

SHORT COMMUNICATION

A common polymorphism in the tissue kallikrein gene is associated with increased urinary excretions of calcium and sodium in Japanese volunteers

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Tissue kallikrein is an enzyme involved in the release of kinin in peripheral tissues. It is believed to regulate hemodynamics and electrolyte transport in the kidney. The present study analyzed polymorphisms of tissue kallikrein in Japanese volunteers and examined the associations between allele H in the promoter region, which has been shown to have decreased promoter activity, and urinary kallikrein activity and physiological parameters in subjects on an *ad libitum* diet. Ninety and 73 volunteers were analyzed for the promoter and coding regions of the tissue kallikrein gene, respectively. The allelic frequency of allele H was found to be 24%. One synonymous and three non-synonymous polymorphisms were found in the coding regions. Urinary kallikrein activity was not significantly decreased in subjects with allele H compared to those without allele H, although they were low in two homozygotes of allele H. Urinary excretions of calcium and sodium were larger in the subjects with allele H than in those without. It is concluded that allele H is a common polymorphism in Japanese and may contribute to decreased reabsorptions of calcium and sodium in the kidney. Further interventional studies are needed to clarify the phenotype of allele H with respect to renal electrolyte handling.

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INTRODUCTION

Human tissue kallikrein is a family of 15 genes (hKLLK1–hKLLK15) located on chromosome 19q13.3–13.4. The product of hKLLK1 is a kinin-forming serine protease and the enzyme is synthesized in many organs including the kidney. The renal kallikrein–kinin system is involved in salt-sensitive hypertension.^{1,2}

hKLLK1 shows polymorphisms in the coding and promoter regions. A loss-of-function coding polymorphism (G230A) was found in Caucasians and Africans.³ An allele H polymorphism, whose promoter activity was decreased by 85% of the control, was found in Asians at an allelic frequency of 25%.⁴ This allelic frequency was higher in hypertensive Chinese patients than in their normotensive counterparts.⁵

hKLLK1 is abundantly synthesized in the renal distal tubules and is secreted in urine. The above loss-of-function polymorphism was demonstrated to associate with reduced urinary hKLLK1 activity in hypertensive patients.³

We analyzed hKLLK1 polymorphisms in healthy Japanese volunteers and examined urinary hKLLK1 activity and physiological phenotypes in these subjects with and without allele H.

MATERIALS AND METHODS

The Ethics Committee of Kitasato University School of Medicine and Kitasato University Hospital approved this study. Healthy Japanese volunteers (age range, 20–45 years, male/female 27/46) participated in the study after informed consent was obtained.

hKLLK1 polymorphisms were identified by a sequencing of genomic DNA including the promoter, five exons, and 10–50 bp of intronic sequences per exon (Applied Biosystems, Foster City, CA, USA). The reference gene sequence was obtained from GenBank (AY094609). Ninety Japanese volunteers were examined for the polymorphic promoter region, –133 to –121 from the transcription initiation site, and 73 were examined for the coding region.

Physiological characteristics were measured in the morning under an uncontrolled diet. Blood samples were collected in a supine position. Urine samples were collected spontaneously (first collection) and by urination after intake of 500 ml of water (second collection). Urinary hKLLK1 activity was measured using a fluorogenic substrate.⁶ Laboratory tests were measured by SRL Inc. (Tokyo, Japan).

Gender distributions between the subjects with and without allele H were analyzed by a χ^2 -test for independence. The physiological characteristics were compared using an unpaired *t*-test.

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Table 1 Allelic frequencies of polymorphisms in the human *KLK1* gene

Location	Position		Alleles	Substitution		Japanese (this study)		Asians ^a		Caucasians ^a				
	from the transcription initiation site	Position from the start codon		Nucleotide	Amino acid	Allele number	Allelic frequency	Allele number	Allelic frequency	Allele number	Allelic frequency			
Promoter	-121 to -133		A			86	0.48	22	0.61	48	0.42			
			B	-128G→C		34	0.19	4	0.11	33	0.29			
			C	-131A→G		0	0.00	0	0.00	0	0.00			
			D	-131A→G, -129G→C		0	0.00	0	0.00	0	0.00			
			E	-127G→C		0	0.00	0	0.00	1	0.01			
			F	-129GC, -130(G)11		0	0.00	0	0.00	0	0.00			
			H	-130(G)11		44	0.24	9	0.25	16	0.14			
			I	-130(G)9		0	0.00	0	0.00	2	0.02			
			K	-130(G)12		16	0.09	1	0.03	14	0.12			
			P	-127G→C, -130(G)9		0	0.00	0	0.00	0	0.00			
				-85			T→C		0	0.00	—	—	—	0.03
				-19			C→G		0	0.00	—	—	—	0.04
			Intron 1	+93			G→A		0	0.00	—	—	—	0.01 ^b
	G→A	Arg→His				0	0.00	—	—	—	—	0.03 ^b		
Exon 3		+230		C→T	Asp→Asp	29	0.20	—	—	—	0.35 ^b			
Exon 3		+405		C→G	Gln→Glu	29	0.20	—	0.11 ^c	—	0.33 ^b			
Exon 3		+433		C→G	Gln→Glu	29	0.20	—	0.11 ^c	—	0.33 ^b			
Exon 4		+514		C→T	Leu→Phe	1	0.01	—	—	—	—			
Exon 4		+556		A→G	Lys→Glu	68	0.47	—	0.70 ^c	—	—			

