Do serum urate-associated genetic variants influence gout risk in people on diuretics? Analysis of the UK Biobank

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Running head: Gene-diuretic interactions for gout

Abstract

Objective: The aim of this study was to determine whether serum urate-associated genetic variants differ in their influence on gout risk in people taking a diuretic compared to those not taking a diuretic.

Methods: This research was conducted using the UK Biobank Resource (n=359,876). Ten serum urate-associated single nucleotide polymorphisms (SNPs) were tested for their association with gout according to diuretic use. Gene-diuretic interactions for gout association were tested using a genetic risk score (GRS) and individual SNPs by logistic regression adjusting for relevant confounders.

Results: After adjustment, use of a loop diuretic was positively associated with prevalent gout (OR 2.34 [2.08-2.63]), but thiazide diuretics were inversely associated with prevalent gout (OR 0.60 [0.55-0.66]). Compared with a lower GRS (< mean), a higher GRS (≥ mean) was positively associated with gout in those not on diuretics (OR 2.63 [2.49-2.79]), in those on loop diuretics (OR 2.04 [1.65-2.53]), in those on thiazide diuretics (OR 2.70 [2.26-3.23]), and in those on thiazide-like diuretics (OR 2.11 [1.37-3.25]). No non-additive gene-diuretic interactions were observed.

Conclusions: In people on diuretics, serum urate-associated genetic variants contribute strongly to gout risk, with a similar effect to that observed in those not taking a diuretic. These findings suggest that the contribution of genetic variants is not restricted to people with 'primary' gout and genetic variants can play an important role in gout susceptibility in the presence of other risk factors.

Introduction

Many factors associate with the development of gout, including genetic variability, comorbid conditions, and medications. Recent cross-sectional studies have identified different phenotypic clusters for gout based on the presence or absence of various co-morbidities and medications (1, 2). Identification of different disease clusters may reflect different pathophysiological processes involved in the development of gout (1, 3). One cluster includes patients with 'isolated gout' in whom few co-morbidities exist. This cluster is often termed 'primary gout' and is presumed to have a strong genetic basis. Genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) associated with serum urate and gout (4-7).

Another phenotypic cluster includes patients with cardiovascular disease and kidney disease, many of whom are on diuretic therapy (1, 2). This cluster is often referred to as 'secondary' gout and is thought to have less of a basis in inherited genetic risk factors. Diuretic agents are widely prescribed, and their main site of action is the kidneys. Loop diuretics inhibit the sodium-potassium-chloride co-transporter at the loop of Henle and are used in fluid overload states (8). Thiazide and thiazide-like diuretics inhibit the sodium-chloride co-transporter at the distal convoluted tubule and their main indication is hypertension (9, 10). An association between diuretic use and gout has been reported by many investigators with most, but not all, early studies reporting an increased risk of gout with diuretic use (11-14). More recently, larger studies have tested for an association between diuretic use and incident gout while attempting to adjust for confounders. All have confirmed a positive association and reported a higher risk of gout with loop diuretics compared to thiazide diuretics (15, 16). Diuretics are thought to increase gout risk by inducing hyperuricaemia through their action on renal urate transporters. A possible mechanism involves competitive inhibition of urate transporters on

renal tubular cells normally involved in urate secretion, such as OAT1 and OAT3 on the basolateral membrane (17), and MRP4 and NPT4 on the apical membrane (17, 18). There is also evidence for diuretic-induced uptake of urate via OAT4 on the basolateral membrane of renal tubular cells (19). Furthermore, diuretics also affect renal urate excretion via indirect mechanisms related to intravascular volume contraction and salt loss which stimulates renal solute (including urate) reabsorption (20).

The aim of this study was to determine whether the genetic risk for gout attributed by serum urate-associated genetic variants differs in people taking a diuretic compared to those not taking a diuretic.

Materials and methods

Study population and diuretic classification

This research was conducted using the UK Biobank Resource (approval number 12611). UK Biobank obtained approval from the North West Multi-Centre Research Ethics Committee (11/NW/0382). Full written informed consent was obtained from all participants prior to the study. Participants of European ancestry aged 40-69 years, and with genome-wide genotypes were included in this study. Exclusion criteria included mismatch between self-reported sex and genetic sex, genotyping quality control failure, and related individuals. Gout was defined using a validated definition of: self-report of gout or urate-lowering therapy (includes allopurinol, febuxostat, sulphinpyrazone) use, and without a hospital diagnosis of leukaemia or lymphoma based on the International Classification of Diseases, Tenth Revision codes C81-C96 (21). For participants who did not meet the gout definition, further exclusion criteria included prescriptions for corticosteroids, non-steroidal anti-inflammatories, or probenecid. This definition has been previously tested in an analysis of the first tranche of

