

ORIGINAL ARTICLE

Influence of genetic variants on renal uric acid handling in response to frusemide: an acute intervention study

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ABSTRACT

Objectives: Genetic variation in the renal urate transporters *SLC2A9* (GLUT9) and *SLC22A11* (OAT4) has been reported to interact with diuretics to increase the risk of developing gout. The aim of this study was to determine whether variation in *SLC2A9* or *SLC22A11* influences acute renal handling of uric acid in response to frusemide.

Methods: Following an overnight fast, healthy participants (n=100) attended a study visit with oral intake of 40 mg frusemide. Blood and urine samples were obtained at baseline and 30, 60, 120 and 180 min after frusemide intake. The primary end point was change in fractional excretion of uric acid (FEUA).

Results: Following intake of frusemide, FEUA initially increased (mean (SD) change from baseline +1.9% (3.0%) at 60 min, p<0.001) and then decreased (mean (SD) change from baseline -1.5% (2.1%) at 180 min, p<0.001). A very small increase in serum urate was observed over the study period (mean (SD) change from baseline 0.007 (0.01) mmol/L at 180 min, p<0.001). The presence of the urate-lowering and gout-protective alleles for *SLC2A9* (*rs11942223* and *rs13129697*) and *SLC22A11* (*rs207826*) did not significantly alter the FEUA following a frusemide load. At both 60 and 180 min, change in fractional excretion of sodium was independently associated with change in FEUA (standardised $\beta \geq 0.40$, p<0.001).

Conclusions: The tested variants in *SLC2A9* and *SLC22A11* do not influence acute changes in renal handling of uric acid in response to frusemide.

Trial registration number: ACTRN12614000871640; Results.

Hyperuricaemia is frequently observed in patients taking diuretics.¹ Short-term intervention studies have shown that oral intake of diuretics reduces uric acid excretion and increases serum urate concentrations.^{2–4} In addition, diuretic use is a risk factor for the development of incident gout.⁵ It has been postulated that the association between diuretics and hyperuricaemia is mediated through

Key messages

What is already known about this subject?

- Diuretic use is a risk factor for hyperuricaemia and gout.
- A recent population-based study reported that variants in *SLC2A9* (encoding GLUT9) and *SLC22A11* (encoding OAT4) interact with diuretic use to increase the risk of developing gout.

What does this study add?

- This study does not support the hypothesis that genetic variation in *SLC2A9* or *SLC22A11* influences acute changes in renal handling of uric acid in response to diuretic intake.

How might this impact on clinical practice?

- Testing for genetic variants in *SLC2A9* or *SLC22A11* is unlikely to predict changes in renal handling of uric acid or serum urate in response to diuretic intake.

interactions between diuretics and urate transporters in the renal proximal tubule.⁶ A variety of urate transporters on the apical and basolateral membranes of the proximal tubule influence uric acid reabsorption from, and secretion into, the tubular lumen to regulate the fractional excretion of uric acid (FEUA) and ultimately determine serum urate concentrations.⁷ In *in vitro* assays, diuretics influence the function of a number of these transporters, particularly the organic anion transporters (OATs).⁸

An analysis of the Atherosclerosis Risk in Communities (ARIC) study reported that specific genetic variants in two urate transporters, specifically *SLC2A9* (encoding GLUT9) and *SLC22A11* (encoding OAT4), interact with diuretic use to increase the risk of developing gout at a population level.⁶ Although not consistently observed,⁹ the ARIC study findings suggest that variation in

SLC2A9 and *SLC22A11* may influence renal uric acid handling in response to diuretics. Genome-wide association studies (GWAS) of serum urate, FEUA and gout have reported a strong association with common variants of *SLC2A9*.¹⁰ Variation in *SLC22A11* has also been associated with hyperuricaemia in a large GWAS of Europeans.¹⁰ Several *SLC2A9* single nucleotide polymorphisms (SNPs) have been implicated in the development of gout in Māori and Pacific people living in New Zealand,¹¹ who have a very high prevalence of early onset and severe gout.^{12–13} *SLC22A11* is also associated with gout in Māori and Pacific people living in New Zealand.¹⁴ The functional mechanisms underlying the GWAS-identified association of these variants on urate transport is not currently understood.¹⁵

The aim of this study was to examine the influence of *SLC2A9* and *SLC22A11* in the FEUA following intake of frusemide.

METHODS

Participants and methods

This acute intervention study was designed to examine the influence of *SLC2A9* and *SLC22A11* variation on diuretic-induced renal urate transport. The protocol was modified from previous short-term studies investigating the acute effect of oral frusemide on serum urate, FEUA and allopurinol dosing.^{2–3–16} Young healthy participants without kidney disease were recruited into the study to allow the examination of gene–frusemide interactions in the context of normal renal tubular function. The primary end point was change in FEUA over 180 min after frusemide ingestion.