^aReported by Song *et al.*⁴ in 18 Asians and 57 Caucasians.

^bReported by Slim *et al.*³ in 46 Caucasians.

^cReported by Zhao *et al.*⁷ in 45 Chinese Han people.

Table 2 Comparison of physiological parameters between subjects with and without allele H

Physiological parameters	Subjects with allele H	Subjects without allele H	P-value
<i>First collection</i>			
Urinary hKLK1 activity (μmol per mg Cr) ^a	0.017 ± 0.012	0.019 ± 0.015	0.53
<i>Second collection</i>			
Urinary hKLK1 activity (μmol per mg Cr)	0.015 ± 0.011	0.019 ± 0.016	0.27
Height (cm)	164.7 ± 7.4	162.4 ± 8.8	0.27
Body weight (kg)	58.5 ± 9.9	56.4 ± 10.4	0.40
Age	27 ± 8	26 ± 8	0.73
Gender (male:female) ^a	10:18	17:28	0.86
	9:12	17:22	0.96
Serum Cr (mg dl ⁻¹)	0.13 ± 0.61	0.27 ± 0.64	0.10
eGFR (ml min ⁻¹ per 1.73 m ²)	99.8 ± 13.1	97.1 ± 19.7	0.51
<i>Supine</i>			
Systolic BP (mm Hg)	116 ± 13	110 ± 13	0.06
Diastolic BP (mm Hg)	67 ± 10	62 ± 9	0.04
Pulse rate (per min)	63 ± 7	60 ± 12	0.22
<i>Sitting</i>			
Systolic BP (mm Hg)	124 ± 13	119 ± 15	0.12
Diastolic BP (mm Hg)	74 ± 12	71 ± 10	0.16
Pulse rate (per min)	67 ± 7	65 ± 10	0.23
Plasma aldosterone (pg ml ⁻¹) ^a	77.5 ± 27.1	87.7 ± 41.5	0.32
Plasma renin activity (ng ml ⁻¹ h ⁻¹) ^a	1.0 ± 0.5	1.3 ± 0.9	0.28
<i>First collection</i>			
FeNa (%) ^a	0.5 ± 0.2	0.5 ± 0.5	0.58
FeK (%) ^a	5.0 ± 2.9	5.4 ± 3.0	0.66
FeCa (%) ^a	0.9 ± 0.5	0.6 ± 0.5	0.03

Table 2 (Continued)

Physiological parameters	Subjects with allele H	Subjects without allele H	P-value
<i>Second collection</i>			
FeNa (%)	1.0±0.3	0.9±0.6	0.36
FeK (%)	7.7±3.7	7.9±4.3	0.78
FeCa (%) ^a	1.0±0.4	0.7±0.6	0.06
<i>First collection</i>			
Na (mmol per mg Cr) ^a	0.11±0.05	0.11±0.08	0.96
K (mmol per mg Cr) ^a	0.03±0.02	0.03±0.02	0.89
Ca (mg per mg Cr) ^a	0.14±0.09	0.09±0.08	0.03
<i>Second collection</i>			
Na (mmol per mg Cr)	0.23±0.08	0.18±0.10	0.03
K (mmol per mg Cr)	0.05±0.03	0.05±0.03	0.96
Ca (mg per mg Cr) ^a	0.15±0.06	0.10±0.09	0.02

Abbreviations: BP, blood pressure; Cr, creatinine; eGFR, estimated glomerular filtration rate; Fe, fractional excretion of.

^aThe number of samples was 60 (21 and 39 for the subjects with and without H-type allele, respectively). The number of the others was 73 (28 and 45, respectively). χ^2 -analysis was performed for the relationship between gender and allele type. Other parameters were analyzed by unpaired *t*-test. $P < 0.05$ was significantly different between the two groups.

