Interaction of the TRIM46 / MUC1 locus with cigarette smoking may influence the risk of gout.

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Short Report

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Abstract

Objectives: Some studies suggest that current-smoking may be protective against gout and smoking cessation associated with higher incidence of gout. Our study assessed potential interactions between smoking, genetic variants and gout prevalence.

Methods: Four loci (ABCG2, GCKR, TRIM46, HNF4G) with evidence of smoking-influenced associations with serum urate were tested for non-additive interaction with current-smoker or ex-smoker status that associates with gout in Aotearoa New Zealand (NZ) East and West Polynesian participants with (n=520) and without (n=629) gout.

Results: Ex-smoker status was associated with higher prevalence of gout in people with East Polynesian but not West Polynesian ancestry. No association was detected between current smoking and gout. An interaction between TRIM46 (rs11264341) and ex-smoker status that associates with gout was observed in meta-analysis of NZ East and West Polynesians [ORInteraction = 0.58 (0.37-0.92)]. Never-smokers who were homozygous for the rs11264341 C-allele had higher odds of gout [OR = 2.43 (1.27; 4.64)], but not never-smoker heterozygotes [1.20 (0.73; 1.97). The C allele was not associated with gout in ex-smokers [0.73 (0.36-1.47)]. No interactions involving current-smoker status were detected.

Conclusions: We provide evidence for a non-additive interaction between TRIM46 (rs11264341) and ex-smoker status that associated with gout prevalence. MUC1, which encodes a transmembrane mucin in the lungs affected by cigarette smoke, is a possible candidate gene at this locus. No interaction involving current-smoker status was observed raising uncertainties about the relevance of an interaction specific to ex-smokers.

Background

Gout arises from an interplay between genetic variants and the environment (1). Genome-wide association studies have identified dozens of genomic loci associated with serum urate concentrations(2, 3) and gout(1).

In Aotearoa New Zealand (NZ), prevalence of gout is 2-3 times greater in people of Eastern (Māori and Cook Island Māori) and Western (Samoan, Tongan, Niuean, Tokelauan) Polynesian ancestry than European (4).

Multiple studies have found associations of current-smoking with lower gout incidence/prevalence (5-7)). However, the evidence is equivocal, with others reporting associations of current-smoking with higher gout prevalence (8, 9). There is also evidence of higher gout incidence in ex- compared to never- and current-smokers (6), and of serum urate increasing following smoking cessation(10).

In a Chinese population GCKR (rs1260326) and TRIM46 (rs11264341) associated with serum urate specifically in current-smokers, and HNF4G (rs2941481) in ex-smokers (11). An interaction between the
genotype combination of $SLC2A9$ (rs3733591), $ABCG2$ (rs2231142) and $PKD2$ (rs2725220) and current-smoking associated with hyperuricaemia in a Korean population (12). Based on evidence of genotype-specific effects of smoking on serum urate, we hypothesised gene-environment (GxE) interactions involving smoking in gout. We explored this possibility focusing on genetic variants with previous evidence of smoking interactions associated with serum urate [$GCKR$ (rs1260326), $TRIM46$ (rs11264341), and $HNF4G$ (rs2941481)], and variants with very strong effects on gout [$SLC2A9$ (rs7442295) and $ABCG2$ (rs2231142)].

Methods

Study participants

Cross-sectional data was from NZ participants aged 18 to 88, recruited 2010-2018. Participants were categorised into East Polynesian (Cook Island and NZ Māori; 381 non-gout and 352 gout) and West Polynesian (Samoa, Tonga, Niue, Tuvalu and Tokelau; 248 non-gout and 168 gout) groups based on self-reported ancestry of their grandparents. Ancestry was confirmed using genotypic principal component (PC) vectors calculated from 2858 ancestry-informative markers that cluster these genetically distinct ancestral groups (13). The study included a group of Māori (44 gout and 33 non-gout) from the rohe (area) of Ngati Porou iwi (tribe) from the Tairawhiti region, recruited in collaboration with Ngati Porou Hauora (health service). Gout was defined by American Rheumatism Association classification criteria (14). All controls self-reported no previous gout diagnosis. Ethical approval was granted by The Northern Y Region Health Research Ethics Committee (Ngati Porou Hauora subset) (NTY07/07/074) and the NZ Multi-Region Ethics Committee (MEC/105/10/130). All participants gave written informed consent. Participant demographic and clinical data are summarised in Table 1.

