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Concise report

# URAT1 inhibition by ALPK1 is associated with uric acid homeostasis

ALPK1 *and* SLC22A12 *in uric acid homeostasis*

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## Abstract

**Objective.** The aim of this study was to identify a protein for urate transporter 1 (URAT1) regulation.

**Methods.** The clinical dataset consisted of 492 case–control samples of Han Chinese (104 gout and 388 controls). Three alpha kinase 1 (*ALPK1*) and *SLC22A12* loci associated with high gout risk and uric acid levels were genotyped. The overexpression of ALPK1 on URAT1 protein expression was evaluated *in vivo* in h*ALPK1* transgenic mice. The *in vitro* protein levels of ALPK1 and URAT1 in *ALPK1* small interfering RNA–transfected human kidney-2 cells with MSU crystal stimulation were examined.

**Results.** *ALPK1*, which is a single nucleotide polymorphism (SNP) of rs11726117 (M861T; T), reduced the risk of gout via the *SLC22A12* gene SNPs rs3825016 and rs475688, as compared with the subject of *ALPK1* rs11726117 (C) allele {rs11726117 [CT + TT] *vs* rs3825016, odds ratio [OR] 0.39 [95% confidence interval (CI) 0.23, 0.67]; rs11726117 [CT + TT] *vs* rs475688, OR 0.39 [95% CI 0.23, 0.67]}. ALPK1-over-expressed mice demonstrated lower levels of URAT1 protein ($P = 0.0045$). Mouse endogenous ALPK1 proteins were detected in renal proximal tubule cells. MSU crystals inhibited URAT1 expressions through an upregulation of ALPK1 in human kidney-2 cells.

**Conclusion.** Elevated ALPK1 expression decreased URAT1 expression. *ALPK1* might prevent the impact of urate reuptake via *SLC22A12* and appeared to be negatively associated with gout. ALPK1 is a potential repressor of URAT1 protein expression.

**Key words:** ALPK1, URAT1, MSU, hyperuricaemia, gout

BASIC SCIENCE

**Rheumatology key messages**

- The interactions between *ALPK1* and *SLC22A12* loci reduce the risk of gout via *SLC22A12*.
- Predominant expression of endogenous ALPK1 is detected in renal proximal tubule cells of mice.
- ALPK1 upregulation decreases the protein levels of URAT1 *in vivo* and *in vitro*.

## Introduction

Gout, a prevalent inflammatory arthritis that results from the intra-articular deposition of MSU crystals, is highly associated with hyperuricaemia. Most patients who develop gout have difficulty in eliminating renal uric acid. Almost all of the circulating urate is filtered by glomeruli, with only a small fraction (~10% of the uric acid) being

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typically excreted in the urine [1]. The amount of excreted uric acid results from a complex interplay between reabsorption and secretion in kidney proximal tubule cells [2].

Urate transporter 1 (URAT1), which is encoded by *SLC22A12* at 11q13 in humans, is a urate/anion exchanger in the apical membranes of renal proximal tubule cells that is responsible for renal urate reabsorption [2]. The loss-of-function mutations in URAT1 causing renal hypouricaemia has been mainly identified in Japanese patients [3]. The rate of uric acid fractional excretion in patients with URAT1 mutation defect reached almost 90% [4]. The association between *SLC22A12* and hyperuricaemia or gout in Han Chinese has already been reported [5]. These data suggest that URAT1 plays an essentially regulatory role in the homeostasis of serum uric acid level. However, the mechanism for the regulation of URAT1 levels remains largely unclear. Sex hormones, such as testosterone, regulate the expressions of URAT1 [6]. Nonetheless, no specific pathway or protein for the regulation of URAT1 levels was identified.

We aimed to identify a protein for URAT1 regulation. Alpha kinase 1 (ALPK1) of the 4q25 region has been linked to the inflammatory process of tophaceous gout [7, 8]. MSU crystals increase ALPK1 levels in the monocytic THP1 cells [8, 9]. ALPK1 inhibits testosterone production [10], which might thereby affect URAT1 levels in the kidney by regulating blood testosterone levels. In the current study, we found that rs11726117 of the *ALPK1* single nucleotide polymorphism (SNP) decreases the gout risk via *SLC22A12*. Therefore, we hypothesized that ALPK1 is a repressor for URAT1 activity in the kidney. Further results using mouse or cultured cell models indicated that ALPK1 inhibits URAT1 expression.

