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Original article

Replication of association of the apolipoprotein A1-C3-A4 gene cluster with the risk of gout

Humaira Rasheed[1,2], Amanda J. Phipps-Green[1], Ruth Topless[1], Malcolm D. Smith[3], Catherine Hill[4], Susan Lester[4], Maureen Rischmueller[4], Matthijs Janssen[5], Timothy L. Jansen[6], Leo A. Joosten[7], Timothy R. Radstake[8], Philip L. Riches[9], Anne-Kathrin Tausche[10], Frederic Lioté[11,12], Alexander So[13], Andre van Rij[14], Gregory T. Jones[14], Sally P. McCormick[1], Andrew A. Harrison[15], Lisa K. Stamp[16], Nicola Dalbeth[17] and Tony R. Merriman[1]

Abstract

Objective. Gout is associated with dyslipidaemia. Association of the apolipoprotein A1-C3-A4 gene cluster with gout has previously been reported in a small study. To investigate a possible causal role for this locus in gout, we tested the association of genetic variants from *APOA1* (*rs670*) and *APOC3* (*rs5128*) with gout.

Methods. We studied data for 2452 controls and 2690 clinically ascertained gout cases of European and New Zealand Polynesian (Māori and Pacific) ancestry. Data were also used from the publicly available Atherosclerosis Risk in Communities study (n = 5367) and the Framingham Heart Study (n = 2984). Multivariate adjusted logistic and linear regression was used to test the association of single-nucleotide polymorphisms with gout risk, serum urate, triglyceride and high-density lipoprotein cholesterol (HDL-C).

Results. In Polynesians, the T-allele of *rs670* (*APOA1*) increased (odds ratio, OR = 1.53, $P = 4.9 \times 10^{-6}$) and the G-allele of *rs5128* (*APOC3*) decreased the risk of gout (OR = 0.86, P = 0.026). In Europeans, there was a strong trend to a risk effect of the T-allele for *rs670* (OR = 1.11, P = 0.055), with a significant protective effect of the G-allele for *rs5128* being observed after adjustment for triglycerides and HDL-C (OR = 0.81, P = 0.039). The effect at *rs5128* was specific to males in both Europeans and Polynesians. Association in Polynesians was independent of any effect of *rs670* and *rs5128* on triglyceride and HDL-C levels. There was no evidence for association of either single-nucleotide polymorphism with serum urate levels ($P \geqslant 0.10$).

Conclusion. Our data, replicating a previous study, supports the hypothesis that the apolipoprotein A1-C3-A4 gene cluster plays a causal role in gout.

Key words: gout, hyperuricaemia, gene, association, apolipoprotein

CLINICAL SCIENCE

Rheumatology key message

- Replicated association of the apolipoprotein A1-C3-A4 gene cluster with gout implicates apolipoprotein metabolism as causal in gout.

[1]Department of Biochemistry, University of Otago, Dunedin, New Zealand, [2]Department of Chemistry, University of Engineering and Technology, Lahore, Pakistan, [3]Department of Medicine, Flinders Medical Centre and Repatriation General Hospital, Adelaide, [4]Rheumatology Department, The Queen Elizabeth Hospital, Woodville South, SA, Australia, [5]Department of Rheumatology, Rijnstate Hospital, Arnhem, [6]Department of IQ HealthCare, VieCuri Medical Centre, Venlo, [7]Department of Internal Medicine and Radboud Institute of Molecular Life Science, Radboud University Medical Center, Nijmegen, [8]Department of Rheumatology and Clinical Immunology, Laboratory of Translational Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands, [9]Rheumatic Diseases Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK, [10]Department of Rheumatology, University Clinic Carl-Gustav-Carus, Dresden, Germany, [11]INSERM, UMR-S 1132, Hospital Lariboisière, [12]University Paris Diderot (UFR de Médecine), Sorbonne Paris Cité, Paris, F-75205, France, [13]DAL, Service of Rheumatology, Laboratory of Rheumatology, University of Lausanne, CHUV, Nestlé 05-5029, Lausanne, Switzerland, [14]Department of Surgery, University of Otago, Dunedin, [15]Department of Medicine, University of Otago, Wellington, [16]Department of Medicine, University of Otago, Christchurch and [17]Department of Medicine, University of Auckland, Auckland, New Zealand

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Correspondence to: Tony R. Merriman, Department of Biochemistry, University of Otago, 710 Cumberland Street, Dunedin 9054, New Zealand. E-mail: tony.merriman@otago.ac.nz

Introduction

Gout typically presents as an acute autoinflammatory response to MSU crystals that form in individuals with hyperuricaemia (HU). While the aetiology of HU is becoming better understood, the genetic and biochemical factors predisposing individuals with HU to symptomatic gout are poorly understood [1]. Gout and HU are associated with an increased level of very low-density lipoprotein triglyceride (VLDL-Tg) [2], with association of VLDL-Tg with gout evident using an asymptomatic hyperuricaemic comparison group [3]. These findings implicate triglyceride and apolipoprotein metabolism in the aetiology of gout.

