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Association analysis of the beta-3 adrenergic receptor Trp64Arg (*rs4994*) polymorphism with urate and gout

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Abstract

The Arg64 allele of variant *rs4994* (Trp64Arg) in the β3-adrenergic receptor gene has been associated with increased serum urate and risk of gout. Our objective was to investigate the relationship of *rs4994* with serum urate and gout in New Zealand European, Māori and Pacific subjects. A total of 1730 clinically ascertained gout cases and 2145 controls were genotyped for *rs4994* by Taqman®. Māori and Pacific subjects were subdivided into Eastern Polynesian (EP) and Western Polynesian (WP) sample sets. Publicly available genotype data from the Atherosclerosis Risk in Communities Study and the Framingham Heart Study were utilized for serum urate association analysis. Multivariate logistic and linear regression adjusted for potential confounders was carried out using R version 2.15.2. No significant association of the minor Arg64 (G) allele of *rs4994* with gout was found in the combined Polynesian cohorts (OR = 0.98, $P = 0.88$), although there was evidence, after adjustment for renal disease, for association in both the WP (OR = 0.53, $P = 0.03$) and the lower Polynesian ancestry EP sample sets (OR = 1.86, $P = 0.05$). There was no evidence for association with gout in the European sample set (OR = 1.11, $P = 0.57$). However, the Arg64 allele was positively associated with urate in the WP data set ($\beta = 0.036$, $P = 0.004$, $P_{\text{Corrected}} = 0.032$). Association of the Arg64 variant with increased urate in the WP sample set was consistent with the previous literature, although the protective effect of this variant with gout in WP was inconsistent. This association provides an etiological link between metabolic syndrome components and urate homeostasis.

Keywords

Gout; Urate; ADRB3; Genetic; Association

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Introduction

Urate is the end product of endogenous and dietary purine metabolism in humans. The most important regulator of serum urate levels is reduced excretion of uric acid in the urine [1]. When supersaturation of urate is reached, mono-sodium urate (MSU) crystals are able to form within synovial fluid. Gout is the extremely painful innate immune response to these crystals. Individual variation in serum urate concentrations is partly explained by genetic factors, with 28 loci associated with serum urate in Europeans at a genome-wide level of significance [2]. The strongest genetic effects are with uric acid transporter genes, which are also associated with gout [2, 3].

Hyperuricemia and gout are closely related to metabolic diatheses like obesity, dyslipidemia, glucose intolerance and hypertension [4, 5]. Renal clearance of uric acid is inversely related to insulin resistance [6], and the evidence that gout is associated with the metabolic syndrome has led to the hypothesis that gout and hyperuricemia may also have a causal relationship with insulin resistance and obesity. This is consistent with Mendelian randomization studies that have demonstrated increased triglyceride levels and body mass index (BMI) as causal of increased urate [7, 8]. Therefore, genes influencing insulin resistance could also contribute to the development of hyperuricemia.

The beta-3 adrenergic receptor (ADRB3) is part of the adrenergic system. It is expressed mainly in adipose tissue in humans and is involved in the regulation of lipid metabolism and glucose homeostasis [9]. The Trp64Arg (*rs4994*) polymorphism in the first transmembrane domain of ADRB3 has been associated with BMI in meta-analysis of 44,833 individuals with Arg64 carriers having increased BMI and a stronger effect in East Asian sample sets [10]. This is consistent with reports of association of type 2 diabetes mellitus and insulin resistance with Arg64 [11, 12] and association of the Arg64 allele with increased adiposity measures, serum urate and blood pressure in older men [13].

The Trp64Arg polymorphism has previously been tested for association with hyperuricemia and gout under the hypothesis that genetic variants contributing to insulin resistance could also contribute to hyperuricemia. A combination of increased BMI and Arg64 increased the risk of developing hyperuricemia by fourfold in a postprandial diabetic group drawn from the Chinese population [14]. Rho et al. [15] and Huang et al. [16] reported association of the Arg64 allele with hyperuricemia in Korean and Chinese sample sets. A similar association was reported by Morcillo et al. [17] who reported that the Arg64 allele predicts the risk of developing hyperuricemia in a prospective study in a population from southern Spain. Wang et al. [18] reported the Arg64 allele was associated with gout in a male Chinese population.

The aim of the study reported here was to further investigate the relationship of the Trp64Arg (*rs4994*) variant of *ADRB3* with hyperuricemia and gout in European and New Zealand Māori and Pacific Island (Polynesian) sample sets. The New Zealand Polynesian population exhibits the highest rate of gout in the world, with considerable comorbidity with type 2 diabetes and cardiovascular disease [19, 20].