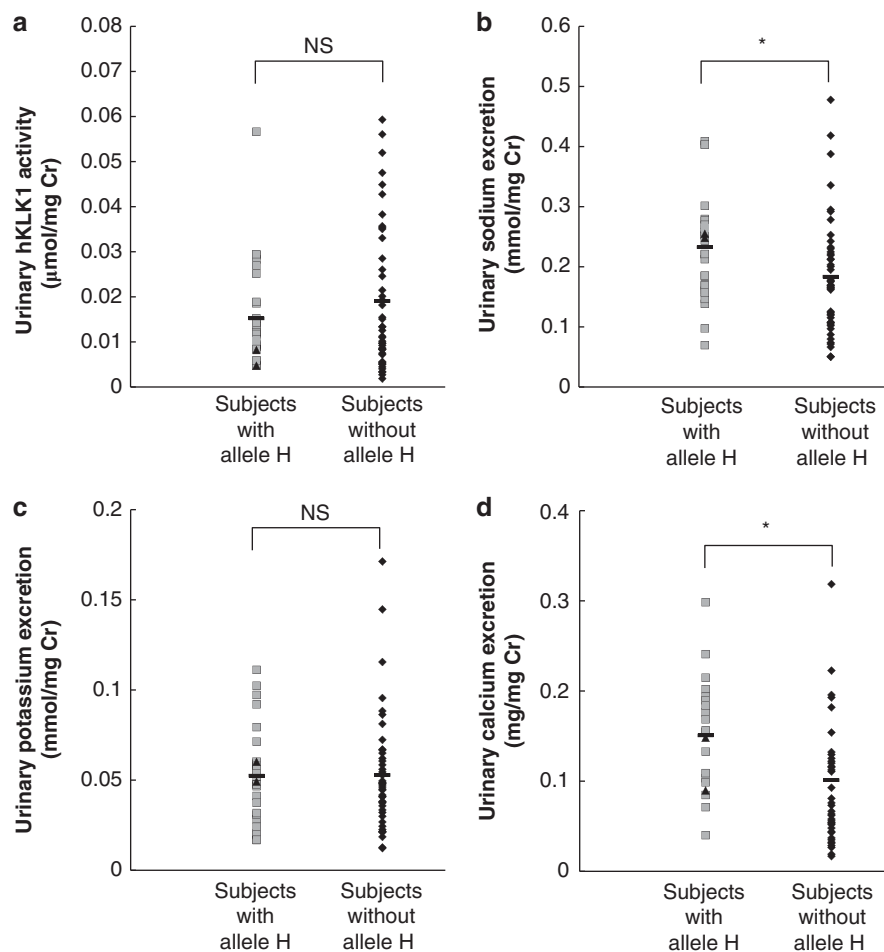


Figure 1 Comparison of urinary hKLK1 activity and urinary excretions of sodium, potassium and calcium between subjects with and without allele H. Graphs show individual urine data from the second collection, which was newly produced urine after water intake. Urinary hKLK1 activity (a) and urinary excretions of sodium (b), potassium (c) and calcium (d) were compared between the subjects with and without allele H. Each symbol indicates the heterozygotes of allele H (gray square) and homozygotes of allele H (black triangle, $n = 2$: male 1, female 1) and subjects without allele H (black diamond). Data were expressed as amounts per milligram urinary creatinine. Bars indicate the mean. The total number of subjects was 73 for a, b and c, and 60 for d. * $P < 0.05$; NS, not significant.

RESULTS

Table 1 presents the allelic frequencies of the hKLK1 polymorphisms as reported in previous studies.^{3,4,7} Alleles A, B, H and K were also identified in Japanese. The frequency of allele H was 24%, which was similar to Asians.

Three subjects carried allele H homozygously. Three non-synonymous polymorphisms, C433G in exon 3 and C514T and A556G in exon 4, were identified. The subjects who had C433G also carried a synonymous polymorphism, C405T. The absence of disequilibrium was indicated between H and C405T/C433G (χ^2 -test, $P = 0.026$) and between K and C405T/C433G (χ^2 -test, $P < 0.0001$) polymorphisms (data not shown).

Table 2 shows a comparison of urinary hKLK1 activity and physiological parameters between the subjects with and without allele H. Although urinary hKLK1 activity was not significantly different, two subjects who were homozygous for allele H displayed low urinary hKLK1 activity (Figure 1a). The fractional excretion of calcium in urine and urinary calcium excretion were larger in the allele-H subjects than in the non-allele-H subjects. Urinary sodium excretion was larger in the second collection in the allele-H subjects.

DISCUSSION

The allele-H polymorphism of hKLK1 promoter was found in Japanese as common as in Asians.⁴ A loss-of-function polymorphism of G230A located on exon 3 was not identified. Other non-synonymous polymorphisms, C433G and A556G, were common, but the former is not responsible for decreased hKLK1 activity.³ The activity of hKLK1 is determined by three specific amino-acid residues for substrate binding and the nucleotide sequences are located on exons 2, 3 and 5. A556G is located on exon 4 and urinary hKLK1 activity was not different between the subjects with and without A556G (data not shown).