Hyperuricaemic-hypertriglyceridaemic patients have an increased ratio of apolipoprotein C3–C2 [4] along with increased VLDL levels [5]. The apolipoprotein molecules are encoded within a gene cluster of ~60 kb on human chromosome 11q23 that encodes for APOA1, APOC3, APOA4 and APOA5 [6]. Among these apolipoproteins, APOA1 is a major contributor to reverse cholesterol transport in the liver by acting as the ligand for the ABCA1 cholesterol transporter and as an obligatory cofactor for lecithin-cholesterol acyltransferase [7]. The major C-allele at the -75 position (*rs670*) in the promoter region has been associated with increased levels of circulating high-density lipoprotein cholesterol (HDL-C) in Han Chinese [8]. APOC3, predominantly produced in the liver (reviewed in [9]), facilitates the exchangeable component of Tg-rich lipoproteins (chylomicron and VLDL) and is an inhibitor of lipoprotein and hepatic lipase [10]. In *APOC3*, the minor allele of *rs5218* in the 3′ non-coding region (3238G/C) has repeatedly been associated with hypertriglyceridaemia [11–14]. However this candidate variant was not associated with gout in a small Spanish case–control sample set [4]. In contrast, supporting a causal role for APOA1 in gout, the C-allele of *rs670* was associated with gout in the same Spanish sample set (odds ratio, OR = 1.99, P = 0.01) [4]. The aim of this study was to test for association of *APOA1* variant *rs670* and *APOC3* variant *rs5128* with gout.

Methods

Subjects

Demographic and clinical details of all study sample sets are summarized in supplementary Table S1, available at *Rheumatology* Online. The New Zealand (NZ) gout participants were mostly recruited from Auckland, Wellington and Christchurch, NZ. Additional cases of European ancestry were recruited by the Eurogout consortium within the European Crystal Network (n = 762) [15] and by the Arthritis Genomics Recruitment Initiative in Australasia (n = 83). All gout cases were clinically ascertained by the ARA preliminary classification criteria [16]. All variables except for biochemical measurements [serum urate (SU), serum triglyceride and HDL-C] and BMI were self-reported.

The NZ control group self-reported their lack of gouty arthritis. It comprised 457 European and 994 Polynesian individuals recruited primarily from the Auckland area, using the same protocol as the cases, and 1001 European controls sampled from the Otago region [17]. Apart from the Otago controls, the NZ participants were not requested to fast. On the basis of the self-reported ancestry of grandparents, the Polynesian participants were divided into three subgroups: Eastern Polynesian (EP)—NZ Māori and Cook Island Māori (489 cases, 626 controls), Western Polynesian (WP)—Tongan, Samoan and Niuean (412 cases, 298 controls) and a small mixed Eastern and Western ancestry group (EP/WP) (34 cases, 70 controls). The stratification of Polynesian subjects into EP and WP was because of genetic diversity between the two groups [18]. The NZ Multi-region Ethics Committee (105/10/130) and the following institutional committees in Europe and Australia granted ethical approval: Research and Ethics Committee, Repatriation General Hospital, South Australia (32/08); Research Ethics Committee, University of New South Wales; Ethikkommission, Technische Universität Dresden (EK 8012012); South East Scotland Research Ethics Committee (04/S1102/41); Commission Cantonale (VD) D'éthique de la Recherche sur l'être Humain, Université de Lausanne; Commissie Mensgebonden Onderzoek regio Arnhem—Nijmegen; Partners Health Care System Institutional Review Board. Written informed consent according to the Declaration of Helsinki was obtained from all participants.

Participants from the Atherosclerosis Risk in Communities (ARIC) study and the Framingham Heart Study (FHS) (Generation 3 only) cohorts were also used as additional controls for the gout risk analysis and for evaluating association with SU (approval number #834; The Genetic Basis of Gout). Subjects from ARIC and FHS who self-reported as taking diuretic medication, who were first-degree related, who were not of European ancestry, who were diagnosed with kidney disease or who reported physician-diagnosed gout, were excluded. The final ARIC and FHS data sets consisted of 5367 and 2984 people of European ancestry, respectively. For all ancestral groups (except the 1001 controls recruited from the Otago region of NZ, for whom SU data were not available), control participants were further stratified into a normouricaemia group (with a SU level <0.41 mmol/l) and a HU group with a SU level of $\geqslant 0.41$ mmol/l.

Genotyping

In the NZ samples, *rs670* and *rs5128* Taqman genotyping was done using a LightCycler 480 Real-Time PCR System (Roche Applied Science, IN, USA) in 384-well plates. The ARIC sample set was genotyped on the Affymetrix SNP 6 platform, and the FHS sample set had been genotyped by the Affymetrix SNP 5 platform and a custom-designed gene-centric 50K+500K array SNP platform. Both single-nucleotide polymorphisms (SNPs) were imputed in the ARIC data set using IMPUTE2 (ver. 2.3.0) [19] to a common reference panel from the 1000 Genomes project [Phase I integrated variant set release (ver. 3), March 2012, NCBI build 37 (hg19)]. Both SNPs were imputed in FHS generation 3 subjects using MACH1 v1.0.15 [20] with the HapMap2 CEU (release 22, build 36) sample set used as reference haplotypes. For the 1001 controls recruited from the Otago region of NZ, 640 participants were

genotyped using the Affymetrix 6 platform and the remainder using the Illumina Omni2.5 platform. *Rs5128* was genotyped by both platforms, whereas *rs670* was imputed (IMPUTE ver. 2.2) to a common reference panel from the 1000 Genomes project [Phase I integrated variant set release (ver. 3), March 2012, NCBI build 37 (hg19)], followed by quality score (*Q* cor.9) filtering. The genotype distributions of all sample sets presented in Table 1 were in Hardy–Weinberg equilibrium ($P > 0.01$).

