6 Dequeker JV. Polymyalgia rheumatica with temporal arteritis, as painted by Jan Van Eyck in 1436. Can Med Assoc J 1981;124:1597–8.

7 Fornaciari G, Fontecchio G, Ventura L *et al*. Rheumatoid arthritis in Cardinal Carlo de' Medici (1595–1666): a confirmed macroscopic, radiologic and molecular diagnosis. Clin Exp Rheumatol 2012;30:12–22.

8 Poma AM, Ventura L, Fontecchio G. Further genomic testing and histological examinations confirm the diagnosis of rheumatoid arthritis in an Italian mummy from the XVI century. Ann Rheum Dis 2012;71:630.

Rheumatology 2020;59:3988–3990
doi:10.1093/rheumatology/keaa461
Advance Access publication 4 October 2020

Identification of a dysfunctional splicing mutation in the *SLC22A12*/*URAT1* gene causing renal hypouricaemia type 1: a report on two families

Rheumatology key message

- Heterozygous as well as homozygous mutations of *SLC22A12* can cause renal hypouricaemia with its complications.

Dear Editor, We herein report two Japanese families with inherited renal hypouricaemia (RHUC) associated with a functionally null variant in an exon–intron boundary of the *urate transporter 1* (*URAT1*, also known as *SLC22A12*) gene. URAT1 dysfunction is reported to cause RHUC type 1 [1, 2] and several variants of *URAT1* have also been reported to be associated with serum uric acid (SUA) level [3, 4]. Glucose transporter 9 (GLUT9, also known as SLC2A9) dysfunction is reported to cause RHUC type 2 [5, 6]. This inherited and heterogeneous disorder is characterized by low SUA levels [$\leqslant$2 mg/dl or 120 μM (normal range 3.0–7.0 mg/dl)] resulting from increased renal urate excretion due to insufficient urate reabsorption: it causes severe complications such as exercise-induced acute kidney injury and urolithiasis [7]. However, we found some patients who have no exonic mutations in *URAT1* that cause RHUC. We consider this study to be the first report of a *URAT1* intronic variant as an aetiologic factor for RHUC.

The pedigrees are described in Fig. 1A. Patient II:1 in family 1 and 2 exhibited extremely low levels of SUA (0.5 mg/dl and 1.0 mg/dl, respectively) and a markedly high level of fractional excretion of uric acid [$FE_{UA,}$ 55.1% and 42.9%, respectively (normal range 5.5–11.1%)], which are typical features of RHUC. These results suggest that dysfunctional variants of *URAT1* or *GLUT9* could be involved in the RHUC seen in our cases. Detailed information on subjects are available in the supplementary note and Table S1, available at *Rheumatology* online. This study was approved by the institutional ethical committees. Written consent was obtained from all participants. All procedures involved were performed in accordance with the Declaration of Helsinki.

To explore the possible causes of these two familial RHUC cases, we conducted genetic analyses targeting *URAT1*. Direct sequencing was initially performed for these cases to seek W258X and R90H in *URAT1*—the first and second most frequent dysfunctional mutations that cause RHUC in the Japanese population. However, we detected only a heterozygous R90H mutation in family 2 and no mutations in family 1. Direct sequencing of all exons of *URAT1* was next performed as shown in supplementary methods and Table S2, available at *Rheumatology* online, which identified an intronic *URAT1* variant (rs58174038, c.506 + 1G>A) in the boundary region between exon 2 and intron 2 in both families (Fig. 1A and B).