## Methods

### Study participants

The study participants (Han Chinese; $n = 492$, 104 gout and 388 controls) and the recruitment criteria for gout, hyperuricaemia and controls were as described previously [7]. The institutional review board (IRB 970200) and ethics committees from Kaohsiung Medical University approved the design of this study and all participants provided written informed consent.

### Genotype determination

Venous whole blood was obtained from all of the participants and DNA was extracted using standard extraction procedures (QIAGEN-Gentra Puregene Blood Kit, Gentra Systems, Minneapolis, MN, USA). Three loci of the *ALPK1* and *SLC22A12* rs3825016 locus were genotyped using TaqMan SNP Genotyping Assays with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA), as previously reported [5, 7]. Two gene loci that were genotyped without any sequence misses were used for all analyses.

### Animals

The ALPK1 transgenic mice were generated as previously described [10]. For this study, 15 homozygous male 10–12-week-old hALPK1 transgenic mice were obtained by backcrossing 19 or 20 filial generations. We used 15 wild-type male age-matched C57BL/6 mice that were purchased from the National Laboratory Animal Center (NLAC; Taipei, Taiwan). Animal procedures conformed to the guidelines published by the National Institutes of Health (publication no. 85-23) and were approved by the Institutional Animal Care and Use Committee of the China Medical University. Because the hALPK1 transgenic mice show moderately low male fertility, the data of murine URAT1 protein detection were combined from three independent experiments. For detection of the integrated transgene, the isolation of total genomic DNA and PCR were as described in the supplementary methods (section Genomic DNA extraction, PCR and real-time quantitative PCR of animals) available at *Rheumatology* Online. The ALPK1 Southern blotting analysis and real-time quantitative PCR were performed to determine the transgene copy number as described in supplementary Fig. S1, available at *Rheumatology* Online.

### Statistical analysis

Quantitative data for URAT1 protein expression were compared in wild-type and hALPK1 transgenic mice using the Wilcoxon rank-sum test. The mean (S.D.) expression levels of *ALPK1* and *URAT1* protein were determined in human kidney-2 (HK-2) cells using a *t*-test. Differences in demographic and clinical information among controls, hyperuricaemia and gout cases were analysed using the chi-square test for categorical variables and a general linear regression model for continuous variables. To evaluate the effects of the gene interaction on hyperuricaemia and gout risk, a multinomial logistic regression was conducted using a recessive genetic model that included the interactions of variants of *ALPK1* and *SLC22A12* after adjusting for covariates, age, sex and alcohol use. To interpret the independent and joint effects of the genes on hyperuricaemia and gout risk, a multinomial logistic regression using a recessive genetic analysis was applied after adjusting for covariates. Data handling and associations were performed using the SAS version 9.3 software package (SAS Institute, Cary, NC, USA). Detailed methodologies for cell culture, real-time quantitative PCR, immunohistochemistry analysis and western blot analysis are described in the supplementary Methods, available at *Rheumatology* Online.

## Results

### *ALPK1* rs11726117 (M861T) wild-type reducing the risk of gout via *SLC22A12* gene SNPs

Graessler *et al.* [11] reported that *SLC22A12* rs3825016 is associated with reduced fractional excretion of uric acid {odds ratio [OR] 2.25 [95% confidence interval (CI) 1.31, 3.88], $P = 0.0035$} for the (C/T) + (T/T) genotype. Furthermore, we demonstrated that the *SLC22A12* C

