The potential pathogenic role of B-cells in sarcoidosis is supported by the presence of B cells surrounding granulomas [2]. B-cell activation factor (BAFF) has been reported to be elevated in the serum of patients with sarcoidosis [3]. BAFF has also been identified on immunohistochemistry in the area of granulomas from skin affected by sarcoidosis [3]. Furthermore, patients with active sarcoidosis had higher serum BAFF levels than patients with inactive disease or healthy controls [4]. BAFF was positively correlated with angiotensin-converting enzyme levels and hypergammaglobulinaemia [3, 4]. Naïve B cells were increased in patients with sarcoidosis [3]. In contrast, plasmablasts and memory B cells were reduced [3]. This differentiation is also seen when comparing patients with active sarcoidosis versus inactive disease or healthy controls [4]. Somatic hypermutations of IgG and IgA transcripts and downstream IgG subclasses occurred more frequently in patients with sarcoidosis, consistent with abnormal maturation of B cells [2].

Efficacy of rituximab in other organ manifestations of sarcoidosis has been described. In a series of four patients treated with rituximab for refractory granulomatous eye disease from sarcoidosis, three had clinical improvement [5]. Following rituximab, a woman with pulmonary disease (mediastinal lymphadenopathy and mild interstitial syndrome) and polyarticular arthritis had improvement in symptoms, as well as normalization of pulmonary function tests [6]. A woman with neurosarcoidosis was successfully treated with rituximab [7].

In a phase I/II study of rituximab (1000-mg infusion twice, 2 weeks apart) of 10 subjects with refractory pulmonary sarcoidosis, 4 had improvement of $>10\%$ in forced vital capacity and 5 had improvement in the 6-min walk of >30 m at week 24, but these results were not statistically significant, likely due to the small sample size [8]. By 52 weeks, the differences in forced vital capacity or the 6-min walk were no longer observed [8]; however, that may reflect the single course of rituximab at baseline without repeat administration.

Overall, the increasing number of reports suggesting efficacy of rituximab in sarcoidosis further underscores the importance of B cells in the pathogenesis of this condition. Our case suggests rituximab may be a potentially beneficial treatment in refractory cardiac sarcoidosis, a condition with high morbidity and mortality. Further studies on rituximab for management of sarcoidosis, including cardiac sarcoidosis, are necessary. Other therapies that target B cells would also warrant investigation.

Funding: No specific funding was received from any agencies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.
Disclosure statement: The authors have declared no conflicts of interest.

Megan L. Krause[1], Leslie T. Cooper[2], Panithaya Chareonthaitawee[3] and Shreyasee Amin[1,4]

[1]Division of Rheumatology, Department of Medicine, Mayo Clinic, Rochester, MN, [2]Department of Cardiovascular Diseases, Mayo Clinic, Jacksonville, FL, [3]Division of Cardiovascular Diseases, Department of Medicine, Mayo Clinic, Rochester, MN and [4]Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA
Revised version accepted 23 July 2015
Correspondence to: Shreyasee Amin, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA.
E-mail: amin.shreyasee@mayo.edu

References

1 Youssef G, Leung E, Mylonas I *et al*. The use of 18F-FDG PET in the diagnosis of cardiac sarcoidosis: a systematic review and metaanalysis including the Ontario experience. J Nucl Med 2012;53:241–8.

2 Kamphuis LS, van Zelm MC, Lam KH *et al*. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? Am J Respir Crit Care Med 2013;187:406–16.

3 Ueda-Hayakawa I, Tanimura H, Osawa M *et al*. Elevated serum BAFF levels in patients with sarcoidosis: association with disease activity. Rheumatology 2013;52:1658–66.

4 Saussine A, Tazi A, Feuillet S *et al*. Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10-producing regulatory B cells and high BAFF levels. PLoS One 2012;7:e43588.

5 Lower EE, Baughman RP, Kaufman AH. Rituximab for refractory granulomatous eye disease. Clin Ophthalmol 2012;6:1613–8.

6 Belkhou A, Younsi R, El Bouchti I, El Hassani S. Rituximab as a treatment alternative in sarcoidosis. Joint Bone Spine 2008;75:511–2.

7 Bomprezzi R, Pati S, Chansakul C, Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. Neurology 2010;75:568–70.

8 Sweiss NJ, Lower EE, Mirsaeidi M *et al*. Rituximab in the treatment of refractory pulmonary sarcoidosis. Eur Respir J 2014;43:1525–8.

