CONCISE REPORT

Hyperuricaemia: contributions of urate transporter ABCG2 and the fractional renal clearance of urate

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ABSTRACT

Objective To investigate the contributions towards hyperuricaemia of known risk factors, focusing on fractional (renal) clearance of urate (FCU) and variation in the ATP-binding cassette transporter, sub-family G 2 (ABCG2) gene.

Methods The contributions of age, sex, ancestry, Q141K genotype for ABCG2, FCU, sugar-sweetened beverage and alcohol consumption, metabolic syndrome disorders and measures of renal function to the risk of hyperuricaemia were evaluated by comparing hyperuricaemic (serum urate≥0.42 mmol/L, n=448) with normouricaemic (serum urate<0.42 mmol/L, n=344) participants using stepwise logistic regression. Model performance was evaluated using the area under the receiver operator characteristic curve (AUROC).

Results ABCG2 genotype, FCU, male sex, body mass index, serum triglyceride concentrations, estimated glomerular filtration rate and consumption of alcohol were the best predictors of hyperuricaemia (AUROC 0.90, 81% accuracy). Homozygosity in the 141K variant for ABCG2 conferred an adjusted OR of 10.5 for hyperuricaemia (95% CI 2.4 to 46.2). For each 1% decrease in FCU, the adjusted OR increased by 51% (OR 1.51, 95% CI 1.37 to 1.66). There was no association between ABCG2 genotype and FCU (r=0.02, p=0.83).

Conclusions The ABCG2 141K variant and the FCU contribute strongly but independently to hyperuricaemia. These findings provide further evidence for a significant contribution of ABCG2 to extra-renal (gut) clearance of urate.

INTRODUCTION

Hyperuricaemia (serum urate (SU)>0.42 mmol/L) is the major risk factor for gout.1 Approximately two-thirds of SU is cleared by the kidneys, while the remainder is largely cleared by the gut.2 It has been widely accepted that in otherwise well individuals with normal glomerular filtration rates, the dominant cause of hyperuricaemia is inefficient clearance of urate via the kidneys, as quantified by a low fractional (renal) clearance of urate (FCU, a.k.a. fractional excretion of uric acid—FEua; renal urate clearance/creatinine clearance) relative to those with normal urate concentrations.3 However, there is considerable overlap in the distribution of FCU between normouricaemic and hyperuricaemic people, and factors other than FCU contribute to the risk of developing hyperuricaemia.

Combinations of partial and complete loss-of-function variants of the urate transporter, sub-family G 2 (ABCG2) gene in a Japanese population sample confer an increased risk of gout (maximum OR: 25.8, 95% CI 10.3 to 64).4 The partial loss-of-function 141K allele is associated with at least a twofold increase in the risk of gout in other ancestral groups.5 ABCG2 protein is expressed on the apical membrane of small intestine and colon epithelium, apical membrane of kidney proximal tubular cells and the canalicular membrane of hepatocytes.6,7 However, renal expression is low in comparison with the small intestine and the liver.5 Reduced and loss-of-function variants in this transporter are associated with significantly decreased extra-renal clearance of urate.8

Further, Ichida et al9 concluded that ABCG2 dysfunction led to extra-renal under-excretion and hence a 'renal overload' as indicated by an increased urinary excretion of urate (increased 24 h output of urate). As ABCG2 dysfunction is largely thought to affect extra-renal clearance, this variant should not affect FCU, a measure of renal tubular efficiency to clear urate. However, in a recent work, Matsuo et al11 observed an increase in FCU associated with ABCG2 dysfunction. This is inconsistent with the findings of Kötten et al12 of an association between the urate-raising 141K allele and a small reduction in FCU. The aim of this study was to further examine the effects of ABCG2 141K variant and FCU on the risk for hyperuricaemia. Other variables known to be associated with hyperuricaemia were also examined.