**Table 1** Minor allele of *ALPK1* rs11726117 (M861T) reducing the risk of gout via *SLC22A12* gene SNPs[a]

| Locus | Gout cases, *n* (%) | Controls, *n* (%) | OR (95% CI) | *P*-value | Adjusted OR (95% CI)[b] | *P*-value |
|---|---|---|---|---|---|---|
| *ALPK1* rs11726117 | | | | | | |
| CC | 70 (67.31) | 197 (50.77) | 1.00 | | 1.00 | |
| CT | 31 (29.81) | 161 (41.49) | 0.54 (0.34, 0.87) | 0.0108 | 0.41 (0.24, 0.71) | 0.0016 |
| TT | 3 (2.88) | 30 (7.73) | 0.28 (0.08, 0.95) | 0.0413 | 0.25 (0.06, 1.05) | 0.0577 |
| *SLC22A12* rs3825016 | | | | | | |
| TT | 2 (1.92) | 10 (2.58) | 1.00 | | 1.00 | |
| CT | 39 (37.5) | 137 (35.31) | 1.31 (0.28, 6.12) | 0.7340 | 1.22 (0.24, 6.33) | 0.8122 |
| CC | 63 (60.58) | 241 (62.11) | 1.42 (0.30, 6.77) | 0.6574 | 1.44 (0.27, 7.63) | 0.6685 |
| *SLC22A12* rs475688 | | | | | | |
| TT | 16 (15.38) | 95 (24.48) | 1.00 | | 1.00 | |
| CT | 42 (40.38) | 193 (49.74) | 1.29 (0.69, 2.42) | 0.4224 | 2.01 (0.97, 4.16) | 0.0605 |
| CC | 46 (44.23) | 100 (25.77) | 2.73 (1.45, 5.15) | 0.0019 | 4.18 (1.97, 8.83) | 0.0002 |
| Combined group 1 | | | | | | |
| *ALPK1* rs11726117 | | | | | | |
| CC and CT + TT | 32 (30.77) | 76 (19.59) | 1.00 | | | |
| CC and CC | 38 (36.54) | 121 (31.19) | 0.75 (0.43, 1.29) | 0.2968 | 0.58 (0.30, 1.09) | 0.0922 |
| CT + TT and CT + TT | 9 (8.65) | 71 (18.30) | 0.30 (0.13, 0.68) | 0.0036 | 0.16 (0.06, 0.43) | 0.0002 |
| CT + TT and CC | 25 (24.04) | 120 (30.93) | 0.50 (0.27, 0.90) | 0.0209 | 0.36 (0.18, 0.72) | 0.0039 |
| Condition: *SLC22A12* rs3825016 | | | | | | |
| CC | 70 (67.3) | 197 (50.8) | 1.00 | | 1.00 | |
| CT + TT | 34 (32.7) | 191 (49.2) | 0.50 (0.32, 0.79) | 0.0029 | 0.39 (0.23, 0.67) | 0.0006 |
| Combined group 2 | | | | | | |
| *ALPK1* rs11726117 | | | | | | |
| CC and CT + TT | 33 (31.73) | 147 (37.89) | 1.00 | | 1.00 | |
| CC and CC | 37 (35.58) | 50 (12.89) | 3.30 (1.87, 5.82) | <0.0001 | 3.42 (1.76, 6.64) | 0.0003 |
| CT + TT and CT + TT | 25 (24.04) | 141 (36.34) | 0.79 (0.45, 1.40) | 0.4161 | 0.60 (0.31, 1.16) | 0.1259 |
| CT + TT and CC | 9 (8.65) | 50 (12.89) | 0.80 (0.36, 1.79) | 0.5902 | 0.73 (0.29, 1.83) | 0.5066 |
| Condition: *SLC22A12* CC rs475688 | | | | | | |
| CC | 70 (67.3) | 197 (50.8) | 1.00 | | 1.00 | |
| CT + TT | 34 (32.7) | 191 (49.2) | 0.50 (0.32, 0.79) | 0.0029 | 0.39 (0.23, 0.67) | 0.0006 |

[a]Two gene loci that were genotyped without any sequence misses were used for all analyses. [b]Adjusted ORs and their *P*-values were calculated after adjusting for age, BMI, total cholesterol, log triglycerides, creatinine, hypertension and alcohol use using a multiple logistic regression model.