Rheumatology 2016;55:191–194
doi:10.1093/rheumatology/kev350
Advance Access publication 30 September 2015

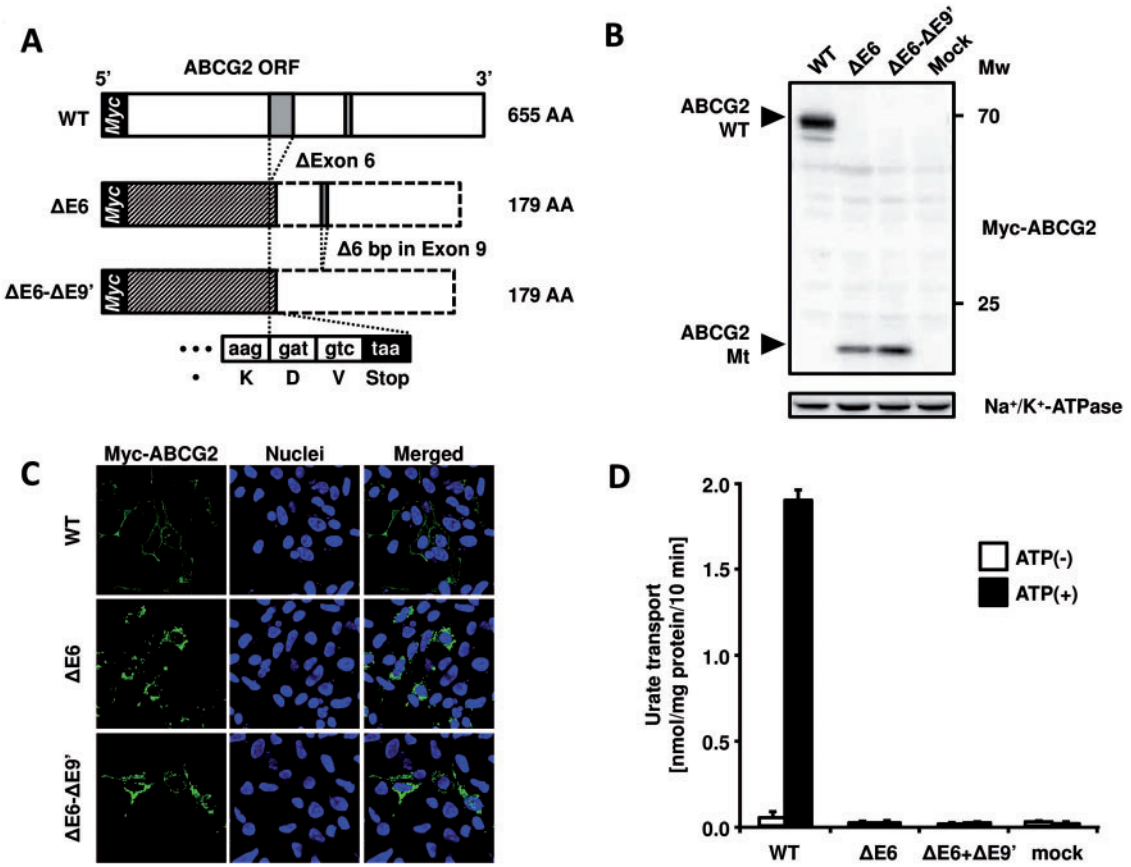
Novel dysfunctional variant in *ABCG2* as a cause of severe tophaceous gout: biochemical, molecular genetics and functional analysis

Rheumatology key message

- Dysfunctional variants of urate transporter *ABCG2* are an important genetic factor in hyperuricaemia and gout.

SIR, Gout is caused by hyperuricaemia. Several genes involved in renal urate transport, such as *SLC2A9*, *ABCG2* and *SLC22A12*, have been identified as risk factors for hyperuricaemia and gout [1–5]. More recently, a