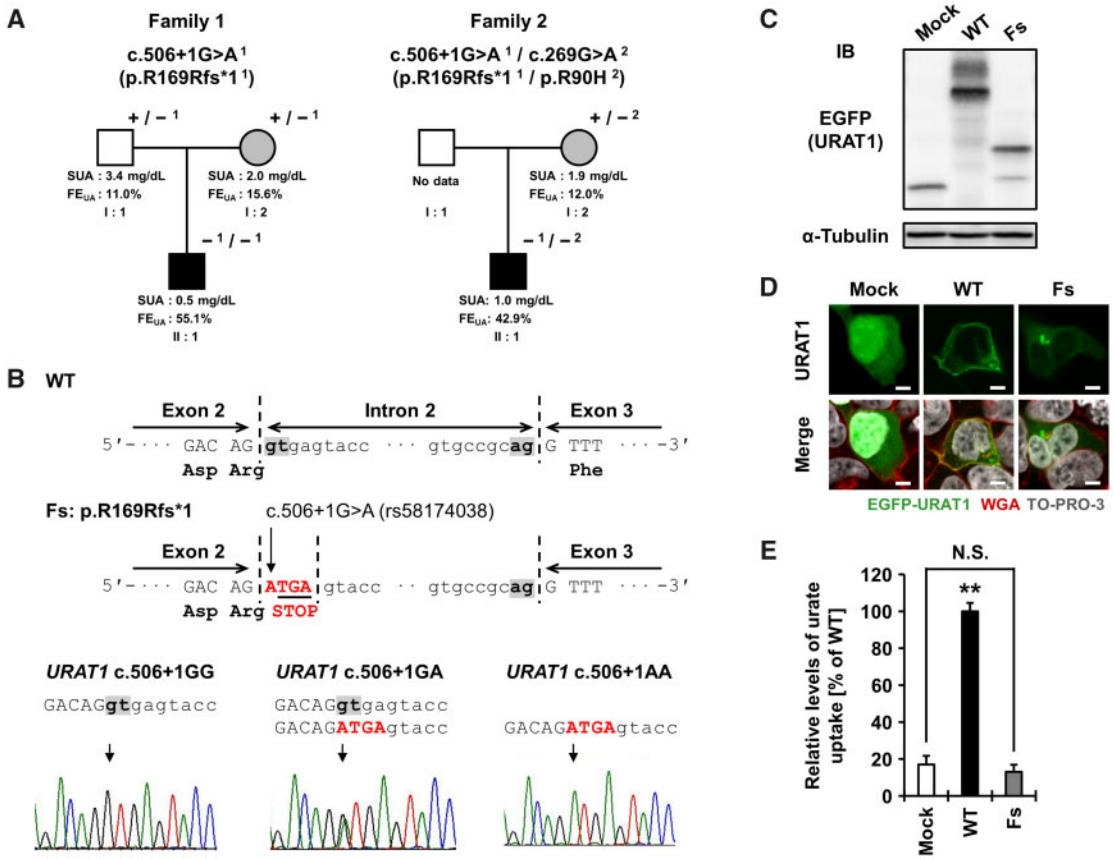
Patient II:1 of family 1 with RHUC presented homozygous mutations, indicating that two alleles of this variant are demonstrably related to the development of RHUC. Notably, subject I:2 (the proband's mother; c.506 + 1G>A heterozygote) of family 1 (SUA 2.0 mg/dl, FE_{UA} 15.6%) also met the diagnostic criteria for RHUC (SUA $\leqslant$2.0 mg/dl) [7] and had a past history of urolithiasis. Whereas subject I:1 (the proband's father; c.506 + 1G>A heterozygote) did not meet the diagnostic criteria for RHUC, the effect of one allele of this splicing mutation was suggested by the slightly lowered SUA for men (3.4 mg/dl) and mild elevation of FE_{UA} (11.0%).

Patient II:1 of family 2 had a non-synonymous variant (R90H), reported as a functionally null mutation [2], in addition to a variant of c.506 + 1G>A (Fig. 1A). When compared with those of his mother (patient I:2) having only a heterozygous R90H in *URAT1* (SUA 1.9 mg/dl, FE_{UA} 12.0%), patient II:1 (the compound heterozygote of c.506 + 1G>A and R90H) exhibited severely low SUA (1.0 mg/dl) and ~3.5-fold higher FE_{UA} (42.9%). It is therefore probable that *URAT1* c.506 + 1G>A is responsible for this familial RHUC due to the disruption of URAT1's function as a urate reabsorption transporter.

We therefore performed functional validation using cell-based assays [8] to examine the effects of c.506 + 1G>A (supplementary methods and Table S3, available at *Rheumatology* online). This variant disrupts the original splice donor site in intron 2 of *URAT1* (Fig. 1B), which appeared to result in the production of a premature stop codon (p.R169Rfs*1). We constructed the expression vector for this frameshift variant using a site-directed mutagenesis technique from pEGFP-C1/URAT1 wild-type plasmid for EGFP-URAT1 expression as a starting material. First, we performed immunoblot analysis using an anti-EGFP antibody to detect EGFP-tagged URAT1 (Fig. 1C). As expected, unlike the URAT1 wild-type, the frameshift variant was expressed as a truncated form. Next, confocal microscopy revealed that under our experimental conditions, URAT1 wild-type was localized on the plasma membrane, while the frameshift variant was rarely observed on the cell surface (Fig. 1D). Finally, our urate transport assay confirmed the frameshift variant to be functionally null (Fig. 1E).