the UK Biobank and was found to detect the highest number of gout cases and had the best precision for genetic association analyses compared to other methods for defining gout status (21). In addition, when compared to gold standard synovial fluid microscopy results, this definition was found to have the best test performance characteristics out of ten different definitions used in epidemiological studies that contributed to the Global Urate Genetics Consortium (22). Medication use, co-morbidities (including renal failure, heart failure and hypertension), alcohol intake, and smoking status data were collected via self-report. Diuretic agents were classified into 4 groups: loop diuretics, thiazide diuretics, thiazide-like diuretics and potassium-sparing diuretics. Participants on two or more diuretics were assigned to the particular diuretic class based on a hierarchy grading whereby loop diuretic > thiazide diuretic > thiazide-like diuretic > potassium-sparing diuretics.

Genotyping analysis

UK Biobank samples were genotyped using an Axiom array (820,967 markers; Affymetrix, Santa Clara, CA, USA) and imputed to approximately 73.3 million SNPs using SHAPEIT3 and IMPUTE2 with a combined UK10K and 1000 Genomes reference panel (23). For quality control, SNPs with a minor allele frequency < 0.001, and Hardy-Weinberg equilibrium < 1×10^{-6} were excluded. Thirty serum urate-associated SNPs have been previously reported (4). However, not all of these SNPs associated with gout in a previous analysis of the UK Biobank (21). Therefore, we analysed the 10 serum urate-associated SNPs with the strongest association for gout (that included renal urate transporters) as reported by Cadzow et al. (21) in the analysis from the first tranche (n=105,421) of the UK Biobank genotyping dataset. These included two loci encoding urate transporters for which a gene-diuretic interaction for gout has previously been reported (*SLC2A9* [encoding GLUT9] and *SLC22A11* [encoding OAT4]) (24), and four loci encoding for other urate transporters

and ancillary genes (*ABCG2* [encoding ABCG2], *SLC17A3* [encoding NPT4], *SLC22A12* [encoding URAT1]) and *PDZK1* [encoding PDZK1]). The SNPs and effect allele for each locus tested in this analysis were the lead SNP at the respective locus as identified by Köttgen et al. (4).

Genetic risk score

A weighted genetic risk score for gout was calculated from the UK Biobank dataset to model the cumulative effects of an individual's risk for gout for the 10 variants. For each of the 10 serum urate-associated SNPs, allelic odds ratios (ORs) were calculated to determine the risk of gout adjusting for age, sex, and body mass index (BMI). The ORs were converted into a logarithmic value and for each individual, these logarithmic values were multiplied by the number of urate-raising alleles and summed into a weighted genetic risk score. Higher scores indicate a greater genetic predisposition for gout.

Study power

Details on study power are provided in the Supplementary Methods.

Statistical analysis

Data were analysed using IBM SPSS Statistics 25 software. Baseline characteristics according to diuretic use were summarised using standard descriptive statistics including means, standard deviations [SD], number and percent, and were compared using unpaired t-tests or Pearson's chi-squared tests where appropriate. Logistic regression of diuretic use with gout as the dependent variable was performed in an unadjusted model, a model adjusted for age, sex, and BMI, and a model adjusted for age, sex, BMI, hypertension, renal failure and heart failure. Genetic risk score-diuretic interactions for gout association were assessed

using logistic regression models that included a genetic risk score by diuretic interaction term. Interaction models were calculated with genetic risk score as a categorised variable (lower [< mean] or higher [≥ mean]), and as a continuous variable. Association of the SNPs with gout according to diuretic use were determined based on the presence or absence of the allele that increased the risk of gout. SNP-diuretic interactions for gout association were analysed using logistic regression models that included a SNP by diuretic interaction term. Age, sex, BMI, hypertension, renal failure and heart failure were included as variables in all interaction analyses. A sensitivity analysis was also performed in which the genetic risk score was modelled using effect sizes for gout from Köttgen et al. (4). Data were reported at experiment-wide significance ($P < 0.005$) to account for multiple testing in the individual SNP analysis.

Results

Clinical features of participants

Data including genome-wide genotypes were available for 359,876 participants. Baseline characteristics according to diuretic use are shown in Table 1. There were 29,711 (8.3%) diuretic users, of whom 3,728 (12.5%) were taking a loop diuretic, 23,623 (78.9%) were taking a thiazide diuretic, and 2,001 (6.7%) were taking a thiazide-like diuretic.