Participants

One hundred healthy participants were recruited by public advertisement. The study protocol prespecified recruitment of 50 participants of Māori or Pacific ancestry and 50 participants of European ancestry. Ancestry was determined by self-report. Inclusion criteria were: age between 18 and 50 years, able to provide written informed consent, and estimated glomerular filtration rate >60 mL/min/1.73 m². Exclusion criteria were history of gout, history of diabetes, fasting capillary glucose >6 mmol/L, existing diuretic use, hypokalaemia (serum potassium <3.5 mmol/L), recurrent episodes of vasovagal syncope or fainting, and previous intolerance to frusemide.

Potential participants attended a screening visit, during which a general health questionnaire was completed and baseline measurements (including weight, height and waist circumference) and physical examination were performed. Screening blood tests were also carried out. The first participant visit was in September 2014 and the last participant completed the study in November 2015. All visits took place at a clinical research facility in a tertiary medical centre. The study was approved by the New Zealand Ministry of Health

Multiregional Ethics Committee (MEC/05/10/130/AM06), and each participant gave written informed consent. The study was registered by the Australian Clinical Trials Registry (ACTRN12614000871640).

Māori and Pacific participants were recruited as a single group of Polynesian ancestry, as the allele frequencies for the *SLC2A9* and *SLC22A11* variants studied are similar in these two groups,^{11–14} and our previous data have shown similar renal responses to hyperuricaemia in Māori and Pacific participants.¹⁷ We included a 1 week period of salt restriction prior to the study visit to standardise the renal urate responses to frusemide and reduce variation due to non-genetic factors.¹⁸

Protocol

The study visit occurred within 2 weeks of the screening visit. Following an overnight fast, participants attended a study visit. Online supplementary table S1 shows the study visit timeline. At this visit, participants were given 500 mL water and a light low-purine breakfast at 08:00. A venous catheter was inserted for blood collection. A single oral dose of frusemide 40 mg was given at 09:00, and blood was obtained for urate, sodium, potassium, creatinine and serum storage, prior to frusemide ingestion and then 30, 60, 120 and 180 min after ingestion. Blood was also obtained for genomic DNA extraction at baseline. Urine volume was measured and urine was obtained at each time point for testing of urate, sodium, potassium and creatinine. Water was also provided at each time point (30, 60, 120 and 180 min) to a volume equivalent to the collected urine volume. Additional details about the study protocol are shown in the online supplementary methods.

Laboratory testing

Serum and urine chemistry were tested using the Roche Modular P (Hitachi) analyser. Urate concentrations are reported in mmol/L (for conversion to mg/dL, multiply value in mmol/L by 16.81). The FEUA was calculated; this is the ratio between the renal clearance of uric acid to the renal clearance of creatinine, expressed as a percentage. The fractional excretion of sodium (FENa) and fractional excretion of potassium (FEK) were also calculated for each time point. Genotyping of the following SNPs: *SLC2A9* rs11942223 and rs13129697, and *SLC22A11* SNP rs2078267 was performed on DNA extracted from whole blood samples by a guanidinium-HCl and chloroform extraction-based method. The rs11942223 variant from *SLC2A9* was genotyped using a TaqMan SNP genotyping assay (Thermo Fisher Scientific, Assay ID: C_1216479_10) in a Lightcycler 480 Real-Time PCR System (Roche Molecular Systems). Genotypes were autocalled by the Lightcycler 480 software, and the reporter dye plots were visually inspected for correct genotype clustering by a trained analyst. The other two SNPs rs13129697 from *SLC2A9* and rs2078267 from *SLC22A11*, were extracted from whole-genome genotyping performed on the Illumina Infinium CoreExome

V.24 bead chip platform at the University of Queensland (Centre for Clinical Genomics). Bead chip genotypes were autoclustered using GenomeStudio V.2011.1 software (Illumina). Clusters were reviewed by a trained analyst following the Illumina GenomeStudio best practice guidelines and quality control protocols of Guo *et al.*¹⁹ to ensure final genotype calls were of the highest possible quality.²⁰

Statistical analysis

The primary end point was a comparison of the FEUA response to frusemide based on the presence or absence of the *SLC2A9* SNP *rs11942223* urate-lowering and gout-protective C allele and independently the presence or absence of the *SLC22A11* SNP *rs2078267* urate-lowering and gout-protective T allele. The key secondary end points were change in serum urate, and a comparison of the change in FEUA between ancestral subgroups. The study protocol prespecified *SLC2A9 rs11942223* as the *SLC2A9* SNP for the primary analysis; this SNP was selected due to the strong association with hyperuricaemia and gout in European and Polynesian populations.^{10 11 21} In addition, the *SLC2A9* SNP *rs13129697* was included in further exploratory analyses, as *rs13129697* was reported in the ARIC study that described the interaction between *SLC2A9* and diuretic use in risk of incident gout in European participants.⁶ Linkage disequilibrium (r^2) between *rs11942223* and *rs13129697* was 89% in European participants and 26% in Māori and Pacific participants.