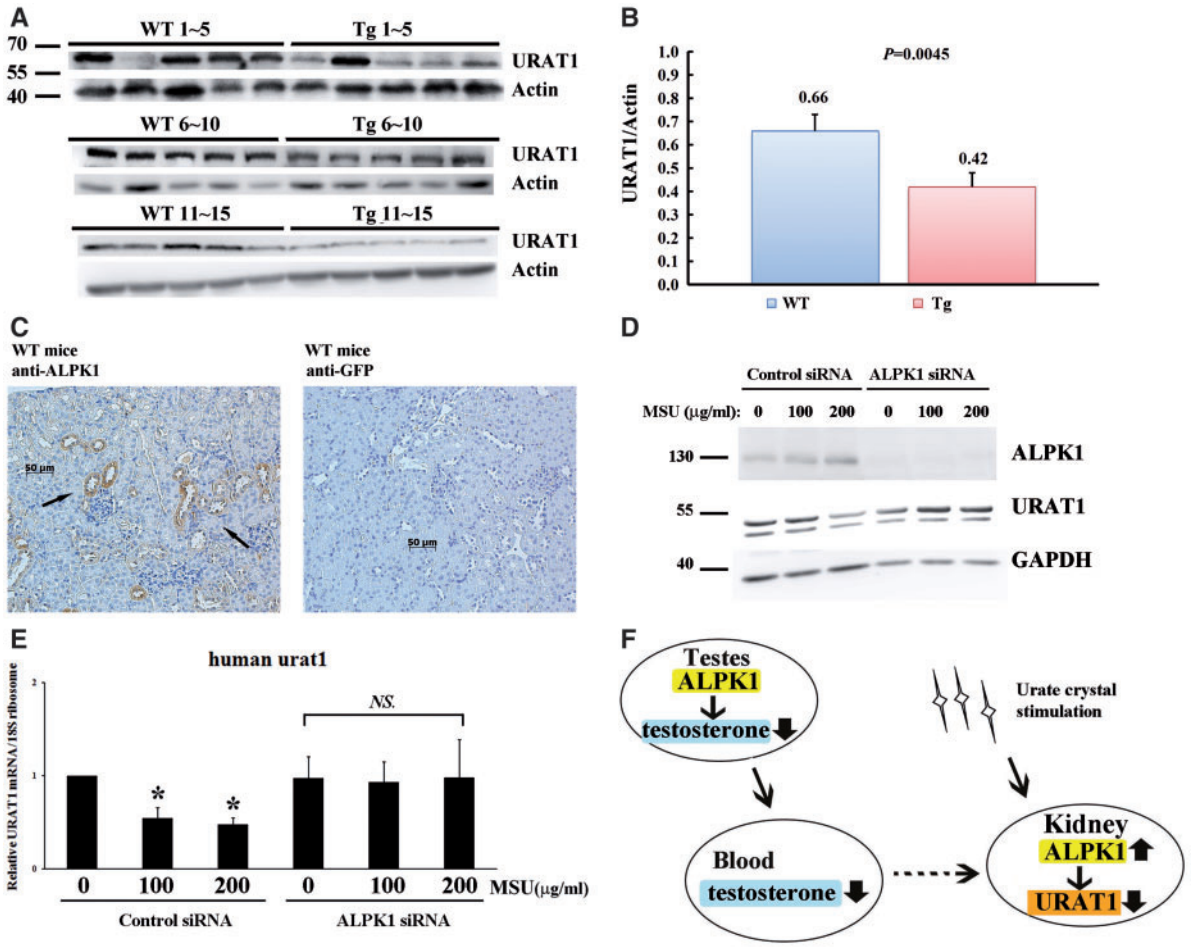
allele of rs475688 is associated with gout risk [in Han Chinese, OR 1.98 (95% CI 1.36, 2.88), $P = 0.0054$] and uric acid levels [5]. An *ALPK1* loci, non-synonymous rs11726117 M861T [C], is highly associated with gout risk [in Han Chinese, OR 2.41 (95% CI 1.41, 4.12), $P = 0.0025$] [7]. We investigated these *SLC22A12* and *ALPK1* loci. The subjects' baseline demographics and clinical characteristics are presented in supplementary Table S1, available at *Rheumatology* Online. Patients with gout had higher mean uric acid levels and a greater proportion of hyperuricaemia or hypertension. The frequency distributions of the *ALPK1* loci and *SLC22A12* rs3825016 or rs475688 are shown in Table 1. To explore the contribution of the *ALPK1* and *SLC22A12* loci to the risk of gout occurrence, a multinomial logistic regression analysis of the gout cases was conducted by introducing into the model an interaction term at *ALPK1* × *SLC22A12* after adjustment for covariates.