Overall, there were 7,342 (2.0%) participants with gout. In participants with gout, those taking any diuretic were older, had a higher BMI, and had a higher prevalence of co-morbidities including hypertension, compared to participants who were not taking a diuretic. For participants with gout on a loop diuretic, those with gout had a higher prevalence of renal failure and heart failure, compared to participants with gout who were not taking a diuretic (Table 1).

Association of diuretic use and gout

Gout was present in 6,145 (1.9%) non-diuretic users, 462 (12.4%) loop diuretic users, 615 (2.6%) thiazide diuretic users, and 102 (5.1%) thiazide-like diuretic users. Supplementary Table 2 shows unadjusted and adjusted ORs for prevalent gout according to diuretic use. Participants taking a loop diuretic had the highest OR for gout in the unadjusted model (OR [95% CI] 7.46 [6.74-8.25]) and this association persisted in the fully adjusted model (OR [95% CI] 2.34 [2.08-2.63]). For participants taking a thiazide diuretic, there was a positive association with gout in the unadjusted model (OR [95% CI] 1.41 [1.30-1.53]); however, in the fully adjusted model there was an inverse association with gout (OR [95% CI] 0.60 [0.55-0.66]). For participants taking a thiazide-like diuretic, an increased OR for gout was also present in the unadjusted model (OR [95% CI] 2.83 [2.32-3.46]). However following adjustment for all confounders, no association with gout was observed (OR [95% CI] 1.05 [0.85-1.29]), (Supplementary Table 2).

Association of genetic risk score and gout

The mean [SD] genetic risk score for all participants, including those with gout, was 1.15 [0.26]. In the entire study population, 174,115 (48.9%) participants had a higher (≥ mean) genetic risk score. Participants with gout had a significantly higher genetic risk score compared to those without gout (mean [SD] 1.30 [0.26] vs 1.15 [0.26], $P < 1x10^{-300}$).

Compared to participants with a lower (< mean) genetic risk score, the unadjusted OR [95% CI] for gout was 2.48 [2.36-2.61] in participants with a higher genetic risk score (Supplementary Table 3). After adjusting for age, sex, BMI, hypertension, renal failure, and heart failure a significant association for gout persisted (OR [95% CI] 2.60 [2.46-2.74]).

Association between genetic risk score and gout, according to diuretic use

The mean genetic risk score was higher in participants with gout compared to participants without gout for non-diuretic users, loop diuretic users, thiazide diuretic users and thiazide-like diuretic users. Data for the prevalence of gout according to genetic risk score category and diuretic use are shown in Figure 1. Compared to participants with a lower genetic risk score, the prevalence [95% CI] of gout was higher in those with a higher genetic risk score in non-diuretic users (1.12% [1.07-1.17] vs 2.79% [2.71-2.87]), loop diuretic users (8.98% [7.67-10.28] vs 15.88% [14.21-17.55]), thiazide diuretic users (1.54% [1.32-1.76] vs 3.76% [3.41-4.11]), and thiazide-like diuretic users (3.52% [2.39-4.65] vs 6.88% [5.27-8.48]), (Figure 1).

For non-diuretic users, a higher genetic risk score was positively associated with gout compared to those with a lower genetic risk score (OR [95% CI] 2.63 [2.49-2.79], P = 8.74×10^{-240}). A higher genetic risk score was also positively associated with gout compared to those with a lower genetic risk score in loop diuretic users (OR [95% CI] 2.04 [1.65-2.53], P = 4.09×10^{-11}), thiazide diuretic users (OR [95% CI] 2.70 [2.26-3.23], P = 1.17×10^{-27}), and thiazide-like diuretic users (OR [95% CI] 2.11 [1.37-3.25], P = 6.48×10^{-4}) with similar ORs and overlapping confidence intervals compared to participants not on diuretics (Table 3).

When the genetic risk score was analysed as a categorical variable, no non-additive genetic risk score-diuretic interactions were observed (Table 3). Similarly, when genetic risk score was analysed as a continuous variable, no non-additive genetic risk score-diuretic interactions were observed for loop diuretic use (P = 0.16), thiazide diuretic use (P = 0.76), and thiazide-

like diuretic use (P = 0.89). Probability and interaction data for genetic risk score (analysed as a continuous variable) and loop diuretic use are shown in Figure 2.