METHODS

Participants
We assembled a sub-cohort of hyperuricaemic participants (SU≥0.42 mmol/L; n=448) not taking medicines known to raise SU (thiazide diuretics and frusemide) from a New Zealand cohort recruited between 2006 and 2011;13 226 of our 448 participants had gout. A convenience sample of normouricaemic participants (SU<0.42 mmol/L; n=344) not taking urate-lowering medicines (allopurinol, probenecid, benzbromarone) was assembled at the same time. Ethical approval was obtained from the New Zealand Multi-Region Ethics Committee (MREC/05/10/130). All participants gave written informed consent.

Data
Age, sex, ancestry, height, weight, lean body weight,14 systolic and diastolic blood pressure, serum and urinary urate and creatinine, serum triglycerides and cholesterol, blood glucose and

Handling editor Tore K Kvien

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Received 21 June 2015
Revised 3 November 2015
Accepted 7 November 2015


Clinical and epidemiological research
ABC2 single nucleotide polymorphism rs2231142 (Q141K) (genotypes determined as previously described) were recorded. Urate and creatinine concentrations were measured using a Roche Modular P (Hitachi) analyser. The FCU is the ratio of urate to creatinine clearances expressed as a percentage.

Continuous variables were analysed as such and also as categorical variables following divisions based on clinical thresholds and population distributions (see online supplementary methods).

Data analysis
Variables differentiating hyperuricaemic from normouricaemic groups were examined using χ² analysis (categorical variable) and unpaired t tests (continuous variables). Associations of variables with SU and also associations between variables were assessed by linear regression, correlation coefficients (r) and analysis of variance with p≤0.05 indicating a significant association. Forward stepwise logistic regression was used when SU was analysed as a binary response variable (SU≥0.42 or <0.42 mmol/L). This was repeated in the hyperuricaemic cohort comparing asymptomatic participants with those with gout (see online supplementary methods). Variables with less than 90% complete data across the whole dataset were excluded from stepwise regression analyses. For independent explanatory variables with more than one categorical level, at least one category required a p≤0.05 to be considered a significant association. Multicollinearity between variables was tested. Variance explained by each stepwise logistic regression model was determined using the area under the receiver operator characteristic curve (AUROC). All data analysis was conducted using SPSS software (V.22.0.0.0; SPSS, Chicago, Illinois, USA).

RESULTS
Complete data were available for ABC2 rs2231142 genotype, FCU, serum triglyceride concentrations, age and sex (see online supplementary table S1) and over 95% complete data with the exception of blood glucose concentrations (70% complete data) were available. The minor allele (T-allele) frequency for the ABC2 141K allele was 14% in the total population.

Differences (p<0.05) were apparent between normouricaemic and hyperuricaemic participants for all factors with the exception of ancestry (p=0.18) (see online supplementary table S1). Mean body mass index (BMI) and serum triglyceride concentrations were higher while FCU and estimated glomerular filtration rate (eGFR) were lower in the hyperuricaemic cohort. Alcohol intake was higher in the hyperuricaemic group when evaluated as a continuous variable but did not reach significance when treated as a categorical variable.

Associations between variables
There was no association between FCU and ABC2 for all participants (r=0.02, p=0.91), Caucasians (r=0.09, p=0.30) or Polynesians (r=0.04, p=0.71) (figure 1). Separate adjustments for ancestry and sex (see online supplementary figure S1) did not alter this finding.

There was no correlation between FCU and eGFR (r=0.02, p=0.66), the mean FCU remaining consistent (~5%) across all eGFR values until greatly increasing at eGFR<15 mL/min/1.73 m² (n=5; see online supplementary figure S2). Caucasians had higher FCUs than East and West Polynesians (6.00 vs 4.87 and 4.71, p=1.3×10⁻⁵ and 1.7×10⁻⁴, respectively; Bonferroni adjusted p values). There was no difference in the FCU between East and West Polynesians (p=0.98).

Univariate risk factor analysis
All factors conferred significant odds ratios (ORs) for association with hyperuricaemia with the exception of alcohol consumption when quantified as a categorical variable (see online supplementary table S1). The strongest associations with hyperuricaemia were FCU, eGFR, BMI, male sex, high-density lipoprotein cholesterol and triglyceride concentrations and ABC2 genotype.

