  and Uua ( $r = -0.393$ ,  $p < 0.0001$ ). The distribution of the UE was spread widely in the horizontal ( $R_2$ ) direction from the lower to the higher  $R_2$  range, and the majority of UE were densely located in the higher  $R_2$  region to the higher part of the normal  $R_2$  range. Uua of these UE in the dense distribution was located in the significantly lower Uua range to the lower half of the normal range. Uua/ $R_2$  of the majority of the UE in the dense distribution was almost the same as that in the NC. These findings suggest that significantly higher  $R_2$  might be one of the main causes of urate underexcretion in the HU, especially in the UE. However, approximately 1/6 of all UE were located in the significantly lower  $R_2$  range, and the majority of these cases were shown to have normal Uua, suggesting that the cause of urate underexcretion in UE might not always be increased reabsorption.

The OP were mainly located in the normal  $R_2$  range, with the majority of these OP shown to have significantly higher Uua than the NC. The OP with significantly lower  $R_2$  represented approximately only 1/4 of all OP, and of all OP there was only 1 case (3.6%) with significantly higher  $R_2$ .

These distribution studies on various combinations of urate transport coefficients as coordinates showed that hyperuricemia could be produced by various combinations of transport abnormalities.



**Fig. 7.** Distribution of each type of HU on a plot evaluating Uua as a function of  $R_2$ . ● = UE; □ = OP; ○ = C; ■ = N; ◇ = NC; ---- = regression equation and line.

## Discussion

In the present study, urate transport and intratubular urate contents in the tubule of nephrons in the subtypes of 172 HU were estimated by employing a previously designed equation,  $C_{ua} = [C_{cr}(1 - R_1) + TSR](1 - R_2)$ , using the BBR-loading urate clearance test [7]. Differences in urate transport between UE and OP were analyzed, and the relationships between urate transport and Uua were compared in the subtypes of the HU. Since the causes of hyperuricemia differ markedly between UE and OP, characteristic features of urate transport in UE and in OP might also differ markedly, reflecting differences in urate excretion and the pathophysiology of intratubular urate contents. Analysis of the differences might prove valuable in understanding the mechanisms underlying urate underexcretion in hyperuricemia.

In the subtypes of the HU, the UE were shown to have the lowest TSR and the highest  $R_2$  in intratubular urate flow. Conversely, the OP had the highest TSR and the lowest  $R_2$ . TSR and Uua showed a significant correlation ( $r = 0.367$ ,  $p < 0.0001$ ), and  $R_2$  and Uua showed a significant negative correlation ( $r = -0.393$ ,  $p < 0.0001$ ). In addition, the majority of UE were located in the significantly lower TSR range area in the TSR-versus-Uua plot and were also located in the significantly higher  $R_2$  range to the higher part of the normal range in the  $R_2$ -versus-Uua plot. The Uua/TSR and Uua/ $R_2$  ratios of these UE in the main distribution of the graph were almost equal to the ratio of the NC. These findings suggest that TSR and  $R_2$  are closely related to Uua and that both decreased TSR and increased  $R_2$  are considered as the main causes of urate underexcretion in UE, although the effects of urate underexcretion were reduced to some extent at the following stage of UTS and  $UR_2$ , respectively.

As shown in table 2, TSR and UTS were highly correlated ( $r = 0.773$ ,  $p < 0.0001$ ). The HU distribution pattern in the TSR-versus-Uua graph (fig. 5) and in the UTS-versus-Uua graph (fig. 4) appeared to be similar except for the distance from the NC distribution. In comparison with HU distribution in the TSR-versus-Uua plot and UTS-versus-Uua plot, the former could be shifted in a higher TSR direction to roughly replicate the latter. This finding could be under-

stood from the following relationship of UTS and TSR:  $UTS = TSR \times Sua$  [7]. The distance between the distribution of HU and NC was reduced in the UTS-versus-Uua plot compared to the TSR-versus-Uua plot by multiplying higher Sua by TSR in the former. These findings suggest that decreased Uua, produced in association with the decrease of TSR in the UE compared to the NC, is improved by the product of TSR and higher Sua in the UE compared to the NC. Hyperuricemia is thus considered to represent a reasonable reaction for reducing the decreased urate excretion that might have been induced by decreased TSR. Decreased TSR can thus be considered as the primary cause of urate underexcretion in UE and offers a better parameter for estimating urate secretion abnormality in tubules than UTS.

$R_2$  and  $UR_2$  are also highly correlated ( $r = 0.655$ ,  $p < 0.0001$ ). Like the relationship between the distribution of the HU in the TSR-versus-Uua and UTS-versus-Uua plots, the distribution of the HU in the  $R_2$ -versus-Uua plot (fig. 7) could be shifted in a higher  $R_2$  direction to roughly replicate that in the  $UR_2$ -versus-Uua plot (fig. 6). Judging from the higher, dense distribution of the UE in the significantly higher  $R_2$  range in the  $R_2$ -versus-Uua plot,  $R_2$  was considered to also be a main cause of urate underexcretion, and  $R_2$  could offer a better parameter of urate underexcretion than  $UR_2$ . Decreased TSR and increased  $R_2$  were thus considered as the main mechanisms of urate underexcretion in UE.