Considering the following three points together with the renal expression pattern of URAT1 [1], we conclude that this splicing mutation in *URAT1* is responsible for RHUC.

FIG. 1 Identification and functional validation for c.506+1G>A of *URAT1* in two families with renal hypouricaemia (RHUC)



(**A**) Two families with RHUC. +, wild-type allele; $-^{1}$, mutant allele of c.506+1G>A (rs58174038); $-^{2}$, mutant allele of R90H. White, unaffected subject; grey, subjects with a mutant allele; black, subjects with two mutant alleles. (**B**) The position and representative sequences of c.506+1G>A in *URAT1*. WT, wild-type; Fs, frameshift. (**C**) Immunoblot detection of URAT1 protein from transfected 293A cells. (**D**) Confocal microscopic observations of URAT1 protein. Bars, 5 μm. (**E**) Urate transport activities by cell-based transport assay. $^{**}P < 0.01$ *vs* the other groups; N.S., not significant. Detailed information for Fig. 1 is described in supplementary material, available at *Rheumatology* online.

First, this splicing mutation in *URAT1* caused almost null function as a urate reabsorption transporter (Fig. 1E). Second, patients with this dysfunctional variant had increased FE_{UA} levels and decreased SUA levels. Third, clinical genetic analyses of the two families with RHUC with this variant revealed consistent results.

In summary, we have identified a functionally null intronic mutation of *URAT1* that causes RHUC in two Japanese pedigrees. These findings contribute to a better understanding of the genetic aetiology of RHUC.

Acknowledgements

We would like to thank all the participants for their generous involvement in this study. We are indebted to M. Miyazawa and K. Morichika (National Defence Medical College) for genetic analysis. We are grateful to H. Ueda (Osaka City General Hospital) for sample collection and clinical analysis. Y.K., Y.T., T.H., T.T. and H.M. conceived and designed the study. T.O., R.H., M.Y., I.K., R.F. and H.M. analysed the clinical data of the cases. Y.T. and T.T. performed functional analyses. Y.K., T.H., A.N., S.S. and H.M. performed the genetic analyses. H.S. and N.S. provided intellectual input and assisted with the preparation of the manuscript. Y.K., Y.T., A.N., T.T. and H.M. wrote the manuscript. H.M. organized clinico-genetic analysis and T.T. supervised functional analysis for our paper.

Funding: This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, including MEXT Kakenhi (17H04128 and 19K22786) and JSPS Kakenhi grants (16H01808, 18KK0247, 19K16441, 20H00566 and 20H00568), The Uehara Memorial Foundation, Mochida Science Foundation for Medical and Pharmaceutical Research, Takeda Medical Foundation, MSD Life Science Foundation, Public Interest Incorporated Foundation, Kawano Masanori Memorial Foundation for Promotion of Pediatrics and the Gout and Uric Acid Foundation of Japan.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Yusuke Kawamura[1,*], Yu Toyoda [2,*], Takuma Ohnishi[3], Ryutaro Hisatomi[4], Toshihide Higashino[1], Akiyoshi Nakayama[1], Seiko Shimizu[1], Masato Yanagi[3], Isamu Kamimaki[3], Rika Fujimaru[4], Hiroshi Suzuki[2], Nariyoshi Shinomiya[1], Tappei Takada[2,*] and Hirotaka Matsuo[1,*]

[1]*Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, Tokorozawa,* [2]*Department of Pharmacy, The University of Tokyo Hospital, Tokyo,* [3]*Department of Pediatrics, National Hospital Organization Saitama Hospital, Wako and* [4]*Department of Pediatrics, Osaka City General Hospital, Osaka, Japan*
Accepted 19 June 2020
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: matsuo29@gmail.com

*Yusuke Kawamura and Yu Toyoda contributed equally to this manuscript. Also, Hirotaka Matsuo and Tappei Takada contributed equally to this manuscript as senior authors.