FIG. 1 Dysfunction of ABCG2 ΔE6 and ΔE6-ΔE9' variants as urate transporters



(**A**) Schematic illustration of myc-tagged ABCG2 ORF and translational region. Shaded box shows the translational region. (**B**) Expression of ΔE6 and ΔE6-ΔE9′ variants as a truncated form of the ABCG2 protein. Forty-eight hours after the transfection of each expression vector with polyethylenimine MAX, HEK293A cells were subjected to immunoblot analysis with myc-specific antibody for detecting myc-tagged ABCG2 proteins. (**C**) Intracellular localization of ΔE6 and ΔE6-ΔE9′ variants in HEK293A cells. ABCG2 proteins were probed with anti-myc antibody and then labelled with green fluorescence dye (Alexa Fluor 488 goat anti-rabbit IgG); the nuclei were labelled with TOPRO3 (blue). (**D**) No transport activity in the ΔE6 and ΔE6-ΔE9′ variants. Bars are mean (S.D.); $n = 3$. $^{**}P < 0.01$ compared with WT by Dunnett's post hoc test. AA: amino acids; bp: base pair; ΔE6 and ΔE6-ΔE9′: mutant variants (see main text); Mt: mutant variants; Mw: molecular weight; Na^+/K^+-ATPase: a control for equal loading; ORF: open reading frame; WT: wild-type.

genome-wide association study of gout uncovered that single-nucleotide polymorphisms (SNPs) of *SLC2A9* and *ABCG2* are associated with two types of gout, renal underexcretion and renal overload, respectively [6]. The genome-wide association study, however, has limitations in establishing causality of disease-associated SNPs. Therefore, experimental validations are essential to determine if interesting variants are indeed responsible for clinical symptoms. As reported in our previous study of common *SLC2A9* allelic variants, we detected variants with no evidence of an association with hyperuricaemia and gout [7]. In this letter we present a biochemical, molecular genetic and functional case study suggesting a causal link between a hitherto undescribed sequence variant in the *ABCG2* gene and a severe gouty phenotype.

The patient, a participant of our study on common *SLC2A9* allelic variants [7], was a 47-year-old male who started having gout attacks at the age of 29 years. His medical history was unremarkable and no close relative was known to have gout or hyperuricaemia. Unfortunately, none of his close relatives agreed to an analysis of their biological material. When first seen in our clinic, he already suffered from chronic debilitating polyarthritis with widespread bulky tophi located mainly on his hands and feet and around the elbows and knees, with intermittent discharge of a chalky substance (supplementary Fig. S1, available at *Rheumatology* Online). Monosodium urate crystals were microscopically verified from the SF aspirated from his knee. Without treatment his serum uric acid level was 647 μmol/l.

To explore the cause of gout, we performed a metabolic investigation. The results suggested that his hyperuricaemia was mainly due to a defect in the urate excretion system and did not result from an excess

production of urate associated with purine metabolic disorders and/or with uromodulin-associated disorders (normal excretion of uromodulin, xanthine and hypoxanthine). Based on two separate measurements of the patient's fractional excretion of urate (FE_{UA}) and urinary urate excretion (UUE) (FE_{UA} 4.21%, UUE 14.8 mg/h/1.73m^2; and FE_{UA} 1.67%, UUE 31 mg/h/1.73 m^2), his hyperuricaemia could be classified as either renal underexcretion or a combined renal underexcretion and renal overload type, which corresponds to an *ABCG2* dysfunction [8].

Next we performed a sequencing analysis focusing on physiologically important urate transporters for further investigation. The analysis of *SLC22A12* revealed two sequence variants in intron regions and two synonymous variants in exon regions. The analysis of *SLC2A9* revealed eight variants in intron regions, three synonymous variants in exon regions and three heterozygous non-synonymous variants: rs2276961 (p.G25R), rs3733591 (p.R294H) and rs2280205 (p.P350L). Our previously reported approach, which used association analysis together with functional and immunohistochemical characterization of these variants in *SLC2A9*, did not show any influence on expression, subcellular localization or urate uptake of GLUT9 [7].

The analysis of *ABCG2* revealed eight variants in intron regions, an unpublished heterozygous intron variant c.689+1G>A and two exon variants, synonymous rs35622453 and heterozygous non-synonymous rs22231137 (p.V12M), which had little effect on the expression and urate transport activity of ABCG2 [6]. Two abnormal ABCG2 splicing variants were identified: r.[532_689del] deletion of exon 6 (ΔE6), and r.[532_689del; 944_949del] deletion of exon 6 and deletion of the first six base pairs of exon 9 (ΔE6-ΔE9′). Predictions for these variants showed an extra stop codon resulting from the frame shift.