URAT1 has been reported as the most potent urate transporter for urate reabsorption and was highly inhibited by BBR [13]. URAT1 is also closely related to Sua in humans [14]. However, in other experiments using cultured *Xenopus laevis* oocytes injected with URAT1 mRNA, inhibition of urate uptake by BBR at a saturated concentration was 54.9%, while the inhibition of URAT1 by BBR at the same saturated concentration level was 95.1% [15]. These findings suggest that almost complete inhibition of URAT1 with BBR could not cause complete inhibition of all urate transport in reabsorption. For the remaining urate transport, numbers of urate transporters such as OAT1 and its families [16–25], including nicotinate transporter hOAT10 (SLC22A13) [26], fructose transporter SLC2A9 [15] and URATv1 [27], might continue to function. Another transporter, ABCG2, mediates urate secretion in proximal tubule cells, and genetic variants with single nucleotide polymorphisms were associated with high Sua [28–30]. These findings suggest that urate transporters play important roles in urate transport in the nephron and urate excretion. However, in the present study, the urate transport was assumed to be parallel with intratubular urate contents as a tentative example, and both increased  $R_2$  and decreased TSR were suggested to be functioning independently as causes of urate underexcretion. Estimation of the grade of low TSR and high  $R_2$  in individual cases with a simple method such as the BBR-loading urate clearance test might be valuable for investigating the pathophysiology of urate transport in nephrons and controlling hyperuricemia in clinical practice.

The distribution of the HU in the  $UR_2$ -versus-Uua plot (fig. 6) showed a positive correlation between  $UR_2$  and Uua. This finding seems to contradict the common concept that the correlation coefficient between  $UR_2$  and Uua would be negative. However, intratubular urate contents of the UE in the significantly higher UTS region were certainly higher than intratubular urate contents in other regions and Uua of the UE in this region was not sufficiently reduced compared to Uua in other regions of the UE after reabsorption by  $UR_2$  (fig. 4), although  $UR_2$  was relatively high because  $UR_2$  and UTS showed an extremely high correlation ( $r = 0.993$ ,  $p < 0.0001$ ). Therefore, increased UTS was considered to exceed the increase in  $UR_2$  and subsequently resulted in higher Uua than commonly expected.

Another 20 UE (approximately 1/6 of the 117 UE) were located in the significantly lower  $UR_2$  range in the  $UR_2$ -versus-Uua plot (fig. 6) and half of these 20 cases were shown to have significantly lower Uua than seen in the NC. The latter cases were shown to have significantly lower Uua despite a low rate of urate reabsorption, suggesting that intratubular urate contents in these UE were sufficiently lower compared to the UE in other regions and the NC at the  $UR_2$

stage of intratubular urate flow. UTS and TSR in the UE thus show a wide variation and represent important factors for determining Uua in the HU in combination with  $R_2$ .

Very high correlation coefficients between Uua and Uex ( $r = 0.939$ ,  $p < 0.0001$ ) suggested that the calculation of urate transport coefficients using the equation  $C_{ua} = [C_{cr}(1 - R_1) + \text{TSR}](1 - R_2)$  is suitable. Using this equation, TSR and  $R_2$  in the NC was calculated as 78.0 ml/min and 0.877 by substitution of  $C_{ua} = 9.76$  ml/min,  $C_{cr} = 133$  ml/min and  $R_1 = 0.99$ , respectively [7]. Urate transporters (UGF, UTS,  $UR_2$ ) and Uex were then calculated as 7,062, 4,142, 3,694 and 519  $\mu\text{g}/\text{min}$ , respectively. Similarly, values in the UE were calculated as 8,660, 4,217, 3,890 and 414  $\mu\text{g}/\text{min}$ , respectively. These values are comparable to those in table 1. For example, in a tentatively assumed condition where in the urate intratubular flow in the NC, only TSR was assumed to be decreased from 78.0 ml/min in the NC to 48.7 ml/min in the UE,  $C_{ua}$  would be calculated as 6.15 ml/min and Uex would be 321  $\mu\text{g}/\text{min}$ . Similarly, if  $R_2$  was assumed to be increased from 0.877 in the NC to 0.904 in the UE,  $C_{ua}$  would be calculated as 7.62 ml/min and Uex would be 404  $\mu\text{g}/\text{min}$ . The marked decreases in Uex by the one transporter change suggest that both decreased TSR and increased  $R_2$  are fundamental factors for urate underexcretion in UE.

## Conclusion

Both TSR and  $R_2$  represent fundamental factors for determining Uua in HU. Significantly lower TSR and significantly higher  $R_2$  were considered as the main causes of urate underexcretion in UE. Neither was apparent in OP. Understanding the difference between UE and OP in terms of the pathophysiology of urate transport in urate underexcretion might be useful for controlling hyperuricemia in clinical practice.

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