References

1 Enomoto A, Kimura H, Chairoungdua A *et al*. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature 2002;417:447–52.

2 Ichida K, Hosoyamada M, Hisatome I *et al*. Clinical and molecular analysis of patients with renal hypouricemia in Japan-influence of URAT1 gene on urinary urate excretion. J Am Soc Nephrol 2004;15:164–73.

3 Nakatochi M, Kanai M, Nakayama A *et al*. Genome-wide meta-analysis identifies multiple novel loci associated with serum uric acid levels in Japanese individuals. Commun Biol 2019;2:115.

4 Misawa K, Hasegawa T, Mishima E *et al*. Contribution of rare variants of the *SLC22A12* gene to the missing heritability of serum urate levels. Genetics 2020;214: 1079–90.

5 Matsuo H, Chiba T, Nagamori S *et al*. Mutations in glucose transporter 9 gene *SLC2A9* cause renal hypouricemia. Am J Hum Genet 2008;83:744–51.

6 Dinour D, Gray NK, Campbell S *et al*. Homozygous *SLC2A9* mutations cause severe renal hypouricemia. J Am Soc Nephrol 2010;21:64–72.

7 Nakayama A, Matsuo H, Ohtahara A *et al*. Clinical practice guideline for renal hypouricemia (1st edition). Hum Cell 2019;32:83–7.

8 Miyata H, Takada T, Toyoda Y *et al*. Identification of Febuxostat as a new strong ABCG2 inhibitor: potential applications and risks in clinical situations. Front Pharmacol 2016;7:518.

Rheumatology 2020;59:3990–3992
doi:10.1093/rheumatology/keaa527
Advance Access publication 21 October 2020

Which disease activity outcome measure discriminates best in axial spondyloarthritis? A systematic literature review and meta-analysis

Rheumatology key message

- ASDAS disease activity response criteria are more discriminative than ASAS response or status criteria.

Dear Editor, Several disease activity response and status criteria are currently used to assess treatment efficacy in Randomized Controlled Trials (RCTs) in axial spondyloarthritis (axSpA). Response criteria include: the Assessment of SpondyloArthritis international Society (ASAS)-based ASAS 20, ASAS 40 and ASAS 5/6; the BASDAI 50; the Ankylosing Spondylitis Disease Activity Score (ASDAS)-based clinically important improvement (ASDAS-CII) and major improvement (ASDAS-MI). Additionally, the following disease activity status are used: ASAS partial remission (ASAS-PR), ASDAS-low DA (ASDAS-LDA) and ASDAS-inactive disease (ASDAS-ID) [1, 2] (Supplementary Table S1, available at *Rheumatology* online). All these nine are variably used in RCTs, but it remains unknown which one is the most discriminative.

The aim of the present study was to compare the ability of different outcome measures to discriminate between active treatment and placebo in axSpA RCTs.

A systematic literature review (SLR) was performed in Medline and Embase to identify RCTs of biological (b) and targeted-synthetic (ts) DMARDs in axSpA. RCTs were first retrieved including data from two previous SLRs identifying RCTs in axSpA [3, 4] and secondly updating literature search, with the same search terms of the SLRs, until 31 December 2019. Placebo-controlled RCTs meeting their primary end point were included, provided they reported $\geqslant 2$ (of the nine) outcomes and were in the English language. Outcomes were collected at the time-point of primary endpoint assessment. Risk of bias was evaluated by the Cochrane tool. Data were pooled and meta-analysed with the Mantel-Haenszel method, calculating the χ^2 between the number of patients (percentage) fulfilling each outcome in the treatment *vs* the placebo arm. Higher χ^2 indicated better discrimination. Per meta-analysis, we pooled RCTs presenting the same sets of outcomes.

Eleven articles fulfilling inclusion criteria were retrieved from a preceding SLR about RCTs in axSpA (2001–2013), and 12 from another SLR (2009–2016) [3, 4]. The search update resulted in six eligible articles out of 130 hits. Thus, 29 RCTs were finally included in the present SLR. In total, 23/29 RCTs with primary endpoint at 12–16 weeks, all at a low risk of bias, could be meta-analysed. The other six RCTs had a later (e.g. 24 weeks) or earlier (e.g. 6 weeks) primary endpoint, thus could not be considered in the meta-analysis due to heterogeneity. Out of the 23 RCTs,