Multivariate risk factor analysis (stepwise logistic regression model)
The key variables identified as predictors for hyperuricaemia were ABC2 genotype, FCU, BMI, serum triglyceride concentration, eGFR and alcohol intake (g/week; table 1). For each 1% decrease in FCU, there was a 50% increase in the odds for hyperuricaemia. For each 10 mL/min/1.73 m² decrease in eGFR, there was a 69% increase in the odds for hyperuricaemia. There was no evidence of multicollinearity between explanatory variables. The logistic regression model was 81% accurate, 83% sensitive (hyperuricaemia) and 79% specific (normouricaemia). The model explained 90% of the variance (AUROC: 0.90, 95% CI 0.88 to 0.92, figure 2).

Excluded from the analysis were age due to a strong correlation with eGFR, serum glucose concentration because of insufficient data (<90%) and ancestry as the distribution of Polynesians was not representative of the New Zealand population. Across all variables, 90% (n=714) of participants had complete data for multivariate analysis.

Figure 1 Relationship between FCU and ABC2 genotype in (A) all participants, (B) Caucasians and (C) Polynesians is shown. All bars are shown in mean±SEM. Association (r) between FCU and ABC2: (A) r=0.02, (B) r=0.09 and (C) r=0.04. ABC2, ATP-binding cassette transporter, sub-family G 2; FCU, fractional clearance of urate.
**Table 1** Stepwise logistic regression model of risk factors for hyperuricaemia (SU≥0.42 mmol/L)

<table>
<thead>
<tr>
<th>Significant variables</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG2 Q141K genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Heterozygous</td>
<td>2.71 (1.60 to 4.60)</td>
<td>2.3×10⁻⁴</td>
</tr>
<tr>
<td>Homozygous</td>
<td>10.15 (2.37 to 43.43)</td>
<td>1.8×10⁻³</td>
</tr>
<tr>
<td>FCU (%)*</td>
<td>1.50 (1.37 to 1.65)</td>
<td>1.0×10⁻¹⁷</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11.06 (6.32 to 19.34)</td>
<td>3.8×10⁻¹⁷</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.17 (1.12 to 1.22)</td>
<td>4.6×10⁻¹⁴</td>
</tr>
<tr>
<td>TG (0.1 mmol/L)</td>
<td>1.02 (1.01 to 1.04)</td>
<td>5.6×10⁻³</td>
</tr>
<tr>
<td>eGFR (10 ml/min/1.73 m²)†</td>
<td>1.69 (1.50 to 1.90)</td>
<td>4.0×10⁻²⁰</td>
</tr>
<tr>
<td>Alcohol (10 g/week)‡</td>
<td>1.03 (1.01 to 1.06)</td>
<td>9.1×10⁻²³</td>
</tr>
</tbody>
</table>

*For each 1% decrease in FCU, there was a 50% increase in the OR for hyperuricaemia.
†For each 10 ml/min/1.73 m² decrease in eGFR, there was a 69% increase in the OR for hyperuricaemia.
‡10 g of alcohol—'standard drink' size in Australia and New Zealand.

Analyses of all variables expressed categorically gave materially similar results (see online supplementary results, online supplementary tables S2, S3, online supplementary figure S3).

**DISCUSSION**

Both FCU and the ABCG2 genotype were independent and highly significant predictors of hyperuricaemia (SU ≥0.42). Additional predictors included eGFR, male sex, serum triglyceride concentration, weekly alcohol consumption and BMI.

Our findings confirm those of Matsuo et al11 who identified Q141K and Q126X polymorphisms (the latter variant not found in our cohort) of the ABCG2 gene that increased the risk of hyperuricaemia in a Japanese population. The effect of the polymorphisms was attributable to a reduction in extra-renal urate clearance. Their results differ, however, in that they observed a statistically significant but very small *increase* of 0.24% in FCU associated with each copy of the ABCG2 141K urate-raising allele. We would expect an increase in FCU to be associated with lower urate concentrations. This suggests the effect of rs2231142 on extra-renal clearance to be even more significant. These data are also consistent with the data from Dalbeth et al16 in Europeans and Polynesians exposed to an acute fructose load who showed a small increase in FCU in those with the 141K allele. By contrast, Köttgen et al13 showed that, while the ABCG2 141K allele raised SU by 0.013 mmol/L (0.217 mg/dL) per risk allele in Europeans, there was a *small decrease* of 0.076% in FCU (p=9.8×10⁻³). However, a Bonferroni correction for multiple comparisons to their data resulted in this decrease to be no longer statistically significant. Overall, these data of Matsuo et al, Dalbeth et al and Köttgen et al indicate very little clinical effect of the Q141K polymorphism on FCU.