Association of serum urate-associated SNPs and gout, according to diuretic use

Genotype distribution of the serum urate-associated SNPs according to diuretic use are shown in Supplementary Table 4. For non-diuretic users, association with gout at experiment-wide significance was observed for all 10 serum urate-associated SNPs (Table 4). For loop diuretic users, experiment-wide association for gout was observed for two SNPs: *ABCG2* (rs2231142) and *SLC2A9* (rs12498742). For thiazide diuretic users, the same two SNPs were associated with gout, as well as *GCKR* (rs1260326), *SLC17A3* (rs1165151), and *SLC22A11* (rs2078267). For thiazide-like diuretic users, *ABCG2* (rs2231142) was associated with gout. For some of the other SNPs tested in the diuretic groups, similar ORs for gout association were found compared to non-diuretic users, however these did not reach experiment-wide significance. The *ABCG2* and *SLC2A9* effect alleles exerted the highest ORs for gout in non-diuretic users and users of each diuretic class, with similar ORs and overlapping confidence intervals for each group. For all SNPs tested, no non-additive SNP-diuretic interactions were observed (Table 4).

Due to the low power to detect an association between some serum urate-associated SNPs and gout in participants on a thiazide-like diuretic (Supplementary Table 1), a permutation test for logistic regression was performed for the serum urate-associated SNPs for which the power to detect an association with gout was < 10%. The results of the permutation test were identical to that in the main analysis (Supplementary Table 5). This is in keeping with evidence suggesting that the permutation test is equivalent to that of asymptotic tests in datasets with > 1,000 observations (25).

Sensitivity analysis

In the sensitivity analysis, the genetic risk score was modelled using effect sizes for gout from Köttgen et al. (4). In this analysis, the mean [SD] genetic risk score for all participants, including those with gout, was 0.78 [0.18]. Participants with gout had a significantly higher genetic risk score compared to those without gout (mean [SD] 0.88 [0.19] vs 0.78 [0.18], $P < 1x10^{-300}$). The mean genetic risk score was higher in participants with gout compared to participants without gout for non-diuretic users, loop diuretic users, thiazide diuretic users and thiazide-like diuretic users (Supplementary Table 6). Similar to the main analysis, a higher genetic risk score was positively associated with gout compared to those with a lower genetic risk score in non-diuretic users, loop diuretic users, thiazide diuretic users and thiazide-like diuretic users (Supplementary Table 7). No non-additive genetic risk score-diuretic interactions were observed.

Discussion

In this large cohort of European ancestry, we have shown that genetic susceptibility plays a significant contribution to gout risk in people on diuretics, with associations of similar magnitude observed between those not taking a diuretic and those taking a diuretic. These data demonstrate that the effects of serum urate-associated genetic variants also contribute to gout susceptibility in diuretic users. Our data also suggest that the influence of serum urate-associated genetic variants is not restricted to people with 'primary' gout, and that genetic variability is an important contributor to gout risk in people who may also have 'secondary' risk factors for gout.

Although a non-additive gene-loop diuretic interaction was not observed, our analysis demonstrated a high prevalence of gout (> 15%) in the presence of both a higher genetic risk score and loop diuretic use. This high prevalence is likely due to the independent and additive effects of both risk factors for gout association and represents a clinically important increase in the prevalence of gout in this group.

The individual serum urate-associated SNP analysis demonstrated an association with gout for all 10 SNPs in participants not taking a diuretic. Associations with gout were also observed for some individual SNPs in those taking a loop, thiazide or thiazide-like diuretic. This includes *ABCG2* (rs2231142) and *SLC2A9* (rs12498742) which, consistent with previous reports (4, 7, 26), exerted the highest association for gout of all SNPs tested and suggests that the effects of a higher genetic risk score for gout risk are primarily driven by these two SNPs. For other SNPs tested, similar ORs for gout association were found compared to the non-diuretic group, and experiment-wide significance may not have been reached due to low power to detect association, most likely explained by a relatively lower number of participants in the diuretic groups and lower effect size.