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Materials and methods

Study participants

All New Zealand (NZ) gout cases and controls included in this study were recruited during the years 2006–2013. Gout cases fulfilled the American Rheumatology Association criteria for gout by clinical examination [21], while controls self-reported no history of gouty arthritis. Except for the biochemical measurements and BMI, all other variables were self-reported. Written informed consent was obtained from all subjects for collection of samples and subsequent analyses. Publicly available genotype and phenotype data from the Framingham Heart Study (FHS) and Atherosclerosis Risk in Communities (ARIC) cohorts were accessed from the ARIC and FHS studies under the project name "The Genetic Basis of Gout" and approval number 834. Table S1 reports the demographic and clinical data for all study groups.

The NZ gout case–control sample set was divided into four ancestral groups [22]: NZ European (648 cases and 877 controls), Eastern Polynesian (EP; Cook Islands and NZ Māori; 491 cases and 696 controls), Western Polynesian (WP; Samoa, Tonga, Tuvalu, Niue and Tokelau; 367 cases and 310 controls) and mixed Eastern and Western Polynesian (EP/WP; 29 cases and 70 controls). Eastern Polynesian participants were further subdivided into EPN (subjects with high EP ancestry; 334 cases and 392 controls) and EPZ (subjects with low EP ancestry; 157 cases and 311 controls) [22]. A separate Māori sample set (NPH; 195 cases and 192 controls) was also included in the study, ascertained with criteria described above. These participants were recruited in collaboration with Ngāti Porou Hauora Charitable Trust (NPHCT) from the Ngāti Porou *rohe* (tribal territory) located in the East Coast (Tairawhiti) region of the North Island of New Zealand. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. The NPHCT study was approved by the Ngāti Porou Hauora Board. The Lower South Ethics Committee (OTA/99/11/098) and New Zealand Multi-region Ethics Committee (MEC/05/10/130) granted ethical approval for this study. The Northern Y Region Health Research Ethics Committee granted ethical approval for the NPHCT study (NTY07/07/074).

Control (non-gout) subjects were recruited from NZ and sourced from ARIC (http://www2.cscc.unc.edu/aric/) and FHS (Offspring and Generation 3) (http://www.framinghamheartstudy.org/) cohorts for the purpose of evaluating the association of *rs4994* with serum urate. The ARIC data set consisted of 4144 subjects and the FHS of 5109 subjects. Subjects who self-reported as taking diuretic medication, or had renal failure, gout, or had first-degree relatives with gout or were not of European ancestry were excluded.

Data collection and genotyping

The uricase oxidation method was applied to measure serum urate levels for NZ subjects using a Roche chemistry modular P/D analyzer. Data for serum urate were obtained from visit 1 (1987–1989) for ARIC and examination 1 (Offspring: 1971–1975 and Generation 3: 2002–2005) for FHS cohorts. Genotyping of NZ samples for *rs4994* was done using a TaqMan® assay (C_2215549_20; Applied Biosystems, Foster City, USA) using a

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Lightcycler® 480 Real-Time Polymerase Chain Reaction (RT-PCR) System (Roche Applied Science, Indianapolis, IN, USA). For the FHS and ARIC cohorts, *rs4994* genotype was imputed using all 1000 Genomes haplotype data (phase 1; 2012) as a reference panel using IMPUTE2 [23].

Statistical analysis

Logistic and linear regression analysis was done using statistical software R (v 2.15.2) [24] to test for an association of *rs4994* (explanatory variable) with gout (binary response variable) and serum urate (continuous response variable), respectively. Any individual with missing data for any variable was excluded from the various analyses. The adjusted odds ratio (OR) and β-coefficients were obtained by including age, sex and BMI as covariates in the regression model. In additional analyses, the presence or absence of type 2 diabetes, hypertension and renal disease was included as an adjustor. Self-reported grandparental ancestry was also included as a covariate in Polynesian data sets. Adherence to Hardy–Weinberg equilibrium (HWE) was calculated using the SHEsis package (http://analysis2.bio-x.cn/myAnalysis.php) with a significant deviation from HWE if $P < 0.004$ (0.05 divided by 12—the number of data sets tested in Table 1). Meta-analysis was done using the meta package (http://CRAN.R-project.org/package=meta, 2014) within R using a fixed-effect model. For analyses showing heterogeneity ($P_{Het} < 0.05$), the fixed-effect model was replaced with a random-effect model. A threshold of $P \leq 0.05$ was used to indicate nominal statistical significance between response and explanatory variables. $P_{Corrected}$ was calculated by dividing the P by the number of tests performed (eight), and a $P_{Corrected} < 0.05$ indicated significance.