To examine the effect of ΔE6 and ΔE6-ΔE9′ variants on the expression and function of the ABCG2 protein, we performed *in vitro* experiments. Fig. 1A shows the structure inserted in pcDNA3.1(+). Fig. 1B shows the immunoblot analysis. ABCG2 WT (wild-type) was estimated to be ~70 kDa (glycosylated form) and 65 kDa (non-glycosylated form), whereas the ΔE6 and ΔE6-ΔE9′ variants were ~18 kDa. In addition, protein expression levels of ΔE6 and ΔE6-ΔE9′ variants were lower than that of the WT version, suggesting that the mutated ABCG2 protein is not stable. Fig. 1C shows the immunofluorescence images of HEK293A cells expressing the ABCG2 protein. Strong ABCG2 signals were observed at the plasma membrane in cells expressing ABCG2 WT; however, the ΔE6 and ΔE6-ΔE9′ variant proteins were only detected within the intracellular compartment. These results indicate that the ΔE6 and ΔE6-ΔE9′ variants could not reach the plasma membrane. Finally, to assess the effect of ΔE6 and ΔE6-ΔE9' mutations on ABCG2 transport activity, ABCG2-mediated urate transport experiments were performed using plasma membrane vesicles prepared from ABCG2-expressing HEK293A cells. As shown in Fig. 1D, the ΔE6 and ΔE6-ΔE9′ variants of ABCG2 had no urate transport activity.

In conclusion, our findings suggest a causal relationship between the hitherto undescribed dysfunctional mutation of the *ABCG2* gene and a severe gouty phenotype, and strongly support the pathophysiological significance of ABCG2 as a risk factor for gout.

Acknowledgements

The authors would like to thank Petr Vyletal, PhD for uromodulin analysis (Institute of Inherited Metabolic Disorders, Prague, Czech Republic). In addition, the authors' thanks go to Dr Hirotaka Matsuo (National Defense Medical College, Saitama, Japan) and Dr Kimiyoshi Ichida (Jikei University School of Medicine, Tokyo, Japan) for engaging in a fruitful discussion on gout risk.

T.T. was supported by Japan Society for the Promotion of Science (JSPS) Kakenhi Grant Number 23689008, Kobayashi International Scholarship Foundation, the Mochida Memorial Foundation for Medical and Pharmaceutical Research and the Gout Research Foundation of Japan. Y.T. was a JSPS research fellow.

All the authors were involved in drafting the article or revising it critically for important intellectual content and all the authors approved the final version for publication. Study conception and design: B.S., H.M., Y.T., T.T., H.S.; clinical observation: J.Z., M.T.; acquisition of data: B.S., H.M.; analysis and interpretation of data: B.S., H.M., G.S., Y.T., T.T., K.P.

Funding: This study was supported by the grants from the Czech Republic Ministry of Health (RVO 023728 Institute of Rheumatology, RVO VFN64165, AZV 15-26693A).

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

Blanka Stiburkova[1,2,*], Hiroshi Miyata[3,*], Jakub Závada[1], Michal Tomčík[1], Karel Pavelka[1], Gabriela Storkanova[2], Yu Toyoda[3], Tappei Takada[3] and Hiroshi Suzuki[3]

[1]*Institute of Rheumatology,* [2]*Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic and* [3]*Department of Pharmacy, University of Tokyo Hospital, Tokyo, Japan*

Revised version accepted 12 August 2015

Correspondence to: Blanka Stiburkova, Institute of Rheumatology, Na Slupi 4, 128 50 Prague 2, Czech Republic. E-mail: stiburkova@revma.cz

*Blanka Stiburkova and Hiroshi Miyata contributed equally to the study.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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 Kolz M, Johnson T, Sanna S *et al*. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet 2009;5:e1000504.

3 Matsuo H, Takada T, Ichida K *et al*. Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. Sci Transl Med 2009;1:5ra11.

4 Ichida K, Matsuo H, Takada T *et al*. Decreased extra-renal urate excretion is a common cause of hyperuricemia. Nat Commun 2012;3:764.

5 Stibůrková B, Pavlíková M, Sokolová J *et al*. Metabolic syndrome, alcohol consumption and genetic factors are associated with serum uric acid concentration. PLoS One 2014;9:e97646.

6 Matsuo H, Yamamoto K, Nakaoka H *et al.* Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. Ann Rheum Dis 2015; doi: 10.1136/annrheumdis-2014-206191 [Epub ahead of print].

7 Hurba O, Mancikova A, Krylov V *et al*. Complex analysis of urate transporters SLC2A9, SLC22A12 and functional characterization of non-synonymous allelic variants of GLUT9 in the Czech population: no evidence of effect on hyperuricemia and gout. PLoS One 2014;9:e107902.

8 Matsuo H, Nakayama A, Sakiyama M *et al*. ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload. Sci Rep 2014;4:3755.