Data collection

Participants were asked if they were a current-smoker (Yes/No) or an ex-smoker. Participants who reported any or no alcohol consumption in the past week were categorised as drinkers or non-drinkers, respectively. Participants missing data for smoking, BMI and alcohol were excluded.

Genotyping

Genotypes of rs7442295 (surrogate for rs11942223), rs11264341, rs1260326, rs2231142, and rs2941481 were determined using the Infinium Human CoreExome-v24 single nucleotide polymorphism (SNP) array (Illumina, Inc., San Diego, CA, USA) (13).
Statistical analysis

All analyses were performed using R statistical software version 4.0.2. (R Core Team 2018) (15). Allele frequencies and results of the Hardy-Weinberg Equilibrium exact test were calculated using SNPassoc (version 1.9.2) (Supplementary File 1: Table S1, S2) (16). Two binary smoking variables were analysed in separate regressions - current-smoker compared to never-smoker and ex-smoker compared to never-smoker. Multivariable logistic regression was used for association analyses of smoking variables and gout, and to estimate interaction between individual SNP genotypes and smoking in gout prevalence, using a SNP x smoker-variable interaction term. Interaction analysis of SLC2A9 (rs7442295) was not performed because the minor allele frequency was too low (<0.03) (Table S1). TRIM46 (rs11264341) was further analysed by stratification of participants according to smoking status (never-smoker or ex-smoker) and minor allele genotype. Multivariable logistic regression was performed with the genotype-smoker stratified independent variable and gout as the dependent variable. All regression models were adjusted for age (continuous), sex (dichotomous), BMI (continuous) and alcohol intake (dichotomous), and the first 10 genotypic PC vectors, to adjust for genetic admixture and population substructure. Adjusted odds ratios (ORs) for gout and their 95% confidence intervals (CIs) were calculated. Meta-analysis was performed using meta package (version 4.13-0) (17). Heterogeneity was calculated using the Q (chi squared, $\chi^2$) test. When $P_{\text{Heterogeneity}} < 0.10$ the random-effect model is shown instead of the fixed-effect. Individual SNP vs gout association analyses are shown in Supplementary File 1: Table S3.

Results

Association analyses of smoking categories with gout.

Ex-smoker status was associated with higher gout prevalence in the East Polynesian subset [OR (95% CI): 2.60 (1.73; 3.92), $p=4.4\times10^{-6}$; Supplementary file 2: Figure S1 A]. An association in the same direction was observed in the West Polynesian subset, but was not statistically significant [1.27 (0.70; 2.31), $p=0.43$], and the meta-analytic effect of East and West Polynesian group regression estimates was not significant [OR$_{\text{Meta}}$: 1.88 (95% CI: 0.94; 3.79), $p=0.08$, Figure S1 A]. No association was detected between current-smoker status and gout, and ORs were in opposite directions, in East [OR: 1.32 (0.80; 2.19), $p=0.28$] and West Polynesian [0.48 (0.20; 1.12), $p=0.09$] subsets [Figure S1 B].

