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 angiotenisin-converting enzyme levels and hypergammaglobulinaemia [3, 4]. Naive 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.

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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
A 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 (FEUA) and urinary urate excretion (UUE) (FEUA 4.21%, UUE 14.8 mg/h/1.73 m²; and FEUA 1.67%, UUE 31 mg/h/1.73 m²), 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 (∆E6), and r.[532_689del; 944_949del] deletion of exon 6 and deletion of the first six base pairs of exon 9 (∆E6-∆E9). Predictions for these variants showed an extra stop codon resulting from the frame shift.

To examine the effect of ∆E6 and ∆E6-∆E9 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 ∆E6 and ∆E6-∆E9 variants were ~18 kDa. In addition, protein expression levels of ∆E6 and ∆E6-∆E9 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 ∆E6 and ∆E6-∆E9 variant proteins were only detected within the intracellular compartment. These results indicate that the ∆E6 and ∆E6-∆E9 variants could not reach the plasma membrane. Finally, to assess the effect of ∆E6 and ∆E6-∆E9 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 ∆E6 and ∆E6-∆E9’ 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.

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Supplementary data
Supplementary data are available at Rheumatology Online.
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