Possible reasons for the slight but insignificant lower urinary hKLK1 activity in subjects with allele H than in those without allele H are as follows; the other allele might compensate for the lower activity, spot urine might not reflect the total biogenesis of hKLK1,⁸ and potassium intake, a known secretagogue of hKLK1, was not controlled.^{6,9} A higher urinary calcium excretion in subjects with allele H than in those without allele H may be explained by reduced renal tubular calcium reabsorption.^{10–12} A difference in urinary sodium excretion in the second collection may reflect reduced activity of renal tubular epithelial sodium channels.¹³ Such ionic transports are known to be controlled by hKLK1 in the renal distal tubules and may not be kinin-mediated.¹⁴

Further studies will be needed to identify the roles of a common polymorphism of allele H in the regulation of urinary excretions of

calcium and sodium under a controlled diet. The present results can be applied to a pharmacogenetic study to examine an inter-individual difference in the response to diuretics acting on the renal distal tubule.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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- 1 Katori, M. & Majima, M. The renal kallikrein-kinin system: its role as a safety valve for excess sodium intake, and its attenuation as a possible etiologic factor in salt-sensitive hypertension. *Crit. Rev. Clin. Lab. Sci.* **40**, 43–115 (2003).
- 2 Katori, M. & Majima, M. A missing link between a high salt intake and blood pressure increase. *J. Pharmacol. Sci.* **100**, 370–390 (2006).
- 3 Slim, R., Torremocha, F., Moreau, T., Pizard, A., Hunt, S. C., Vuagnat, A. *et al.* Loss-of-function polymorphism of the human kallikrein gene with reduced urinary kallikrein activity. *J. Am. Soc. Nephrol.* **13**, 968–976 (2002).
- 4 Song, Q., Chao, J. & Chao, L. DNA polymorphisms in the 5'-flanking region of the human tissue kallikrein gene. *Hum. Genet.* **99**, 727–734 (1997).
- 5 Hua, H., Zhou, S., Liu, Y., Wang, Z., Wan, C., Li, H. *et al.* Relationship between the regulatory region polymorphism of human tissue kallikrein gene and essential hypertension. *J. Hum. Hypertens.* **19**, 715–721 (2005).
- 6 Fujita, T., Ogino, M., Daigo, F., Yamaguchi, T. & Majima, M. Intracellular Ca²⁺ contributes to K⁺-induced increase in renal kallikrein secretion. *Int. Immunopharmacol.* **6**, 1487–1495 (2006).
- 7 Zhao, W., Wang, L., Lu, X., Yang, W., Huang, J., Chen, S. *et al.* A coding polymorphism of the kallikrein 1 gene is associated with essential hypertension: a tagging SNP-based association study in a Chinese Han population. *J. Hypertens.* **25**, 1821–1827 (2007).
- 8 Tsai, T. J., Chen, Y. M., Hsieh, B. S. & Chen, W. Y. Comparison between spot urine and overnight urine in the estimation of 24-hour excretion of urine protein, sodium and kallikrein. *J. Formos. Med. Assoc.* **90**, 755–759 (1991).
- 9 Suzuki, T., Katori, M., Fujita, T., Kumagai, Y. & Majima, M. Involvement of the renal kallikrein-kinin system in K(+)-induced diuresis and natriuresis in anesthetized rats. *Eur. J. Pharmacol.* **399**, 223–227 (2000).
- 10 Picard, N., Van Abel, M., Campone, C., Seiler, M., Bloch-Faure, M., Hoenderop, J. G. *et al.* Tissue kallikrein-deficient mice display a defect in renal tubular calcium absorption. *J. Am. Soc. Nephrol.* **16**, 3602–3610 (2005).
- 11 Gkiika, D., Topala, C. N., Chang, Q., Picard, N., Thébaud, S., Houillier, P. *et al.* (2006) Tissue kallikrein stimulates Ca(2+) reabsorption via PKC-dependent plasma membrane accumulation of TRPV5. *EMBO. J* **25**, 4707–4716 (2006).
- 12 Blanchard, A., Azizi, M., Peyrard, S., Stern, N., Alhenc-Gelas, F., Houillier, P. *et al.* Partial human genetic deficiency in tissue kallikrein activity and renal calcium handling. *Clin. J. Am. Soc. Nephrol.* **2**, 320–325 (2007).
- 13 Picard, N., Eladari, D., El Moghrabi, S., Planès, C., Bourgeois, S., Houillier, P. *et al.* Defective ENaC processing and function in tissue kallikrein-deficient mice. *J. Biol. Chem.* **283**, 4602–4611 (2008).
- 14 Pizard, A., Richer, C., Bouby, N., Picard, N., Meneton, P., Azizi, M. *et al.* Genetic deficiency in tissue kallikrein activity in mouse and man: effect on arteries, heart and kidney. *Biol. Chem.* **389**, 701–706 (2008).