Previous studies testing for non-additive interactions between serum urate-associated genetic variants and diuretics for incident gout risk have reported conflicting results. McAdams-DeMarco et al. (24) reported differential effects of diuretic use (loop or thiazide) on incident gout risk according to genetic urate score (GUS). An increased risk of gout was observed with loop or thiazide diuretic use in those with a GUS above the median, but no change in risk was observed in those with a GUS below the median. Further analysis demonstrated this interaction was driven by two specific genetic variants (*SLC22A11* [encoding OAT4] and *SLC2A9* [encoding GLUT9]). Nine-year cumulative incidence of gout was higher in

participants taking a diuretic who had two *SLC22A11* risk alleles compared to those with one or no risk allele, with a significant non-additive interaction. Similar findings were also seen for the *SLC2A9* risk allele (24). However these interaction findings were not replicated in a subsequent analysis of the Health Professionals Follow-up Study and Nurses' Health Study which tested for non-additive gene-diuretic interactions for incident gout using 29 serum urate-associated SNPs (27) that included the 10 (or their surrogates) studied here. The lack of non-additive gene-diuretic interactions in this larger analysis suggest that the risk of gout associated with loop or thiazide diuretics does not vary according to the genetic risk for hyperuricaemia (27). Our study of prevalent gout also did not demonstrate non-additive gene-diuretic interactions for gout, consistent with the findings of the Health Professionals Follow-up Study and Nurses' Health Study.

Our data show that genetic susceptibility to gout is important in people taking diuretics. However, our study did not address the causal relationship between exposure to diuretics and incident gout. Causality of diuretic exposure for gout has yet to be shown and the strong association reported from previous studies might have resulted from indication bias. This has been demonstrated in a case-control study based in a Dutch primary healthcare centre in which diuretic use was associated with gout in an unadjusted logistic regression model, but after adjustment for hypertension, heart failure and myocardial infarction, there was a lack of association between diuretic use and incident gout (13).

In contrast to prior studies of incident gout which reported that loop, thiazide and thiazide-like diuretics were associated with an increased risk of developing gout (15, 16, 28), we have identified variable associations for prevalent gout according to diuretic class. Following adjustment for relevant confounders, use of a loop diuretic was positively associated with

gout. However, use of a thiazide diuretic was associated with a lower odds ratio for gout, and no association was found with thiazide-like diuretics. These contrasting findings may be due to differences in study design, as our cross-sectional study reports prevalent gout compared to longitudinal studies that reported incident gout. The inverse association found in our study for thiazide diuretic use and the lack of association for thiazide-like diuretic use may therefore reflect physicians' prescribing behaviour with avoidance of these diuretic agents in people with gout, consistent with the current guidance for hypertension management (9, 10, 29, 30). It is also important to note that the inverse association for thiazide diuretic use was observed after adjustment for relevant confounders, including hypertension, which also suggests that physicians' prescribing behaviour may explain the inverse association.

We acknowledge the limitations of this study. Firstly, our analysis was restricted to participants of European ancestry and our results may not be generalizable to populations of non-European ancestry. The age range for recruitment into UK Biobank means that younger people with early-onset gout, and participants over the age of 70 years were not included in the analysis. Despite the large size of the UK Biobank, power to detect association between some serum urate-associated SNPs was low. This is likely due to a relatively lower number of participants in the diuretic groups, and a high or low effect allele frequency for some SNPs. Co-morbidity and medication use data collected via the UK Biobank resource was through self-report. This method of data collection may not accurately represent the true prevalence of co-morbidities and medication use. However, this imprecision is likely to have applied systemically to all groups in the analysis. A genetic risk score modelled using effect sizes from the same dataset used for analysis may introduce bias. However, in our sensitivity analysis we modelled a genetic risk score using effect sizes from an external dataset and demonstrated similar findings to the main analysis. Strengths of this study include the large

sample size with consistent methods of data collection, and comprehensive assessment including patient interviews, hospitalisation records and medical information.

Conclusion

In people on diuretics, serum urate-associated genetic variants contribute strongly to gout risk, with a similar effect to that observed in those not taking a diuretic. This suggests that the contribution of genetic variants is not restricted to people with 'primary' gout and can play an important role in gout susceptibility in the presence of other risk factors.

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Figure legends

Figure 1: Prevalence of gout according to genetic risk score category and diuretic use. CI, confidence interval.

Figure 2: Probability of gout according to genetic risk score and no diuretic use or loop diuretic use. Genetic risk score is shown as a continuous variable in this analysis. Solid lines represent probability of gout and shaded areas represent 95% confidence intervals. Data are adjusted by age, sex, body mass index, hypertension, renal failure, and heart failure. Genetic risk score-loop diuretic interaction P = 0.16.

Table 1: Baseline characteristics of participants according to diuretic use.