Sample size calculations were based on the previous study by Yu *et al.*³ Using this estimate of variability, a sample size of 100 participants had 90% power at the $\alpha=0.05$ significance level to detect a biologically relevant absolute mean difference of at least 1 FEUA unit between those with ($n=30$) and without ($n=70$) the protective *SLC2A9 rs11942223* allele,¹¹ and between those with ($n=50$) and without ($n=50$) the protective *SLC22A11 rs2078267* allele,¹⁴ 3 hours after frusemide (the primary end point). See online supplementary methods for further details.

Analyses were performed using SAS (SAS Institute V.9.2) on an intention-to-treat basis. Data are presented as mean (SD) or n (%) for descriptive purposes; however, measures of effect are presented with the appropriate 95% CI. A mixed-model approach to repeated measures (analysis of covariance, ANCOVA) was employed, modelling the change from baseline in FEUA as the dependent variable and including baseline FEUA as a covariate. Time, the presence or absence of the protective allele and their interaction was modelled. Unstructured, compound symmetry and first-order autoregressive covariance structures were examined and unstructured, which had the smallest Akaike information criterion, was subsequently used in all analyses. Significant main and/or interaction effects were explored using the method of Tukey to preserve the overall 5% significance level within each analysis. Age, sex and ancestry were adjusted for

within the models. Prespecified comparison of change from baseline between alleles at 30, 60, 120 and 180 min was made using error terms calculated from the mixed model, an overall 5% significance level was maintained using a false discovery rate protected p value for these comparisons. Stepwise linear regression was used to identify variables that were independently associated with changes in FEUA and serum urate. See online supplementary methods for further details.

RESULTS

Participants

There were 131 prospective participants screened. Thirty-one prospective participants were excluded following the screening visit for the following reasons: 14 had serum potassium <3.5 mmol/L, 12 did not meet ancestry criteria, 2 had a history of gout, 2 had fasting capillary glucose >6 mmol/L and 1 had veins unsuitable for cannulation. All other participants ($n=100$) attended the study visit.

Characteristics of participants attending the study visit at baseline are shown in table 1. The ancestral groups were generally well matched, although Māori or Pacific participants were older, and had higher baseline serum urate and lower baseline serum potassium. The protective *SLC22A11* SNP *rs2078267T* allele was less frequent in the Māori or Pacific group.

Adverse events during the study visit were noted in four participants; two participants experienced hypotension and nausea which resolved following intravenous infusion of 500 mL 0.9% NaCl, one participant experienced severe nausea and bradycardia during a blood draw which resolved by supine positioning, and one participant experienced residual arm pain following venesection. All participants ($n=100$) completed the study visit.

The effects of frusemide in the entire group

In the entire group ($n=100$), oral intake of 40 mg frusemide led to a marked diuresis, with mean (SD) urine volume 2161.9 (717.37) mL over 180 min (figure 1). Following intake of frusemide, FEUA initially increased (mean (SD) change from baseline +1.9% (3.0%) at 60 min, $p<0.001$) and then decreased (mean (SD) change from baseline -1.5% (2.1%) at 180 min, $p<0.001$; figure 2). A very small increase in serum urate was observed over the study period (mean (SD) change from baseline 0.007 (0.01) mmol/L at 180 min, $p<0.001$). There was also an increase in FENa and FEK over the 180 min study period ($p<0.0001$ for all), peaking 120 min after frusemide intake. There was a small increase in serum sodium and potassium over the study period, peaking at 60 min post frusemide intake.

Co-primary end point: *SLC2A9*

In the entire group, the presence of the *SLC2A9 rs11942223* protective C allele did not significantly