Statistical analyses

Associations with gout risk and blood metabolites (SU, serum triglyceride and HDL-C) were determined using logistic and linear regression, respectively, using STATA ver. 8.0 (StataCorp, College Station, TX, USA). All associations were adjusted for age and sex with additional adjustors being serum triglyceride, HDL-C and BMI. In Polynesians, a STRUCTURE [21] ancestral estimate was also included as an adjustor, as previously described [22], in order to account for admixture, primarily with Europeans. Meta-analysis of allele counts was performed in R within STATA, using rmeta to calculate combined ORs and to evaluate heterogeneity between studies, with a random effect model being used when $P_{Het} < 0.05$. Using rmeta, the OR is a ratio of frequency ref/alt alleles in cases *vs* the frequency of ref/alt alleles in controls, whereas the OR derived from logistic regression measures the relationship between the binary dependent variable (gout) and independent variable (genotype) by estimating probabilities using a logistic function. Coefficients with $P \leqslant 0.05$ were considered to indicate a nominally significant association. Because there was little linkage disequilibrium between *rs670* and *rs5128* (r^2 in NZ Europeans = 0.01, r^2 in NZ Polynesians = 0.08), association analysis of haplotypes was not done; instead, epistatic interaction was tested by incorporating an interaction term in the logistic regression analysis.

Power calculations for the individual sample sets are presented in supplementary Table S2, available at *Rheumatology* Online. For *rs670*, using all controls, there was adequate power in the European sample set to detect an allelic effect size of $OR > 1.2$, and adequate power in the combined Polynesian sample set to detect an allelic effect size of $OR > 1.3$. For *rs5128*, power was adequate in both sample sets to detect an effect size of $OR > 1.2$.

Models of inheritance were estimated by formulating the genotype predictor in the logistic regression model in different ways: full genotype model (CC, CT, TT)—two ORs; additive model (0, 0.5, 1) or (0, 1, 2)—one OR (this should be approximately the same as the allelic OR but may differ if there is lack of fit to Hardy–Weinberg equilibrium in either cases or controls); dominant model (0, 1, 1)—one OR; or recessive model (0, 0, 1)—one OR. A model selection tool (Akaike Information Criterion [23]) was used to select the most likely model.

Results

Association of *rs670* and *rs5128* was first evaluated with serum triglyceride and HDL-C levels in the combined gout and non-gout European and Polynesian sample sets (supplementary Table S3, available at *Rheumatology* Online). Evidence for association of the G-allele of *rs5128* (*APOC3*) with serum triglyceride and both variants with HDL-C (C-allele of *rs5128* and T-allele of *rs670*) was observed in both ancestral groups, with a consistent direction of association. For example, for *rs5128* in serum triglyceride the effect size was, per copy of the minor G allele, 0.150 mmol/l in Europeans and 0.149 mmol/l in Polynesians, a direction of effect consistent with previous reports (ref. [14] and citations therein). In Polynesians and Europeans, the minor T allele of *rs670* was associated with increased HDL-C, an opposing effect to that previously observed in Han Chinese [8].

Increased risk of gout was associated with the minor T-allele of *rs670* in the EP sample set (Table 1; OR = 1.61, P = 0.001). When the three Polynesian sample sets were combined by meta-analysis, increased risk of gout was associated with the T-allele (Fig. 1A; $OR_{meta} = 1.53$, $P_{meta} = 4.88 \times 10^{-6}$). A strong trend towards association of the T-allele with gout risk was observed in Europeans (OR = 1.11, P = 0.055) (Table 1). Combined with the Polynesian sample sets and the previously published Cardona *et al.* [4] data in meta-analysis, there was also evidence for association of *rs670* with the risk of gout (Fig. 1B; $OR_{meta} = 1.47$, $P_{meta} = 0.003$). The minor G-allele of *rs5128* was associated with reduced risk of gout in the WP sample set (Table 1; OR = 0.65, P = 0.001) as well as in meta-analysis of the combined Polynesian sample set (Fig. 2A; $OR_{meta} = 0.86$, $P_{meta} = 0.026$). No evidence of association of the G-allele with gout risk was observed in Europeans (OR = 0.91, P = 0.22), although adjustment by serum triglyceride and HDL-C level provided nominal evidence for association (OR = 0.81, P = 0.039). When the European, Cardona *et al.* [4] and Polynesian samples were combined by meta-analysis for *rs5128* there was evidence for association with gout (Fig. 2B; $OR_{meta} = 0.86$, $P_{meta} = 0.036$). There was no evidence for epistatic interaction between *rs670* and *rs5128* in determining the risk of gout in Europeans or Polynesians ($OR_{Interaction} = 0.81$, P = 0.40 and $OR_{Interaction} = 0.64$, P = 0.11, respectively).