	No diuretic n=330165		Loop diuretic* n=3728		Thiazide diuretic* n=23623		Thiazide-like diuretic* n=2001	
	Control n=324020	Gout n=6145	Control n=3266	Gout n=462	Control n=23008	Gout n=615	Control n=1899	Gout n=102
Age, years (SD)	56.5 (8.0)	59.5 (7.0)	62.2 (5.8)	62.8 (5.6)	61.7 (5.8)	62.2 (5.9)	61.5 (5.7)	62.7 (5.0)
BMI, kg/m^2 (SD)	26.9 (4.5)	30.3 (4.7)	32.1 (6.6)	33.2 (6.1)	29.6 (5.1)	32.5 (5.2)	29.9 (5.2)	32.8 (6.4)
Sex, n (%)								
Male	152311 (47.0%)	5771 (93.9%)	1520 (46.5)	368 (79.7%)	9927 (43.1%)	529 (86.0%)	957 (50.4%)	86 (84.3%)
Female	171709 (53.0%)	374 (6.1%)	1746 (53.5%)	94 (20.3%)	13081 (56.9%)	86 (14.0%)	942 (49.6)	16 (15.7%)
Smoker, n (%)*	33351 (10.3%)	554 (9.1%)	293 (9.0%)	30 (6.5%)	1430 (6.2%)	41 (6.7%)	96 (5.1%)	3 (2.9%)
Alcohol frequency, n (%)*								
Daily or almost daily	68557 (21.2%)	2135 (34.8%)	484 (14.8%)	113 (24.5)	4759 (20.7%)	197 (32.1%)	441 (23.2%)	25 (24.5%)
Three to four times a week	79423 (24.5%)	1755 (28.6%)	482 (14.8%)	87 (18.9%)	4886 (21.3%)	155 (25.2%)	387 (20.4%)	28 (27.5%)
Once or twice a week	86186 (26.6%)	1375 (22.4%)	725 (22.2%)	121 (26.2%)	5702 (24.8%)	141 (23.0%)	467 (24.6%)	26 (25.5%)
Infrequent[#]	68961 (21.3%)	644 (10.5%)	1010 (30.9%)	92 (20.0%)	568 (24.7%)	83 (13.5%)	437 (23.0%)	14 (13.7%)
Never	20685 (6.4%)	230 (3.7%)	563 (17.2%)	48 (10.4%)	1962 (8.5%)	38 (6.2%)	167 (8.8%)	9 (8.8%)
Co-morbidities, n (%)*								
Hypercholesterolaemia	34034 (14.9%)	1563 (25.6%)	1083 (33.4%)	192 (41.6%)	7067 (30.8%)	249 (40.5%)	664 (35.0%)	44 (43.1%)
Hypertension	63644 (27.8%)	3113 (51.0%)	2199 (67.9%)	360 (77.9%)	21707 (94.6%)	578 (94.0%)	1806 (95.3%)	96 (94.1%)
Angina	9033 (4.0%)	451 (7.4%)	839 (25.9%)	136 (29.4%)	1210 (5.3%)	65 (10.6%)	141 (7.4%)	7 (6.9%)
Myocardial infarction	6687 (2.9%)	343 (5.6%)	757 (23.4%)	130 (28.1%)	675 (2.9%)	42 (6.8%)	82 (4.3%)	4 (3.9%)
Heart failure	75 (<0.1%)	10 (0.2%)	90 (2.8%)	32 (6.9%)	17 (0.1%)	2 (0.3%)	2 (0.1%)	0 (0.0%)
Stroke	1707 (0.7%)	42 (0.7%)	214 (6.6%)	43 (9.3%)	849 (3.7%)	42 (6.8%)	126 (6.6%)	11 (10.8%)
Transient ischaemic attack	3527 (1.5%)	156 (2.6%)	43 (1.3%)	8 (1.7%)	225 (1.0%)	6 (1.0%)	16 (0.8%)	2 (2.0%)
Renal failure	336 (0.1%)	69 (1.1%)	58 (1.8%)	28 (6.1%)	41 (0.2%)	9 (1.5%)	4 (0.2%)	1 (1.0%)
Diabetes mellitus	10374 (4.5%)	570 (9.3%)	775 (23.9%)	143 (31.0%)	2136 (9.3%)	116 (18.9%)	296 (15.6%)	32 (31.4%)

*Smoking status, alcohol frequency, diuretic use and co-morbidity data collected via self-report. [#]Infrequent alcohol frequency defined as: one to three times a month, or special occasions only.

Table 2: Mean genetic risk scores according to diuretic use.

	Genetic risk score, mean (SD)		
	Control	Gout	Control vs gout P
No diuretic	1.15 (0.26)	1.30 (0.26)	$<1x10^{-300}$
Loop diuretic	1.14 (0.26)	1.25 (0.26)	$1.26x10^{-16}$
Thiazide diuretic	1.14 (0.26)	1.28 (0.25)	$1.34x10^{-41}$
Thiazide-like diuretic	1.14 (0.25)	1.29 (0.28)	$2.10x10^{-8}$

In this analysis, the genetic risk score was modelled using effect sizes for gout from the UK Biobank dataset. SD, standard deviation.