Table 1 Characteristics of participants at baseline

	All (n=100)	Māori or Pacific ancestry (n=50)	European ancestry (n=50)	p Value
Age, years	28 (9)	26 (8)	31 (10)	0.0026
Male sex, n (%)	29 (29%)	13 (26%)	16 (32%)	0.66
Body mass index, kg/m ²	26 (5)	27 (5)	25 (5)	0.14
Waist circumference, cm	80 (25)	81 (23)	79 (27)	0.64
Systolic blood pressure, mm Hg	121 (12)	123 (11)	120 (13)	0.34
Diastolic blood pressure, mm Hg	70 (9)	68 (8)	71 (9)	0.13
Serum urate, mmol/L	0.31 (0.07)	0.32 (0.07)	0.29 (0.08)	0.044
Serum urate, mg/dL	5.2 (1.2)	5.3 (1.2)	4.8 (1.3)	0.044
Serum sodium, mmol/L (reference range 135–145 mmol/L)	138 (2)	138 (2)	138 (2)	0.68
Serum potassium, mmol/L (reference range 3.5–5.2 mmol/L)	3.8 (0.3)	3.7 (0.2)	3.9 (0.3)	0.027
Serum creatinine, µmol/L (female reference range 45–90 µmol/L, male reference range 60–105 µmol/L)	74 (13)	74 (12)	74 (13)	0.98
Fractional excretion of urate (%)	6.3 (2.2)	6.1 (2.2)	6.5 (2.2)	0.40
Fractional excretion of sodium (%)	0.4 (0.3)	0.4 (0.3)	0.4 (0.2)	0.56
Fractional excretion of potassium (%)	13 (17)	13 (23)	12 (8)	0.83
<i>SLC2A9</i> rs11942223 protective C allele present, n (%)	30 (30%)	11 (22%)	19 (38%)	0.13
<i>SLC2A9</i> rs13129697 protective G allele present, n (%)	50 (50%)	29 (58%)	21 (42%)	0.16
<i>SLC22A11</i> rs2078267 protective T allele present, n (%)	53 (53%)	17 (34%)	36 (72%)	0.0003

Unless stated, data are presented as mean (SD).

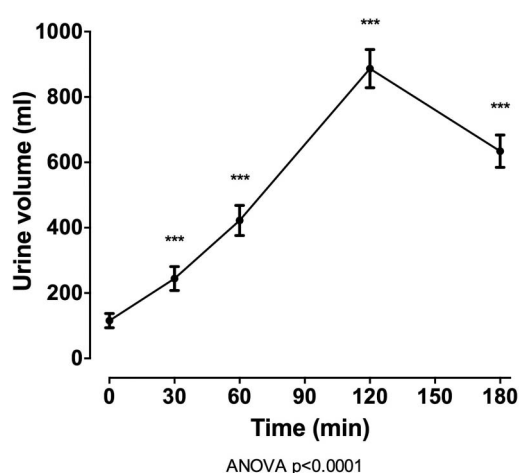


Figure 1 Urine volume over the 180 min study period in the entire group (n=100). Data are presented as mean (95% CI). ANOVA, analysis of variance.

influence changes in FEUA or serum urate following the frusemide load (ANCOVA $p_{\text{SNP}} \geq 0.23$, figure 3). At baseline and throughout the study period, the serum urate concentration was higher in those without the *rs11942223* protective allele (false discovery rate $p \leq 0.013$ for all time points, figure 3). However, the changes in FEUA and serum urate over the study period were similar in those with and without the *rs11942223* protective allele. Similarly, in the additional *SLC2A9* rs13129697 exploratory analysis, the presence of the protective G allele did not influence change in FEUA following the frusemide load (ANCOVA $p_{\text{SNP}} = 0.54$, see online supplementary figure S1). Furthermore, there was a greater increase in serum urate in those with the

rs13129697 protective allele present (ANCOVA $p_{\text{SNP}} = 0.03$), but no difference in the absolute values in serum urate over the study period (false discovery rate $p > 0.49$ for all time points, see online supplementary figure S1).

Co-primary end point: *SLC22A11*

In the entire group, there was no significant difference in baseline FEUA or serum urate in those with and without the *SLC22A11* rs2078267 protective T allele (false discovery rate $p > 0.58$). Furthermore, the changes in FEUA over the study period were similar in those with and without the *rs2078267* protective allele (ANCOVA $p_{\text{SNP}} = 0.46$, figure 4). Despite the lack of effect on FEUA, there was a difference in the direction of the change in serum urate depending on *rs2078267* genotype, with flattening of the serum urate increase in those with the protective T allele (ANCOVA $p_{\text{time} \times \text{SNP}} = 0.0029$, figure 4). However, the change in serum urate from baseline was very small, and absolute serum urate concentrations did not differ in the *SLC22A11* groups at the end of the study period (false discovery rate $p > 0.98$ at the 180 min time point, figure 4).