Patients have been classified as ‘overproducers’ when the amount of urate excreted in the urine over 24 h exceeds 600 mg/day in patients maintained on a low purine diet.17 Data from Matsuo et al11 and Ichida et al10 indicate that some people with hyperuricaemia would be erroneously labelled ‘overproducers’, not because of abnormally high synthesis of uric acid but because they have a risk allele for ABCG2. These individuals have, in fact, a significantly reduced total body clearance of urate compared with those without the risk allele and, therefore, a greater risk of hyperuricaemia. This is because of decreased secretion of urate from serum into the gastrointestinal tract. Because the kidneys in these people are, therefore, exposed to higher concentrations of urate in the blood, there is a relatively greater daily output of urate in urine compared with those without the risk allele. However, there is no change in FCU, concordant with the independence of FCU from ABCG2. Ichida et al10 have recommended a new category of hyperuricaemia, namely ‘renal overload’ of urate, encompassing subjects with either reduced ‘extra-renal urate clearance’, as found in patients with risk alleles for ABCG2, or true ‘overproduction’ of urate via the purine metabolic pathway. Some individuals may manifest both mechanisms.

Limitations to our study include a modest sample size that may be underpowered to detect a small association of Q141K with FCU. Information on the use of low-dose aspirin that is known to increase SU was not collected. The disproportionate ratio of male to female participants in the hyperuricaemic group (367 vs 81) was expected. There is a uricosuric effect of oestrogen18 and also, ABCG2 expression may be upregulated via the oestrogen-response and progesterone-response elements in the promoter region of the ABCG2 gene.19 20

In conclusion, we have shown the major, independent influences of ABCG2 and FCU on hyperuricaemia. Our work emphasises that low extra-renal clearance of urate needs to be considered as an important risk factor in the aetiology of hyperuricaemia. Furthermore, we have confirmed additional factors that are important determinants of the risk of hyperuricaemia, many of which contribute to, or are part of, the group of metabolic syndrome disorders. Finally, our work suggests new opportunities for improved diagnosis and treatment and future therapeutic approaches to treat hyperuricaemia and thereby reduce the risk of gout.

**Acknowledgements** The authors would like to thank Jill Drake, Christopher Franklin, Meaghan House, Roddi Laurence and Gabrielle Sexton for their assistance in recruitment.

**Contributors** Design of study protocol: DRWK, KMW, ROD and TRM. Acquisition of study data: AJP-G, ND, LKS and TRM. Data analysis: DRWK. Interpretation of data: DRWK, KMW, GGG, ROD and TRM. Drafting of manuscript: all authors. Final approval of manuscript: all authors.

**Funding** This work was supported by the Health Research Council of New Zealand Programme Grants 08/075, 11/1075 and 14/527, and the National Health and Medical Research Programme Grants nos 568612 and 1054146.
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Competing interests ND has received consulting fees, speaker fees and grants from the following companies: Takeda, Teijin, Menarini, Pfizer, Ardea, AstraZeneca and Fonterra. LKS has received speaker and consulting fees from AstraZeneca. TRM has received grants and consulting fees from AstraZeneca. Menarini and Astra Zeneca have agreed to be partners along with University of NSW, Western Sydney University, Macquarie University, George Institute of Global Health and University of Sydney, University of South Australia, Arthritis Australia, St Vincent’s Hospital, Arthritis and Rheumatology Association of Australasia, Pharmacy Guild of Australia and the Lexy Davies Trust in a National Health and Medical Research Council Partnership Grant # APP1094708 ‘Examining gout in the community’, 2015 through 2019, ROD being chief investigator.

Ethics approval New Zealand Multi-Region Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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Ann Rheum Dis published online December 1, 2015

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