The results of epistatic interactions of the *ALPK1* loci and *SLC22A12* variant in gout cases *vs* controls are summarized in Table 1. The *ALPK1* rs11726117 risk allele (CC) and *SLC22A12* rs3825016 or rs475688 together produced a high risk of gout, whereas the *ALPK1* rs11726117 non-risk allele (CT + TT) and *SLC22A12* significantly decreased the risk of gout [rs11726117 (CT + TT) *vs* rs3825016, adjusted $P = 0.0006$, OR 0.39 (95% CI 0.23, 0.67); rs11726117 (CT + TT) *vs* rs475688, adjusted $P = 0.0006$, OR 0.39 (95% CI 0.23, 0.67)] compared with the *ALPK1* rs11726117 risk (CC) allele. The associations of these SNPs and urate levels are shown in supplementary Table S2, available at *Rheumatology* Online. Because *ALPK1* loci non-synonymous M861T rs11726117 (C) is associated with gout risk, the results indicated that a minor allele of *ALPK1* [rs11726117 (T)] reduces the risk of gout via *SLC22A12* gene SNPs.

## ALPK1-overexpressed mice show lower levels of URAT1 protein

The effects of ALPK1 on levels of URAT1 protein were confirmed experimentally using male transgenic (h*ALPK1* Tg) mice [10]. Mice carrying the transgene were identified by PCR of genomic DNA from kidney (supplementary Fig. S2A, available at *Rheumatology* Online). Expression of

FIG. 1 Upregulation of ALPK1 mediates URAT1 inhibition




(**A**) URAT1 protein expression in the mouse kidney. (**B**) Quantification of URAT1 protein levels in 15 mice per group. (**C**) Endogenous ALPK1 expression by immunohistochemistry on wild-type mice kidney sections (black arrow, left panel; negative control, right panel). Knockdown of ALPK1 reversed URAT1-inhibited effect of MSU, confirmed by (**D**) western blotting and (**E**) real-time quantitative PCR of ALPK1 or URAT1 (*$P < 0.05$ compared with the control small interfering RNA–transfected cells without MSU treatment). (**F**) Schematic model indicating the regulation of URAT1 expression by ALPK1. Dotted arrow indicates the speculated pathway. Both lower testosterone levels and MSU stimulation increase ALPK1 levels, which might lead to inhibition of URAT1 levels. NS: non-significant.

EGFP-hALPK1 protein in whole kidney lysate was confirmed (supplementary Fig. S2B, available at *Rheumatology* Online). High expression of endogenous URAT1 in wild-type mice was also detected in the kidney (supplementary Fig. S2C, available at *Rheumatology* Online).

We investigated whether the ability of ALPK1 to modify URAT1 protein expression was diminished in h*ALPK1* Tg mice compared with non-h*ALPK1* Tg (wild-type C57BL/6) mice (Fig. 1A). Levels of renal URAT1 protein were significantly lower ($P = 0.0045$) in h*ALPK1* Tg mice than in wild-type mice (Fig. 1B). These results suggest that higher ALPK1 levels correlate with lower URAT1 expression, which confirms the possibility of ALPK1's regulatory effects on URAT1 in mice.

## Endogenous ALPK1 expressed in renal proximal tubule cells

To confirm the expression of endogenous ALPK1 in renal tubule epithelial cells in mice, kidney sections from wild-type mice were immunostained with anti-ALPK1 antibodies. Abundant expression of ALPK1 was detected in the proximal tubule cells, indicating the possibility of a functional interaction between ALPK1 and URAT1 within these cells (Fig. 1C).

## MSU crystals decrease URAT1 through ALPK1 upregulation

To test the effects of increasing endogenous ALPK1 on URAT1 levels, HK-2, a human normal proximal tubule cell

line, was used to examine the effect of MSU crystals. We used *ALPK1* small interfering RNA to knock down endogenous *ALPK1* gene expression in HK-2 cells. MSU crystals increased ALPK1 protein levels in HK-2 cells, whereas URAT1 protein levels were decreased. MSU crystals failed to reduce URAT1 mRNA levels once ALPK1 was knocked down (Fig. 1D and E and supplementary Fig. S3, available at *Rheumatology* Online). These results show that MSU crystals decrease the URAT1 level through ALPK1 upregulation in HK-2 cells.