Ancestral stratification

At baseline, the European and Polynesian ancestral groups had similar FEUA (false discovery rate $p = 0.37$, figure 5). The biphasic FEUA response to frusemide was observed in both ancestral groups, although the Māori or Pacific group had a smaller increase in FEUA following intake of frusemide overall (ANCOVA $p_{\text{ancestry}} = 0.012$). At baseline, the serum urate concentration was higher in those of Māori or Pacific ancestry (false discovery rate $p = 0.001$). Change in serum urate from baseline was small

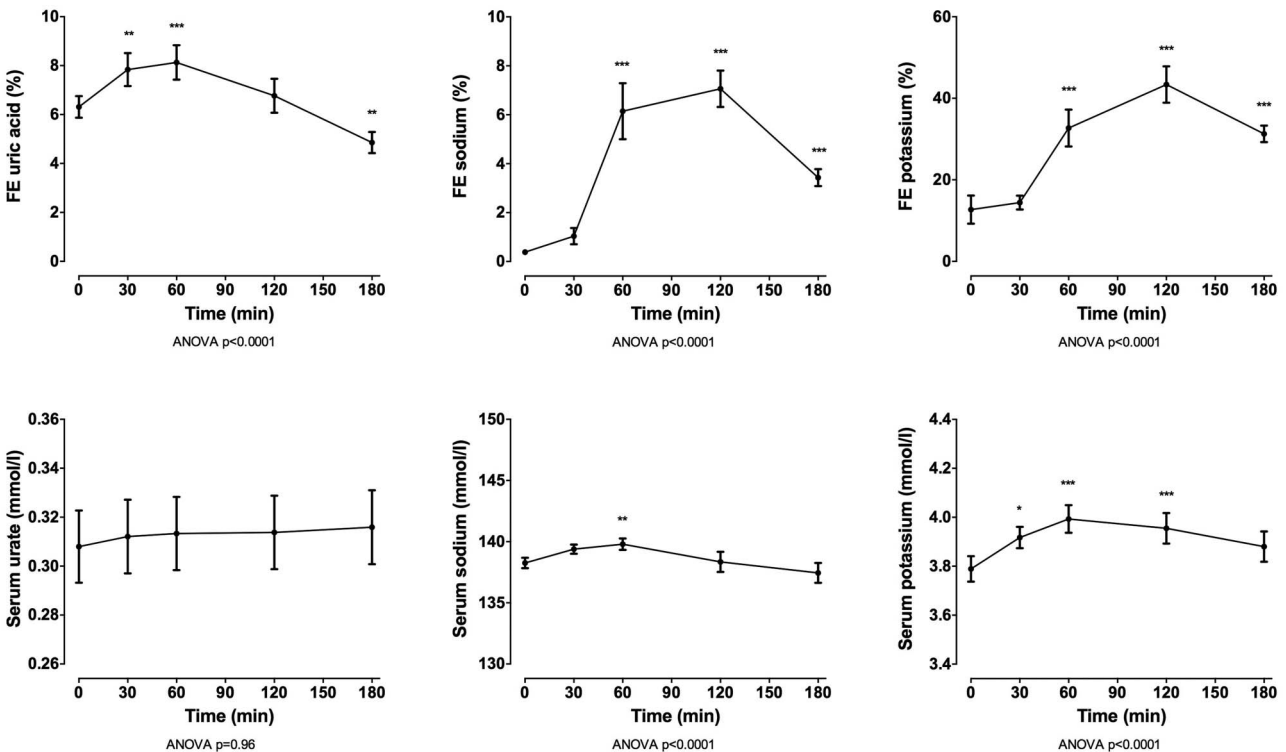


Figure 2 FE and serum concentrations over the 180 min study period for the entire group (n=100). Data are presented as mean (95% CI). Top panels show urate, middle panels show sodium and bottom panels show potassium. ANOVA, analysis of variance; FE, fractional excretion.