Table 3: Association and interaction between genetic risk score and diuretic use for gout.

	OR (95% CI) for gout if genetic risk score ≥ mean*#	P	Interaction P^
No diuretic	2.63 (2.49-2.79)	8.74×10^{-240}	-
Loop diuretic	2.04 (1.65-2.53)	4.09×10^{-11}	0.32
Thiazide diuretic	2.70 (2.26-3.23)	1.17×10^{-27}	0.71
Thiazide-like diuretic	2.11 (1.37-3.25)	6.48×10^{-4}	0.39

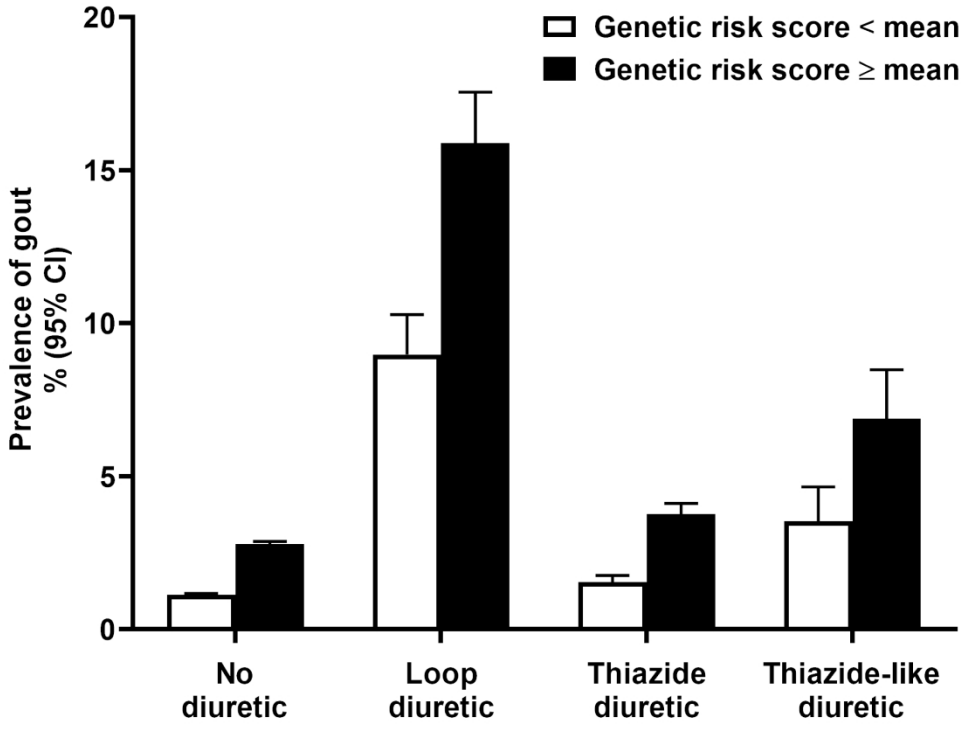
In this analysis, the genetic risk score was modelled using effect sizes for gout from the UK Biobank dataset. *Genetic risk score categorised according to the mean genetic risk score for the entire study population; mean genetic risk score = 1.15. #Data are adjusted by age, sex, body mass index, hypertension, renal failure and heart failure, and the association analysis was performed using genetic risk score < mean as the referent group. ^Interaction P determined using a genetic risk score by diuretic interaction term with comparison to no diuretic use. CI, confidence interval; OR, odds ratio.

Table 4: Association and interaction of serum urate-associated single nucleotide polymorphisms with gout according to diuretic use.