Smoking and SNP interaction

There was interaction between ex-smoker status and TRIM46 (rs11264341) associated with gout prevalence in the combined East and West Polynesian cohort [OR$_{\text{Meta-Interaction}}$ (95% CI): 0.58 (0.37; 0.92),
p=0.021, Table 2]. Main effects for both rs11264341 [OR\textsubscript{Meta}: 1.48 (1.08; 2.02), p=0.015] and ex-smoker status [5.93 (2.25; 15.61), p=3.1x10^{-4}] were in the direction of higher odds of gout (Supplementary File 2: Figure S2). No interaction was observed for rs11264341 with current-smoker status [OR\textsubscript{Meta}: 0.77 (0.43; 1.40), p=0.40, Table 3]. No interaction was observed at any of the other loci tested.

We stratified participants according to \textit{TRIM46} (rs11264341) genotype and smoking status, and looked at the relationship of each genotype-smoker combination with gout (Supplementary File 3: Figure S3 and S4). Gout prevalence was higher in never-smokers with the C/C genotype [OR\textsubscript{Meta} (95% CI): 2.43 (1.27; 4.64), p=0.007, Figure S3 B], but not in never-smoker heterozygotes [1.20 (0.73; 1.97), p=0.47, Figure S3 A], compared to the never-smoker T/T reference group. Prevalence of gout was higher in T/T ex-smoker [OR\textsubscript{Meta}: 3.94 (2.07; 7.51), p<1x10^{-4}] and C/C ex-smoker [2.75 (1.45; 5.21), p=0.002] genotype subgroups, relative to the never-smoker T/T reference subgroup (Figure S3 C and E). There was heterogeneity (P\textsubscript{Het}=0.06) between East and West Polynesian T/C ex-smoker genotype subgroups, where gout prevalence was increased in the East Polynesian [OR: 3.30 (1.68; 6.50), p=0.001], but not in the West Polynesian subgroup [1.10 (0.45; 2.70), p=0.84], compared to the T/T never-smoker reference category (Figure S3 D). Meta-analysis of T/C ex-smoker effects showed no association [OR\textsubscript{Meta} (95% CI): 1.99 (0.68; 5.82), p=0.21]. Additionally, analysis of rs11264341 genotype specifically within the ex-smoker subgroup found no association of T/C [OR\textsubscript{Meta}: 0.65 (0.35; 1.22), P=0.18] or C/C genotype [0.73 (0.36; 1.47), p=0.38] with gout, compared to the T/T reference group (Figure S4).

\textbf{Discussion}

We identified a GxE interaction of the \textit{TRIM46} (rs11264341) locus with ex-smoker status associated with gout prevalence in a NZ population of East and West Polynesian ancestry, independent of age, alcohol consumption, BMI and sex.

In main effect analysis ex-smoker status was independently associated with higher prevalence of gout in East Polynesians (6, 7). No association was observed between current-smoking and gout prevalence in East or West Polynesian cohorts. It is unclear why ex-smoker status is strongly associated with gout in East Polynesians and interacts with \textit{TRIM46} (rs11264341) to associate with gout in the combined Polynesian cohort, while current-smoker status does not. The smaller number of current-smokers compared to ex-smokers in the study cohort may in part explain why an association was not detected. Previous associations have been found for current-smoking with lower serum urate and gout prevalence (5-7). It is possible that greater genetic predisposition to gout in people with Polynesian ancestry negates any protective effect of current-smoking. Higher prevalence of gout in ex-smokers may be due to an increase in serum urate following smoking cessation (10). It is possible that ex-smokers gained weight following smoking cessation and/or are more likely to be older, however our results were
adjusted for age and BMI. It is also possible that higher prevalence in ex-smokers reflects gout onset that occurred before smoking cessation.

When considering the potential nature of the \textit{TRIM46} (rs11264341) interaction, never-smokers homozygous for the C allele had higher prevalence of gout than never-smokers without the C allele. Ex-smokers had higher prevalence of gout compared to T/T never-smokers irrespective of genotype and there was no association of rs11264341 genotype with gout within the ex-smoker subgroup. These results could suggest that rs11264341 genotype influences gout risk in never-smokers, whereas the risk effect of past smoking overrides the effect of genotype.