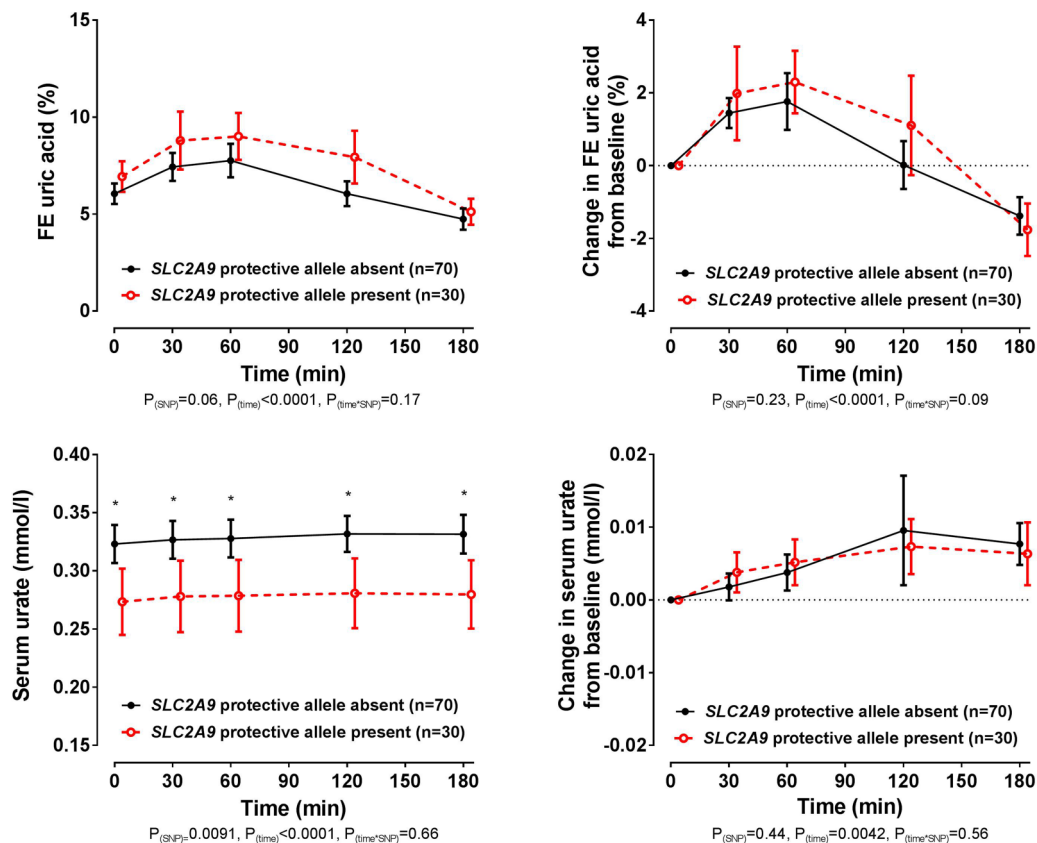


Figure 3 Fractional excretion (FE) of uric acid (FEUA) and serum urate concentrations over the study period, based on the presence or absence of the *SLC2A9* rs11942223 protective C allele. Data are presented as mean (95% CI). Left panel shows absolute values and right panel shows change in values. Age-adjusted, sex-adjusted and ancestry-adjusted p values are shown. *False discovery rate p<0.05, for comparison between *SLC2A9* rs11942223 allele groups at the specified time point.

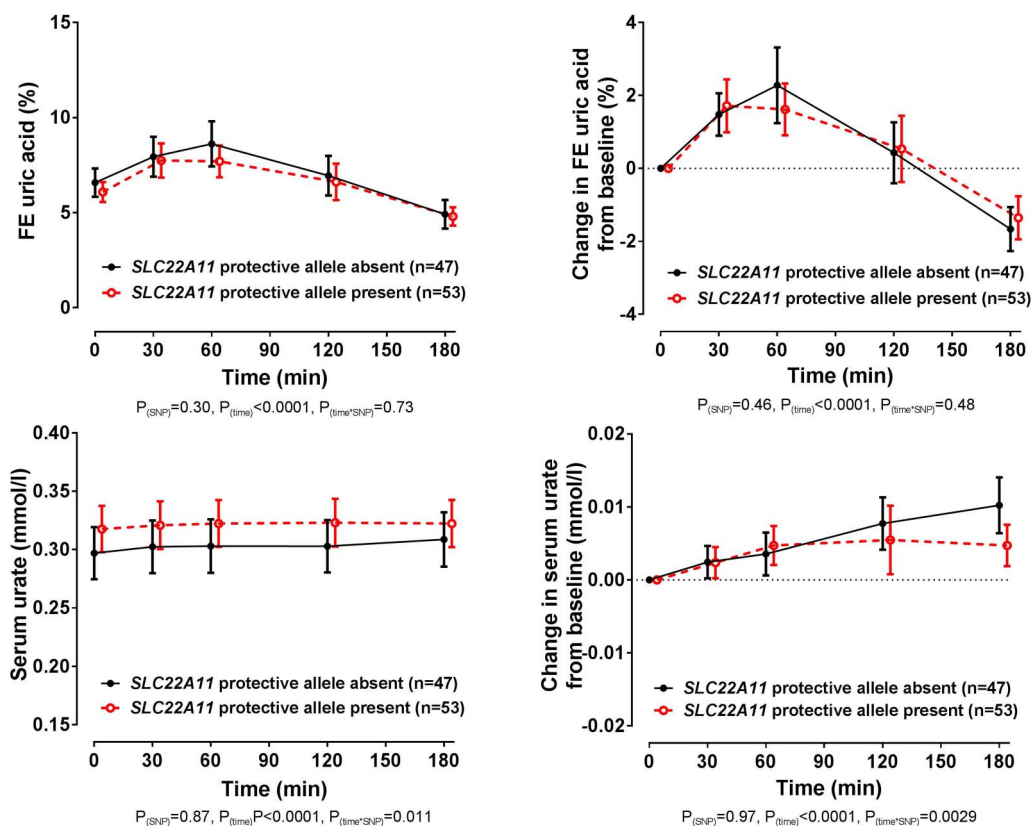


Figure 4 Fractional excretion (FE) of uric acid (FEUA) and serum urate concentrations over the study period, based on the presence or absence of the *SLC22A11* rs2078267 protective T allele. Data are presented as mean (95% CI). Left panel shows absolute values and right panel shows change in values. Age-adjusted, sex-adjusted and ancestry-adjusted p values are shown.