## Discussion

Previous studies have used genome-wide linkage analysis to show that the *Gout Susceptibility 1* gene lies on chromosome 4q21–25, and a region-wide association study fine-mapped *ALPK1* to 4q25 [7]. Mechanisms underlying the synergistic effect of ALPK1 on MSU crystal–induced inflammatory responses have been proposed [8] and suggest that ALPK1 contributes to the inflammatory process associated with the development of gout [7, 8]. Nonetheless, an association between *ALPK1* and gout was not found in a study of Japanese subjects [12]. In addition to gout, ALPK1 is associated with chronic kidney disease and other chronic diseases [13]. Indeed, increased expression of ALPK1 in the kidney of patients with diabetic glomerulosclerosis or leucocytes of gout patients has been observed [8, 13]. ALPK1 is also involved in sorting proteins into apical transport vesicles or the trans-Golgi network [14]. Nevertheless, the cellular function of ALPK1 has not been clearly characterized.

A number of studies have reported that patients, most of whom originate from Asia, have loss-of-function mutations (in either the compound heterozygous and/or homozygous state) in the *SLC22A12* gene [Online Mendelian Inheritance in Man (OMIM) 220150; renal hypouricaemia-1] [15]. Nonetheless, several studies have also suggested that renal hypouricaemia is not necessarily restricted to East Asian populations [4, 16]. The *SLC22A12* SNPs rs3825016 and rs475688 are associated with gout risk and uric acid levels [5, 11]. A study reported that the synonymous variant rs7932775 and intron polymorphism ss161109885 in *SLC22A12* show a joint additive effect on hyperuricaemia in the Han Chinese [17]. However, another study indicated that there was no statistically significant association between rs7932775, rs3825016 and serum uric acid concentrations in mutant allele carriers in the Czech population [18]. Further investigation is required to study whether an enhanced reabsorption of urate causes hyperuricaemia. In addition to promotion of MSU formation resulting from hyperuricaemia, the increase in uric acid level also enhances inflammatory response. Therefore these specific *SLC22A12* genotypes could be implicated in the gain-of-function role in urate reabosorption. In the present study we found that *ALPK1* variant rs11726117 (T) reduces the risk of gout via these *SLC22A12* SNPs. Because rs11726117 may impact the serine/threonine activity of ALPK1 [7], the kinase activity of ALPK1 may be involved in regulation of URAT1 levels in uric acid homeostasis.

Stimulation with MSU crystals increases the expression of CMV promoter-driven URAT1 expression in HEK293 cells [19]. Here we found that MSU crystals decrease endogenous URAT1 in HK-2 cells. Deposition of urate crystals in the kidney has been observed in patients with chronic gouty arthritis [20], which also raises the possibility of expression changes on urate transporters by urate crystal stimulation in the kidney. Testosterone increases the expression of URAT1. ALPK1 inhibits testosterone production *in vivo* and *in vitro*, whereas testosterone reduces ALPK1 levels in murine primary kidney cells [10]. Therefore, lower testosterone levels might be involved in URAT1 regulation by ALPK1. Based on previous and present findings, we propose a regulatory model of URAT1 by ALPK1 (Fig. 1F). In the testes, ALPK1 decreases testosterone production, which is a normal physiological and molecular mechanism in the dynamic regulation of testosterone, and also contributes to the regulation of this hormone throughout the whole body by regulating testosterone levels in the blood [10]. Lower testosterone in the blood then diminishes ALPK1 suppression in the kidney cells, which also might be involved in the inhibition of *URAT1* expression. Urate crystal deposition in the kidney also causes ALPK1 upregulation and URAT1 repression. *ALPK1* variants result in the differential ability to effectively inhibit URAT1, which might be related to uric acid homeostasis.

In conclusion, *ALPK1* inhibits the impact of urate reuptake via *SLC22A12* and appears to be negatively associated with gout. ALPK1 upregulation decreases the expression level of URAT1 in the kidney. The effect of *ALPK1* rs11726117 on URAT1 levels might be an indirect result of altered testosterone levels. We do not exclude that other variations of the *ALPK1* gene with differential activities upon MSU stimulation may contribute to gout susceptibility. These results also add to our understanding of the regulation of uric acid homeostasis and the development of uric acid–lowering therapies.

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## Supplementary data

Supplementary data are available at *Rheumatology* Online.

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