Causal candidate genes were identified at the \textit{TRIM46} (rs11264341) locus (18). Current knowledge would suggest \textit{MUC1} as the most likely candidate to interact with smoking. \textit{MUC1} encodes a transmembrane mucin, expressed on lung epithelium, whose expression, localisation and function is affected by cigarette smoke (19).

Limitations of this study include the relatively small number of participants. We did not know when smoking was commenced or ceased in relation to gout onset. Furthermore, information on smoking duration, intensity or a biochemical measure of smoking were not available for the cohort.

Conclusions

In this study of NZ Polynesian people we identified an interaction of the \textit{TRIM46} (rs1126434) locus and past smoking that is associated with gout prevalence, supporting our hypothesis that GxE interactions involving smoking associate with gout.

Declarations

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Conflict of interest
Prof Nicola Dalbeth reports grants and personal fees from AstraZeneca, grants from Amgen, personal fees from Dyve BioSciences, personal fees from Hengrui, personal fees from Selecta, personal fees from Arthrosi, personal fees from Horizon, personal fees from Abbvie, personal fees from Janssen, outside the submitted work. The remaining authors declare that they have no competing interests.

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**Tables**

**Table 1. Demographic and clinical data of NZ and UK Biobank datasets**
|                          | NZ East Polynesian | NZ West Polynesian |
|--------------------------|--------------------|--------------------|
|                          | Control            | Gout               | Control            | Gout               |
| n                        | 381                | 352                | 248                | 168                |
| Males, n (%)             |                    |                    |                    |
|                          | 164 (43)           | 271 (77)           | 116 (46.8)         | 130 (77.4)         |
| Age                     | 48.12 ± 16.83      | 59.07 ± 11.57      | 39.69 ± 16.74      | 53.89 ± 12.87      |
| BMI, kg/m²               | 32.49 ± 8.09       | 36.01 ± 8.01       | 33.00 ± 6.83       | 34.44 ± 5.92       |
| Serum urate, mmol/L     | 0.359 ± 0.109      | 0.382 ± 0.136      | 0.363 ± 0.111      | 0.400 ± 0.154      |
| Never-smoker, n (%)     | 176 (46.2)         | 102 (29)           | 164 (66.1)         | 89 (53)            |
| Ex-smoker, n (%)        | 108 (28.3)         | 191 (54.3)         | 44 (17.7)          | 66 (39.3)          |
| Current-smoker, n (%)   | 97 (25.5)          | 59 (16.8)          | 40 (16.1)          | 13 (7.7)           |
| Drinkers, n (%)         | 131 (34.4)         | 137 (38.9)         | 56 (22.6)          | 50 (29.8)          |
| Hypertension, n (%)     | 147 (41.1)         | 246 (71.3)         | 55 (23.3)          | 102 (62.6)         |
| Lipid problems, n (%)   | 74 (22.1)          | 178 (58.4)         | 41 (19)            | 78 (50.3)          |
| eGFR, ml/min/1.73 m²    | 74.48 ± 28.69      | 60.9 ± 27.48       | 78.67 ± 31.15      | 55.06 ± 27.25      |
| Diabetes, n (%)         | 104 (27.8)         | 138 (39.8)         | 67 (28)            | 69 (41.8)          |

Serum urate, age, BMI, eGFR and smoking pack years values are expressed as mean ± standard deviation.

Data was missing for serum urate, hypertension, diabetes, lipids and eGFR in 5.9%, 4.1% 2.1%, 12.0% and 6.3% of NZ Polynesian participants, respectively.

BMI, body mass index; eGFR, estimated glomerular filtration rate; NA, not available; NZ, New Zealand; ULT, urate-lowering therapy.

Smoker status, drinker status, hypertension, lipid problems and diabetes were self-reported.