in both ancestral groups, although the Māori or Pacific group had a greater increase in serum urate concentration over the study period (ANCOVA $p_{ancestry}=0.0003$), with a significantly greater increase between ancestral groups at the 180 min time point (false discovery rate $p=0.011$). The presence of the protective alleles for *SLC2A9* or *SLC22A11* did not significantly alter the FEUA or serum urate responses to the frusemide load in either ancestral group (ANCOVA $p_{SNP}\geq 0.10$ for all comparisons, see online supplementary tables S2–S4).

Predictors of change in FEUA and serum urate

At the 60 min time point, change in FEUA was independently associated with change in FENa (standardised $\beta=0.40$, $p<0.001$, see online supplementary tables S5 and S6). Similarly, at the 60 min time point, change in serum urate was independently associated with change in FENa (standardised $\beta=0.44$, $p<0.001$). At the 180 min time point, change in FEUA was also independently associated with change in FENa (standardised $\beta=0.46$, $p<0.001$), and change in serum urate was positively associated with change in serum urate at 60 min (standardised $\beta=0.59$, $p<0.001$) and inversely associated with change in FEUA at 180 min (standardised $\beta=-0.21$, $p=0.009$). Urine volume was not independently associated with change in FEUA or change in serum urate at either time point.

DISCUSSION

This short-term intervention study does not support the hypothesis that genetic variation in *SLC2A9* or *SLC22A11* influences acute changes in renal handling of uric acid in response to diuretic intake. It is conceivable that genetic variants in other urate transporters (either renal or extrarenal) not tested in this analysis do influence these responses. However, in the ARIC population-based study, the effects of genetic risk scores (that included variants in the *ABCG2* and *SLC17A1* uric acid transporter genes in addition to *SLC2A9* and *SLC22A11*) on diuretic-associated gout were driven by *SLC2A9* and *SLC22A11* variants.⁶ The conclusions of our analysis are consistent with another population-based study that failed to replicate the ARIC study findings.⁹

The pattern of FEUA changes over time following intake of oral frusemide was biphasic, with initial increase and subsequent reduction in FEUA. The initial increase in FEUA does not explain the observed increase in serum urate over the study period, suggesting that acute serum urate-increasing effects of diuretics are not directly related to interactions with urate transporters. The changes in FEUA were independently associated with changes in FENa. A number of urate transporters, including URAT1, OAT1, OAT, and OAT10 are influenced by substrates transported by sodium-dependent anion co-transporters,⁷ and our data support the concept that