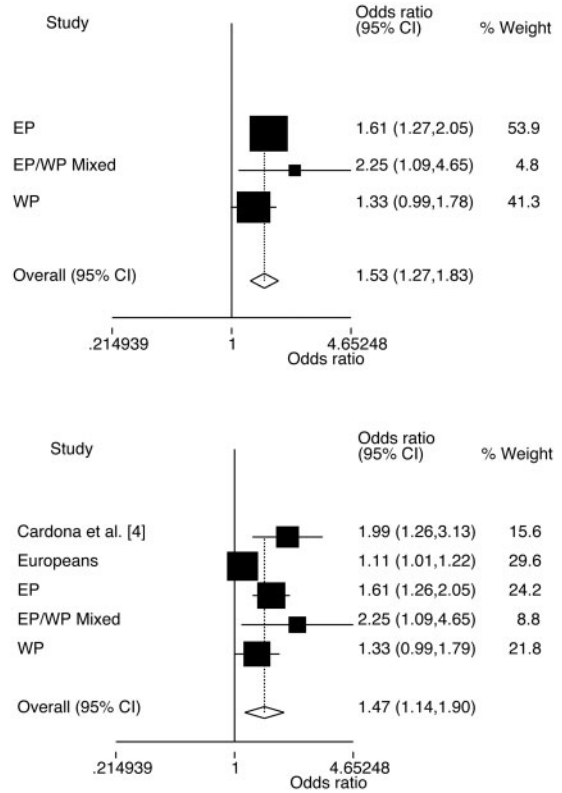
To investigate the inheritance pattern for each of *rs670* and *rs5128*, four models were estimated by conditional logistic regression (with strata defined by population): full genotype model, additive model (that should approximate the allelic OR), dominant model and recessive model. The Akaike Information Criteria [23] were used to select the most favourable model: a recessive model for *rs670* and an additive model for *rs5128*. On this basis, ORs were calculated for *rs670* under a recessive model (comparing the TT-genotype group to the combined CC/CT-genotype group) using all controls and adjusting for age and sex and, for Polynesians, ancestry. This revealed significant evidence for association in Europeans [OR = 1.62 (1.17, 2.23), P = 0.001] compared with OR = 1.11 (P = 0.055) under an additive model and strengthened evidence for association in EP [OR = 21.40 (6.51, 70.35), $P = 4.4 \times 10^{-7}$] compared with OR = 1.61, P = 0.001 under an additive model. For WP, evidence for association was not significant [OR = 1.72 (0.51, 5.80), P = 0.38] compared with OR = 1.38, P = 0.074

Table 1 Association analysis of *rs670* and *rs5128* with risk of gout using all controls

	Case genotypes, n (freq)				Control genotypes, n (freq)				Unadjusted		Adjusted[a]	
	CC	CT	TT	T Freq	CC	CT	TT	T Freq	Allelic OR (T-Allele), (95% CI)	Allelic P	Allelic OR (T-Allele), (95% CI)	Allelic P
rs670 (*APOA1*)												
European	1204 (0.693)	470 (0.270)	64 (0.037)	598 (0.172)	6911 (0.708)	2629 (0.269)	227 (0.023)	3083 (0.158)	1.11 (1.01, 1.22)	0.035	1.11 (1.00, 1.24) 1.07 (0.93, 1.25) 1.14 (1.00, 1.30)	0.055 0.34 0.051
Eastern Polynesian	356 (0.739)	87 (0.180)	39 (0.081)	165 (0.171)	487 (0.780)	132 (0.212)	05 (0.008)	142 (0.114)	1.50 (1.20, 1.88)	0.0004	1.61 (1.12, 2.15) 1.74 (1.26, 2.42) 1.56 (1.16, 2.10)	0.001 0.001 0.003
EP/WP	20 (0.588)	10 (0.294)	04 (0.118)	18 (0.265)	51 (0.739)	17 (0.246)	01 (0.015)	19 (0.138)	2.11 (1.04, 4.28)	0.039	1.97 (0.88, 4.39) 1.98 (0.84, 4.67) 2.23 (0.91, 5.43)	0.097 0.12 0.078
Western Polynesian	266 (0.670)	121 (0.305)	10 (0.025)	141 (0.178)	221 (0.742)	71 (0.238)	06 (0.020)	83 (0.139)	1.34 (1.00, 1.81)	0.053	1.38 (0.97, 1.95) 1.49 (1.01, 2.21) 1.47 (1.02, 2.12)	0.074 0.045 0.039
	CC	CG	GG	G Freq	CC	CG	GG	G Freq	Allelic OR (G-Allele), (95% CI)	Allelic P	Allelic OR (G-Allele), (95% CI)	Allelic P
rs5128 (*APOC3*)												
European	1464 (0.849)	245 (0.142)	16 (0.009)	277 (0.080)	8165 (0.833)	1542 (0.157)	94 (0.010)	1730 (0.088)	0.90 (0.79, 1.03)	0.13	0.91 (0.79, 1.06) 0.81 (0.66, 0.99) 0.89 (0.75, 1.06)	0.22 0.039 0.20
Eastern Polynesian	192 (0.400)	241 (0.502)	47 (0.098)	335 (0.349)	268 (0.432)	271 (0.436)	82 (0.132)	435 (0.350)	0.99 (0.83, 1.19)	0.95	0.98 (0.78, 1.24) 0.93 (0.71, 1.20) 0.96 (0.76, 1.22)	0.91 0.57 0.74
EP/WP	15 (0.441)	14 (0.412)	05 (0.147)	24 (0.353)	26 (0.371)	30 (0.429)	14 (0.200)	58 (0.414)	0.79 (0.45, 1.40)	0.42	0.83 (0.42, 1.63) 1.04 (0.50, 2.17) 0.74 (0.37, 1.50)	0.60 0.92 0.40
Western Polynesian	163 (0.405)	177 (0.439)	63 (0.156)	303 (0.376)	82 (0.275)	157 (0.527)	59 (0.198)	275 (0.461)	0.71 (0.57, 0.87)	0.002	0.65 (0.50, 0.84) 0.62 (0.46, 0.82) 0.66 (0.50, 0.86)	0.001 0.001 0.003