Gene SNP	Effect allele	No diuretic n=330165	Loop diuretic n=3728		Thiazide diuretic n=23623		Thiazide-like diuretic n=2001	
		OR (95% CI), P	OR (95% CI), P	Interaction P^	OR (95% CI), P	Interaction P^	OR (95% CI), P	Interaction P^
ABCG2 rs2231142	T	2.37 (2.24-2.50), $3.10x10^{-209}$	1.93 (1.54-2.43), $1.50x10^{-8}$	0.59	2.17 (1.82-2.59), $5.60x10^{-18}$	0.40	1.98 (1.26-3.10), $3.04x10^{-3}$	0.43
SLC2A9 rs12498742	A	3.06 (2.54-3.69), $7.73x10^{-32}$	4.19 (2.02-8.71), $1.23x10^{-4}$	0.15	2.59 (1.51-4.44), $5.41x10^{-4}$	0.61	3.92 (0.92-16.81), 0.07	0.67
GCKR rs1260326	T	1.38 (1.30-1.46), $9.94x10^{-29}$	1.13 (0.91-1.40), 0.26	0.14	1.51 (1.26-1.81), $7.23x10^{-6}$	0.31	1.61 (1.01-2.57), 0.04	0.46
SLC22A12 rs478607	A	0.72 (0.62-0.85), $4.74x10^{-5}$	1.55 (0.70-3.43), 0.28	0.08	0.77 (0.46-1.28), 0.31	0.85	1.35 (0.31-5.77), 0.69	0.36
MLXIPL rs1178977	A	1.31 (1.13-1.53), $4.03x10^{-4}$	1.31 (0.71-2.44), 0.39	0.99	1.02 (0.65-1.60), 0.91	0.26	1.29 (0.39-4.26), 0.67	0.98
PDZK1 rs1471633	A	1.24 (1.16-1.31), $8.02x10^{-12}$	1.26 (1.00-1.59), 0.05	0.49	1.29 (1.07-1.57), 0.01	0.63	1.80 (1.07-3.03), 0.03	0.13
SLC16A9 rs1171614	T	0.81 (0.77-0.86), $1.50x10^{-13}$	0.87 (0.70-1.07), 0.18	0.61	0.98 (0.83-1.16), 0.81	0.04	0.69 (0.45-1.07), 0.09	0.37
SLC17A3 rs1165151	T	0.80 (0.76-0.85), $3.99x10^{-15}$	0.91 (0.73-1.13), 0.38	0.52	0.75 (0.63-0.89), $1.02x10^{-3}$	0.44	1.21 (0.77-1.90), 0.42	0.08
INHBE rs3741414	T	0.83 (0.79-0.87), $8.24x10^{-12}$	0.81 (0.65-0.99), 0.04	0.51	0.80 (0.67-0.94), 0.01	0.66	0.75 (0.49-1.14), 0.18	0.46
SLC22A11 rs2078267	T	0.77 (0.72-0.82), $3.12x10^{-17}$	0.92 (0.72-1.18), 0.51	0.15	0.75 (0.62-0.90), $2.74x10^{-3}$	0.73	0.86 (0.52-1.41), 0.54	0.85

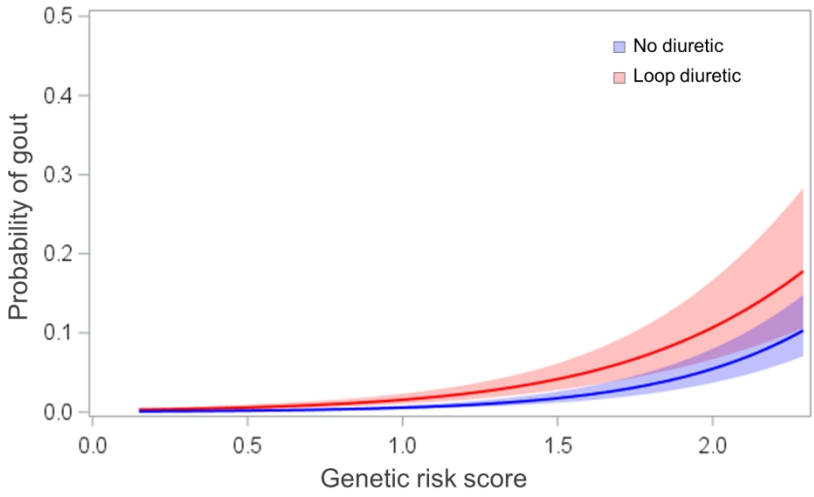
Data are adjusted by age, sex, body mass index, hypertension, renal failure and heart failure. ^Interaction P determined using a SNP by diuretic interaction term with comparison to no diuretic use. CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Experiment-wide significance was defined as $P < 0.005$.



Prevalence of gout according to genetic risk score category and diuretic use. CI, confidence interval.

166x129mm (300 x 300 DPI)



Probability of gout according to genetic risk score and no diuretic use or loop diuretic use. Genetic risk score is shown as a continuous variable in this analysis. Solid lines represent probability of gout and shaded areas represent 95% confidence intervals. Data are adjusted by age, sex, body mass index, hypertension, renal failure, and heart failure. Genetic risk score-loop diuretic interaction P = 0.16.

602x451mm (96 x 96 DPI)