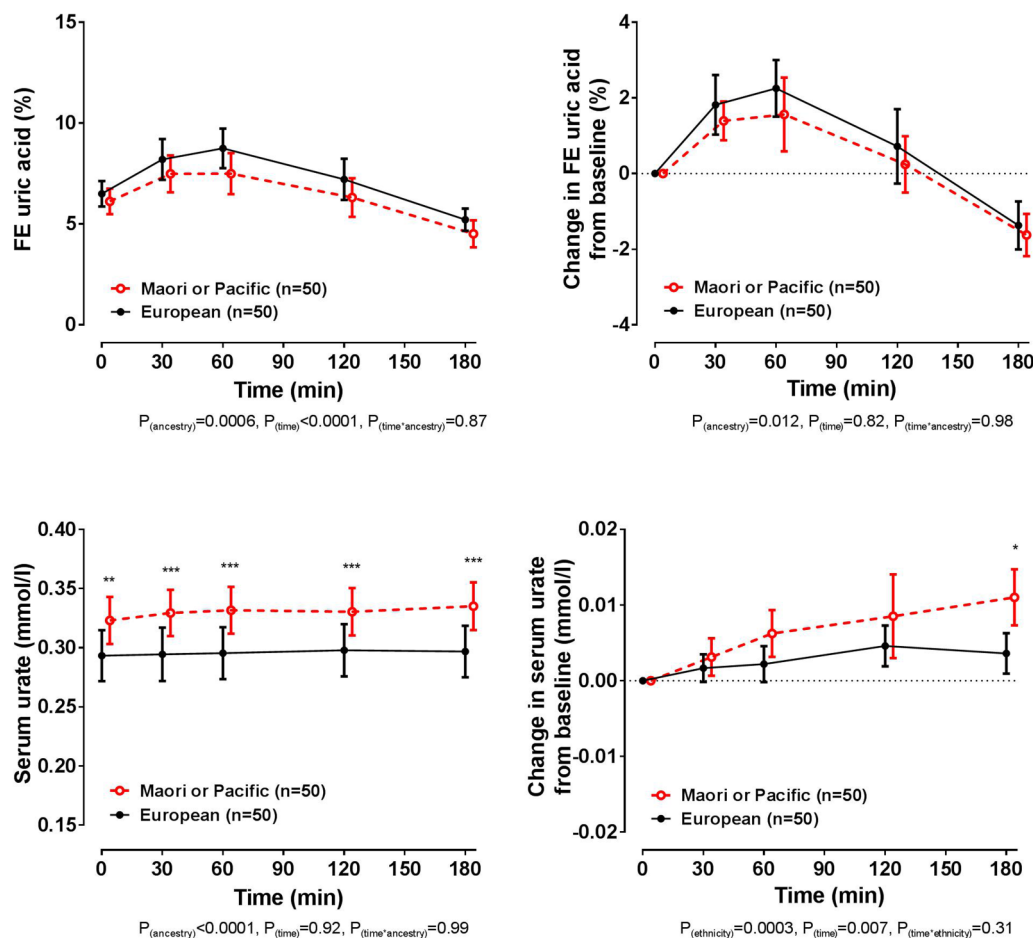


Figure 5 Fractional excretion (FE) of uric acid (FEUA) and serum urate concentrations over the study period, based on ancestral group. Data are presented as mean (95% CI). Left panel shows absolute values and right panel shows change in values. Age-adjusted and sex-adjusted p values are shown. *False discovery rate $p<0.05$, **false discovery rate $p<0.01$ and ***false discovery rate $p<0.001$, for comparison between ancestral groups at the specified time point.

diuretics may influence renal uric acid handling indirectly, through effects on sodium transport.

Our results showing the biphasic FEUA response differ from prior analysis of oral frusemide dosing⁴ but are similar to some studies of intravenous frusemide, which also showed a similar initial increase, then reduction, in FEUA.^{18–22} In the Steele and Oppenheimer's¹⁸ study of intravenous frusemide, late reduction in FEUA was attenuated by volume repletion, suggesting that the effects of frusemide on tubular function were related to volume depletion. In our analysis, changes in FEUA at 60 min and 180 min after frusemide were not independently associated with urine volume, but rather with changes in FENa. The relationship between renal clearance of uric acid and FENa has also been reported in patients with gout.²³ Collectively, these data suggest an interplay between uric acid and sodium clearance.

Māori and Pacific people have very high rates of hyperuricaemia and early-onset gout,¹² with reported differences in renal handling of urate.^{24–25} Consistent with a previous short-term intervention study of fructose intake,¹⁷ we observed differences between ancestral groups in this short-term intervention study of

frusemide, with higher baseline serum urate, lower increase in FEUA and greater increase in serum urate over the 180 min follow-up period in renal urate handling in Māori and Pacific participants, compared with New Zealand European participants. However, these differences were not due to genetic variants tested in *SLC2A9* or *SLC22A11*.

This study has some limitations; the study was designed to examine the effects of *SLC2A9* or *SLC22A11*, based on prior report of these genes contributing to diuretic-associated gout. We cannot exclude that other variants in *SLC2A9* and *SLC22A11* or in other genes (urate, sodium or potassium transporters) may influence renal responses to frusemide. A validation study would be of great interest, to confirm the observed lack of effect of the tested variants. The observed acute serum urate increase in response to a single frusemide in these healthy volunteers was very small and the clinical relevance of these findings is uncertain. The study was designed to recruit participants with normal tubular function, and most participants lacked other factors associated with diuretic use such as kidney or cardiovascular disease; it is possible that these comorbidities

interact with genetic variants and/or diuretics to influence FEUA, serum urate or gout risk. This study was designed to examine the short-term effects of frusemide only; long-term administration may have different effects on FEUA or serum urate.³

Nevertheless, these data do not support the hypothesis that genetic variation in *SLC2A9* or *SLC22A11* plays a role in diuretic-associated gout. Testing for these variants is unlikely to predict changes in renal handling of uric acid or serum urate in response to diuretic intake.

Contributors ND (the guarantor) accepts full responsibility for the work and the conduct of the study, had access to the data and controlled the decision to publish. ND conceived the study, contributed to the data interpretation, and drafted the manuscript. JA and AH recruited participants, coordinated study visits and contributed to data acquisition. JA and BM managed clinical data management, AP-G and TJF contributed to data acquisition and genetic data management. GDG analysed the data. RD, LKS and TRM conceived of the study, contributed to the data interpretation and drafted the manuscript. All authors read and approved the final manuscript.

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Ethics approval New Zealand Ministry of Health Multiregional Ethics Committee (MEC/05/10/130/AM06).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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