Table 2. Genetic interaction effects for ex-smokers compared to never-smokers to gout risk
| SNP           | Gene   | Effect allele | East Polynesian | West Polynesian | Meta Polynesian |
|---------------|--------|---------------|-----------------|-----------------|-----------------|
|               |        |               | OR (95% CI)     | P               | OR (95% CI)     | P               | OR_{Meta} (95% CI) | P               |
| rs2331142     | ABCG2  | T             | 0.80 (0.27-2.42) | 0.699           | 1.05 (0.43-2.57)| 0.920           | 0.94 (0.47; 1.89) | 0.868           |
| rs2941481     | HFN4G  | A             | 0.94 (0.51-1.71) | 0.832           | 0.92 (0.42-2.01)| 0.825           | 0.93 (0.58; 1.5)  | 0.762           |
| rs11264341    | TRIM46 | C             | 0.63 (0.36-1.12) | 0.115           | 0.49 (0.22-1.08)| 0.078           | 0.58 (0.37; 0.92) | 0.021           |
| rs1260326     | GCKR   | T             | 1.19 (0.63-2.23) | 0.597           | 0.94 (0.38-2.32)| 0.891           | 1.10 (0.66; 1.84) | 0.721           |

Shown are odds ratios of the interaction effect model: SNP*ex-smoker status (interaction term) vs gout (response variable). Additive genetic model was assumed. Meta Polynesian column shows odds ratios from meta-analysis of East and West Polynesian population effect estimates. Fixed effects meta-analysis is shown. Heterogeneity was not detected (P_{Heterogeneity} >0.25 for all markers). Odds ratios are adjusted for age, sex, drinker status and BMI and PC1 to PC10. Current-smokers were excluded from models comparing ex- to never-smokers. BMI, body mass index; CI, confidence interval; OR, odds ratio; PC, principal component; SNP, single nucleotide polymorphism.

Table 3. Genetic interaction effects for current-smokers compared to never-smokers on gout risk

| SNP           | Gene   | Effect allele | East Polynesian | West Polynesian | NZ Polynesian |
|---------------|--------|---------------|-----------------|-----------------|--------------|
|               |        |               | OR (95% CI)     | P               | OR (95% CI)  | P           | OR_{Meta} (95% CI) | P               |
| rs2331142     | ABCG2  | T             | 0.82 (0.24-2.81) | 0.757           | 0.71 (0.19-2.59)| 0.599       | 0.77 (0.31; 1.87) | 0.557           |
| rs2941481     | HFN4G  | A             | 1.12 (0.53-2.36) | 0.763           | 0.36 (0.09-1.45)| 0.150       | 0.87 (0.45; 1.67) | 0.677           |
| rs11264341    | TRIM46 | C             | 0.82 (0.41-1.63) | 0.571           | 0.65 (0.2-2.1)  | 0.475       | 0.77 (0.43; 1.40) | 0.395           |
| rs1260326     | GCKR   | T             | 1.04 (0.49-2.21) | 0.919           | 0.42 (0.12-1.48)| 0.176       | 0.82 (0.43; 1.56) | 0.543           |

Shown are odds ratios of the interaction effect model: SNP*current-smoker status (interaction term) vs gout (response variable). Additive genetic model was assumed. Fixed effects meta-analysis is shown. Heterogeneity was detected for GCKR (rs1260326) (P_{Heterogeneity} = 0.23, random effects model OR (95% CI) : 0.76 (0.33-1.78), p=0.53) and HFN4G (rs2941481) (P_{Heterogeneity} = 0.16, random effects model OR (95% CI) : 0.75 (0.26-2.16), p=0.59), P_{Heterogeneity} >0.25 for other markers. Odds ratios are adjusted for age, sex, drinker status, BMI and PC1 to PC10. Ex-smokers were excluded from models comparing current to never-smokers. BMI, body mass index; CI, confidence interval; OR, odds ratio; PC, principal component; SNP, single nucleotide polymorphism.

**Supplementary Files**
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