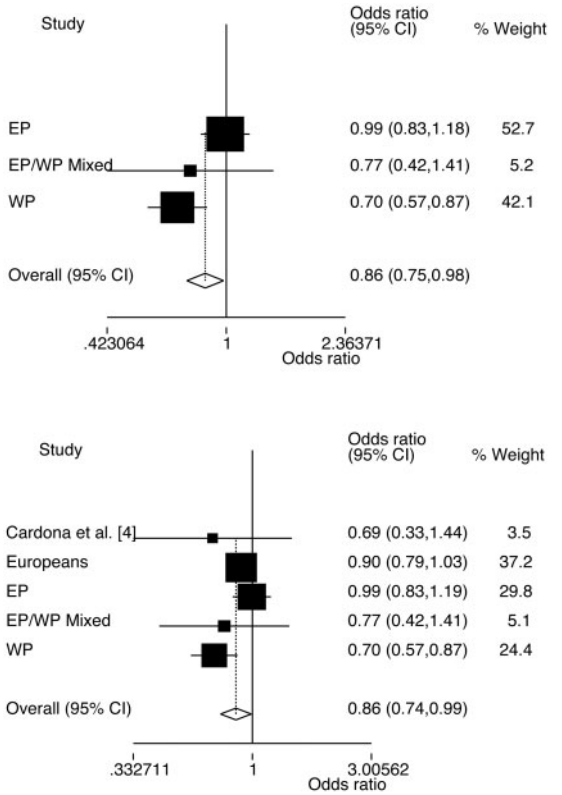
[a]Top value adjusted for age and sex; middle value adjusted for age, sex, triglyceride and HDL-C; bottom value adjusted for age, sex and BMI. Polynesian sample sets were additionally adjusted for STRUCTURE ancestry estimate. WP: Western Polynesian; EP: Eastern Polynesian. HDL-C: high-density lipoprotein cholesterol; freq: frequency.

Fig. 1 Meta-analysis of Polynesian, and Polynesian and European subgroups using all controls for *rs670*



Top, Polynesian subgroups: P_{Het} = 0.34 and P for OR is 4.9×10^{-6}. Bottom, Polynesian and European subgroups: P_{Het} = 0.003 and P for OR is 0.003. WP: Western Polynesian; EP: Eastern Polynesian.

Fig. 2 Meta-analysis of Polynesian, and Polynesian and European subgroups using all controls for *rs5128*



Top, Polynesian subgroups: P_{Het} = 0.048 and P for OR is 0.026. Bottom, Polynesian and European subgroups: P_{Het} = 0.15 and P for OR is 0.036. WP: Western Polynesian; EP: Eastern Polynesian.

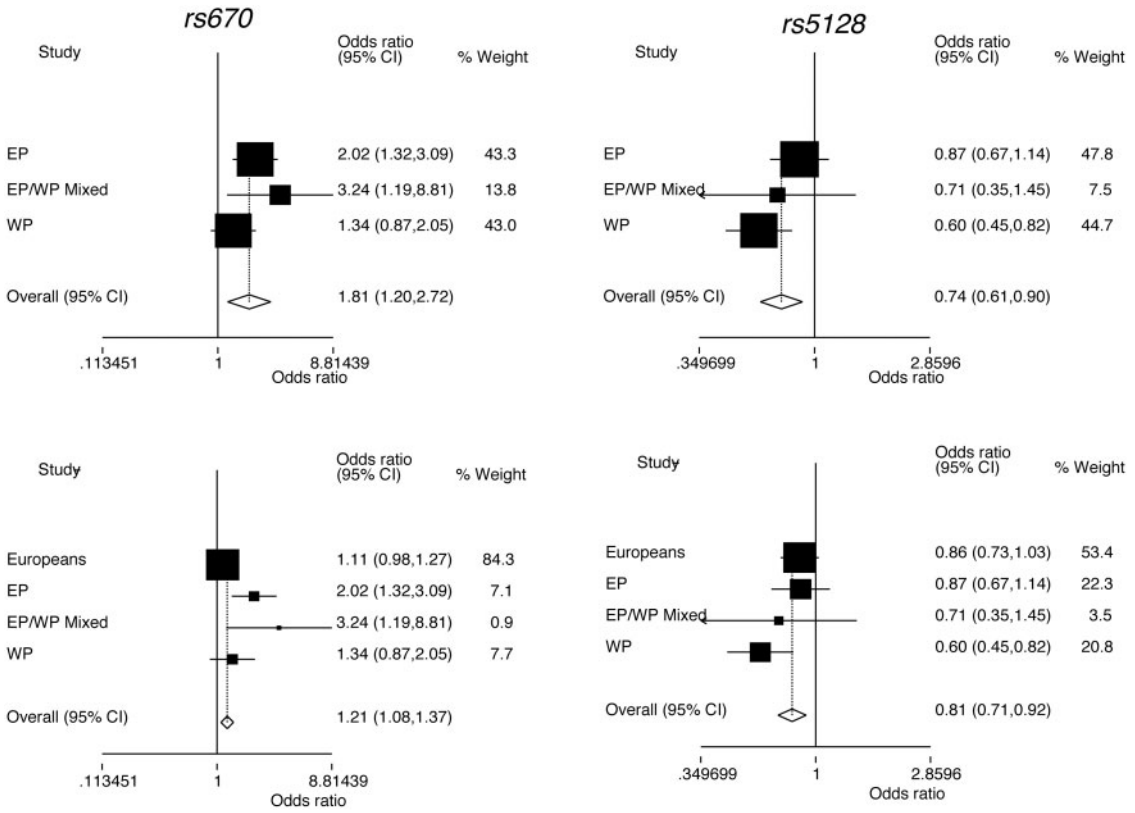
under an additive model with statistical evidence for association similar in the sample set of mixed Eastern and WP ancestry [OR = 6.81 (0.67, 68.82), P = 0.10] to the additive model (OR = 1.97, P = 0.097).

Association analyses were adjusted by serum triglyceride and HDL-C (in addition to age and sex), with no substantial changes in effect size or P-values in the Polynesian analyses (Table 1), indicating that the observed associations with gout were independent of serum triglyceride and HDL-C levels. However, in Europeans, the serum triglyceride and HDL-C adjustment reduced the evidence for association of *rs670* with gout [OR = 1.11, P = 0.055 to OR = 1.07, P = 0.34, although a recessive model provided significant evidence for association; OR = 1.63 (1.06, 2.50), P = 0.026] and provided evidence for association of *rs5128* with gout (OR = 0.81, P = 0.039). However, in Europeans, as was also observed in Polynesians, adjustment for BMI did not substantially influence the effect size or P-values at either SNP.

Analysis was conducted using HU controls (SU $\geqslant$ 0.41 mmol/l) (supplementary Table S4, available at *Rheumatology* Online). Consistent with the unstratified controls analysis, for the T-allele of *rs670* variant, increased gout risk was observed in the EP sample set (OR = 1.84, P = 0.004) and in combined Polynesians (Fig. 3; OR = 1.81, $P = 1.2 \times 10^{-4}$). A recessive model was not used owing to the scarcity of the TT-genotype in the Polynesian HU controls. Evidence for a significant protective role of the G-allele of *rs5128* was observed in the WP sample set (OR = 0.63, P = 0.007) and in combined Polynesians (Fig. 3; OR = 0.74, P = 0.002). In Europeans, there was no significant evidence for association of *rs670* with gout using HU controls, although there was significant association at *rs5128* after adjustment for serum triglyceride and HDL-C (supplementary Table S4, available at *Rheumatology* Online; OR = 0.77, P = 0.045). However, for *rs670* under a recessive model, there was significant evidence for association in Europeans [OR = 1.66 (1.02, 2.70), P = 0.040]. Meta-analysis with Polynesians strengthened the evidence for association of *rs5128* with gout (Fig. 3; OR = 0.81, P = 0.001), but weakened the evidence for association of *rs670* with gout (OR = 1.21, P = 0.001).

Using all controls, stratification by sex did not reveal significant association of *rs670* with either sex in Europeans, using either an allelic or recessive model, with significant association observed in males and

FIG. 3 Meta-analysis of Polynesian, and European and Polynesian sample sets using HU controls



Top, Polynesian sample set: for *rs670* (*APOA1*) $P_{Het} = 0.18$ and P for OR is 1.2×10^{-4} and for *rs5128* (*APOC3*) $P_{Het} = 0.20$ and P for OR is 0.002. Bottom, Polynesian and European sample set: for *rs670* (*APOA1*) $P_{Het} = 0.011$ and P for OR is 0.001 and for *rs5128* (*APOC3*) $P_{Het} = 0.20$ and P for OR is 0.001. WP: Western Polynesian; EP: Eastern Polynesian.

females in combined Polynesians using both models (Table 2). However, at *rs5128*, the association was restricted to males in both the European and combined Polynesian sample sets (Table 2; OR = 0.78, P = 0.027 and OR = 0.69, P = 0.001, respectively). Similar data were observed using HU controls for *rs670* (OR = 1.56, P = 0.012 and OR = 2.86, P = 0.006 for combined Polynesian males and females, respectively, using the allelic model) and for *rs5128* (OR = 0.75, P = 0.032 and OR = 0.66, P = 0.003 for European and combined Polynesian males, respectively).

Neither of the SNPs showed any evidence for association with SU in European and Polynesian non-gout controls (Table 3; all age- and sex-adjusted $P > 0.10$), even when *rs670* was analysed under a recessive model ($\beta = -0.0025$, P = 0.61 and $\beta = -0.0050$, P = 0.84, respectively). Meta-analysis of European and Polynesian data also provided no support for association of the tested SNPs with SU ($\beta_{meta} = -0.001$ mmol/l, $P_{meta} = 0.62$, $P_{Het} = 0.12$ for *rs670* and $\beta_{meta} = 0.003$ mmol/l, $P_{meta} = 0.097$, $P_{Het} = 0.29$ for *rs5128*).

Discussion

We demonstrated association of the minor T-allele of *rs670* with increased risk of gout in Polynesians (Fig. 1; OR = 1.53, $P = 4.9 \times 10^{-6}$). This replicates the finding of Cardona *et al.* [4] in a small Spanish sample set of 68 cases and 165 controls (OR = 1.99, P = 0.0031). Additionally, there was a very strong trend to association in the European sample set (OR = 1.11, P = 0.055). Thus, these *rs670* data are notable in that they represent the strongest replicated evidence to date for association of an apparently non-urate-controlling genetic variant with gout (the *TLR4* gene is to our knowledge the only other non-urate-controlling gene having replicated association with gout [24, 25]). There was no evidence for association of *rs670* with SU; therefore, these data suggest that this variant causes gout in the presence of HU. The relationship between hypertriglyceridaemia and gout is likely to be complex, and the interpretation of data such as these is complicated by the established observational association between HU/gout and hypertriglyceridaemia. There is some evidence that *rs670* is associated with HDL-C, and *rs5128* has been consistently associated with triglyceride levels, including in the data sets studied here with the triglyceride-raising G-allele of *rs5128* protecting from gout in Polynesians (Table 1 and supplementary Table S3, available at *Rheumatology* Online). However, because the gout associations observed here were independent of HDL-C and triglyceride levels (Tables 2 and 3), the

Table 2 Association analysis of *rs670* and *rs5128* with gout using all controls in males and females

		Case genotypes, n (freq)				Control genotypes, n (freq)				Unadjusted		Adjusted	
		CC	CT	TT	T Freq	CC	CT	TT	T Freq	Allelic OR (T-Allele), (95% CI)	Allelic P	OR, (95% CI)	P-values
rs670 (APOA1)													
Males	Combined Europeans	1018 (0.694)	393 (0.268)	55 (0.037)	503 (0.171)	3471 (0.711)	1299 (0.266)	110 (0.022)	1519 (0.156)	1.12 (1.01, 1.25)	0.039	1.09 (0.92, 1.28)	0.056
												1.58 (0.99, 2.54)	0.31
	Combined Polynesians	536 (0.713)	172 (0.229)	44 (0.058)	260 (0.173)	344 (0.763)	102 (0.226)	05 (0.011)	112 (0.124)	1.41 (1.13, 1.77)	3×10^{-3}	1.63 (1.23, 2.16)	1×10^{-3}
												6.54 (2.34, 18.28)	3×10^{-4}
Females	Combined Europeans	184 (0.684)	76 (0.282)	09 (0.033)	94 (0.174)	3440 (0.704)	1330 (0.272)	117 (0.024)	1564 (0.160)	1.11 (0.88, 1.40)	0.36	1.00 (0.67, 1.50)	0.99
												1.82 (0.62, 5.31)	0.27
	Combined Polynesians	106 (0.658)	46 (0.286)	09 (0.056)	64 (0.199)	413 (0.770)	117 (0.218)	06 (0.011)	129 (0.120)	1.80 (1.29, 2.51)	5×10^{-4}	1.67 (1.05, 2.67)	0.031
												14.69 (2.83, 76.24)	1×10^{-3}
		CC	CG	GG	G Freq	CC	CG	GG	G Freq	Allelic OR (G-Allele), (95% CI)	Allelic P	Allelic OR (G-Allele), (95% CI)	Allelic P
rs5128 (APOC3)													
Males	Combined Europeans	1232 (0.846)	210 (0.144)	14 (0.010)	238 (0.082)	4093 (0.835)	759 (0.155)	52 (0.011)	863 (0.088)	0.92 (0.80, 1.07)	0.30	0.78 (0.62, 0.97)	0.027
	Combined Polynesians	304 (0.402)	363 (0.480)	89 (0.112)	541 (0.358)	165 (0.366)	213 (0.472)	73 (0.162)	359 (0.398)	0.84 (0.71, 1.00)	0.046	0.69 (0.55, 0.85)	1×10^{-3}
Females	Combined Europeans	230 (0.865)	34 (0.128)	02 (0.007)	38 (0.071)	4072 (0.831)	783 (0.160)	42 (0.009)	867 (0.088)	0.79 (0.57, 1.11)	0.18	0.94 (0.56, 1.60)	0.83
	Combined Polynesians	66 (0.410)	69 (0.429)	26 (0.161)	121 (0.376)	209 (0.391)	243 (0.455)	82 (0.154)	407 (0.381)	0.98 (0.76, 1.26)	0.87	1.14 (0.80, 1.60)	0.47

Adjusted for age, triglyceride and HDL-C. Polynesians are additionally adjusted for STRUCTURE ancestry estimate. For *rs670* in the adjusted column, the allelic OR is top and the recessive model OR is bottom. HDL-C: high-density lipoprotein cholesterol; freq: frequency.

Table 3 Association analysis of *rs670* and *rs5128* with SU (mmol/l) in controls

	Number	Unadjusted β (95% CI)	P-values	Adjusted β (95% CI)	P-values
rs670 (*APO A1*)					
European non-gout	8755	−0.0016 (−0.0050, 0.0019)	0.37	−0.0002 (−0.0030, 0.0025)	0.87
Polynesian non-gout	821	−0.0131 (−0.0265, 0.00004)	0.057	−0.0098 (−0.0219, 0.0023)	0.11
rs5128 (*APO C3*)					
European non-gout	8764	0.0024 (−0.0019, 0.0068)	0.28	0.0020 (−0.0015, 0.0055)	0.26
Polynesian non-gout	821	0.0131 (0.0043, 0.0220)	4×10^{-3}	0.0068 (−0.0014, 0.0149)	0.10

Adjusted for age and sex. The Polynesian sample set is additionally adjusted for STRUCTURE ancestry estimate and ancestral group. SU: serum urate.

simplest interpretation is that the functional effects (marked by *rs670* and *rs5128*) on apolipoprotein metabolism are directly causal of gout.

The association of *rs5128* (*APOC3*) with gout was restricted to males in both Europeans and Polynesians (Table 2). As the existing literature is scant in the area of apolipoprotein metabolism and the risk of gout, it is not possible to speculate on a biological basis for this effect. Elucidating the basis for this male-specific effect will be important in understanding both the molecular basis of risk of gout at *APOC3* and identifying differences in the pathogenesis of gout between men and women. A recessive model was the most favourable for *rs670*, and use of this model provided significant evidence for association with gout in Europeans using all controls and HU controls (OR = 1.62, P = 0.001 and OR = 1.66, P = 0.040, respectively).

The *rs670* variant maps to the -75 nucleotide position in the promoter region of the *APOA1* gene [26]. In the published literature, whether or not this variant influences APOA1 expression is unclear, with two studies yielding conflicting results [27, 28]. However the Genotype-Tissue Expression database (www.gtexportal.org; ver. 6) associates the gout risk T-allele of *rs670* with increased expression of *APOA1* in heart left-ventricle ($P = 3.3 \times 10^{-16}$) but in no other tissues. Thus, the association of *rs670* with gout may be mediated by an influence on the expression of *APOA1.* There is evidence that APOA1 is involved in gout inflammatory pathways. APOA1 inhibits IL-1β production [29], which is a key factor for monocyte recruitment in gouty inflammation [30]. Moreover, the APOA1 mimetic peptide 4F can inhibit proinflammatory gene expression by altering the assembly of toll-like receptor-ligand complexes in cell membranes [31]. Finally, an increased level of MSU crystal-bound APOA1 has been reported in acute gout [32]. Chiang *et al.* [32] speculated that APOA1 could be involved in the resolution of acute gout attacks, whereas our genetic association data are consistent with an additional role for APOA1 in the initiation of gout. As far as we are aware, APOA1 has not been directly implicated in VLDL metabolism, although evidence exists that increased levels of triglyceride-enriched VLDL particles have a positive association with HDL-APOA1 catabolism (ref. [33] and citations therein).

We detected stronger association of *rs670* (*APOA1*) in Polynesians than in Europeans. Our evidence suggests that this effect is independent of control of urate, although it will be necessary to retest association of these variants with urate levels in a larger Polynesian sample set. If the replicated association of *rs670* with gout in Polynesians was due to contribution to the formation and accumulation of MSU crystals and/or inflammatory processes in gout, then this would indicate a pathogenic process, outside the inherent HU present in Polynesians [34], contributing to the increased prevalence of gout in NZ Polynesians (Māori and Pacific) [35]. It is possible that the pathogenic pathway is related to the presence of Tg-rich VLDL1 particles in Māori and Pacific people with gout [3].

The minor G-allele of *rs5128* decreased the risk of gout by nearly 15% in Polynesian cases compared with normouricaemic controls (Fig. 1B) and by nearly 25% compared with HU controls (Fig. 2B). The G-allele is associated with increased expression of *APOC3* in a range of non-immune tissues (www.gtexportal.org), meaning that the *rs5128*-associated effect on gout is likely to be mediated by an influence on the expression of *APOC3.* The association is genetically independent of *rs670* (r^2 between the two SNPs is 0.08 in Polynesians), and there was no epistasis apparent between the two variants. There was also evidence for association with gout in Europeans after adjustment by serum triglyceride and HDL-C levels, also with a protective effect of the minor allele. The lower frequency of the G-allele of *rs5128* in gout cases compared with in non-gout controls (Table 1) is consistent in direction of association with the observation of Cardona *et al.* [4], in which they reported non-significant association (OR = 0.75, P = 0.42). The association with *rs5128* in our study was independent of serum triglycerides and HDL-C. As is the case for *rs670*, a possible role for *rs5128* in regulation of urate levels will need to be evaluated in a larger sample set, especially given the established association of *rs5128* with triglyceride levels (supplementary Table S3, available at *Rheumatology* Online, and refs [11–14]) and the report that, in Europeans at least, there is evidence from a Mendelian randomization study that increased triglycerides are causal of increased serum urate [36]. However, we note that the triglyceride-raising G-allele of *rs5128* is protective against gout.

APOC3 is a non-competitive inhibitor of lipoprotein lipase and thus reduces the lipolysis of Tg-rich

lipoproteins, VLDL and chylomicron [37]. Animal studies show that upregulation of APOC3 can induce hypertriglyceridaemia due to delayed clearance of VLDL particles [38], and downregulation can ameliorate the hypertriglyceridaemia [39]. This delayed hydrolysis of VLDL can increase the life span of these particles in the circulation and can result in formation of Tg-rich VLDL particles (VLDL1), which are present in Polynesian gout cases but not in European gout cases in which over-production of VLDL has been observed [3]. The high frequency of the G-allele of *rs5128* in Polynesians (>4-fold higher than in Europeans) might ultimately result in lower activity of lipoprotein lipase and thus promote the presence of Tg-rich VLDL1 particles in Polynesian gout cases. It is also possible that *rs5128* could influence the inflammatory response; for example, APOC3 and APOC3-rich VLDL can each induce expression of adhesion molecules in endothelial cells and enhance binding and activation of monocytes [40].

Our findings confirm association of *APOA1* with gout and are the first to associate *APOC3* with gout, although the association in Polynesians does require replication. No evidence for association with urate points towards a role for APOA1 and APOC3 in either formation of MSU or, more likely, in the inflammatory response to these crystals. However, it is not possible, until larger association studies are undertaken with SU as outcome, to eliminate the possibility that the variants studied here are also associated with urate levels. Nevertheless, these data strongly support the value of further genetic, observational and clinical studies of gout in connection with the pathways involving the apolipoprotein A1-C3-A4 locus; such research may reveal dyslipidaemia-focused approaches that could be applied to the management of gout.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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