Results

There was nominally significant association between the *ADRB3* variant *rs4994* and gout only in the WP sample set where the minor Arg64 (G) allele was associated with reduced risk of gout (OR = 0.62, $P = 0.04$) although this association became nonsignificant when adjusted for confounding variables (OR = 0.61, $P = 0.08$) (Table 1). A strong trend of association of the Arg64 allele toward increased risk of gout was observed in EPZ (OR = 1.49, $P = 0.07$) (Table 1). To increase the power of the analysis, all NZ case–control groups were combined with the Wang et al. [18] data set by meta-analysis. No evidence of association was found (Fig. 1; OR = 1.11, $P = 0.52$), although P_{Het} was 0.03 which suggested some heterogeneity. Considering ancestry as a possible source of heterogeneity, Polynesian data sets only were combined in meta-analysis. Again the association was not significant (Fig. 1; OR = 0.98, $P = 0.88$, $P_{Het} = 0.10$). Significant protective association of the *rs4994* Arg64 allele was still observed in the WP sample set when adjusted for diabetes (OR = 0.57, $P = 0.05$), hypertension (OR = 0.56, $P = 0.05$) and renal dysfunction (OR = 0.53, $P = 0.03$), whereas there was evidence for a risk effect of Arg64 on gout in EPZ after adjustment for renal dysfunction (OR = 1.86, $P = 0.05$) (Table 2).

Linear regression analysis was performed to test for association of *rs4994* with urate in the NZ and ARIC/FHS data sets. A significant association of the Arg64 allele with urate was found in the WP sample set ($\beta = 0.036$, $P = 0.004$), which retained significance after

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correction for the eight sample sets examined ($P_{Corrected}$ = 0.03). This indicates that each copy of the Arg64 allele increases serum urate by 0.036 mmol/L (Table 3). To increase the power of the analysis, the various Polynesian and European (including ARIC and FHS) data sets were combined separately by meta-analysis. No significant association was found in either analysis (Arg64 allele: $\beta = 0.01$, $P = 0.16$ and $\beta = 2.99 \times 10^{-5}$, $P = 0.98$, respectively) (Fig. 2).

Discussion

Our study reports association of the Arg64 allele of the *rs4994* polymorphism of *ADRB3* with increased serum urate in WP individuals ($\beta = 0.04$, $P = 0.004$, $P_{Corrected} = 0.03$). This is consistent with the previously reported findings of Morcillo et al. [17] who demonstrated that the Arg64 allele predicts the development of hyperuricemia in the population of southern Spain and with studies in Chinese and Korean sample sets that also associated the Arg64 allele with increased serum urate and risk of gout in Asian subjects [14–16, 18]. Collectively our and previous studies increase the support for a causal role of *ADRB3* and the adrenergic system in urate control. We could not meta-analyze our serum urate findings with previously published studies as they were described as a secondary finding in conjunction with other metabolic conditions, or from a population subgroup, or as a binary outcome [13–15]. It is important to note that the genome-wide association study of Köttgen et al. [2] using ~140,000 individuals would not have been able to test variants in the ADRB3 region for association with urate because of the absence of any Hap-Map2 data including *rs4994* that could be used for imputation (http://hapmap.ncbi.nlm.nih.gov). Using more recently available data, it should be possible to impute this region in the Kottgen et al. data using 1000 Genomes haplotype data in which the *ADRB3* region is adequately covered by common variants and to test for association with urate.

The direction of association of the Arg64 allele with urate opposed that observed in gout in WP, where it was associated with reduced risk of gout. This direction of association also conflicted with the Arg64-mediated increased risk of gout reported by Wang et al. [18] and observed by us in the EPZ sample set (Table 2). Adjusting for the effect of comorbidities type 2 diabetes, hypertension and renal dysfunction did not influence the protective effect of Arg64 with gout in WP (Table 2). Acknowledging that this could be a false-positive finding, the opposing direction of association of Arg64 with gout and hyperuricemia could represent a pleiotropic effect of this allele in the WP population, perhaps having a role both in determining hyperuricemia and in the inflammatory processes leading to gout. The opposing direction to EPZ may reflect different ancestral haplotypes—we have previously observed differential effects at *ABCG2* between Eastern and Western Polynesian sample sets [25]. Most importantly, however, the association of *rs4994* with gout needs to be studied in larger sample sets of diverse ancestries.

The *ADRB3* gene is expressed mainly in adipose tissue and encodes for the beta-3 adrenergic receptor. The activation of this receptor induces lipolysis in adipose tissue and thermogenesis in skeletal muscles. It is also responsible for the delivery of free fatty acids into the portal vein. These free fatty acids and other products of lipolysis disrupt the insulin receptor signaling pathway, thereby leading to insulin resistance [26]. The Arg64 allele of

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rs4994 has been reported to be associated with the development of obesity, increased BMI and insulin resistance [10, 27, 28]. Decreased activity of the Arg64 variant receptor could lead to a decline in lipolysis and increased deposition of adipose tissue. Furthermore, ADRB3 is proposed to be a part of the "leptin-sympathetic-leptin feedback loop," whereby decreased activity of this receptor causes an increase in lep-tin secretion from the adipose tissue [29]. The observation of elevated leptin levels in hyperuricemic patients [30] is consistent with the association of Arg64 with hyperuricemia. Insulin resistance is a possible link between hyperuricemia and obesity [31, 32], which supports the hypothesis that the Arg64 allele could promote hyperuricemia in the obese.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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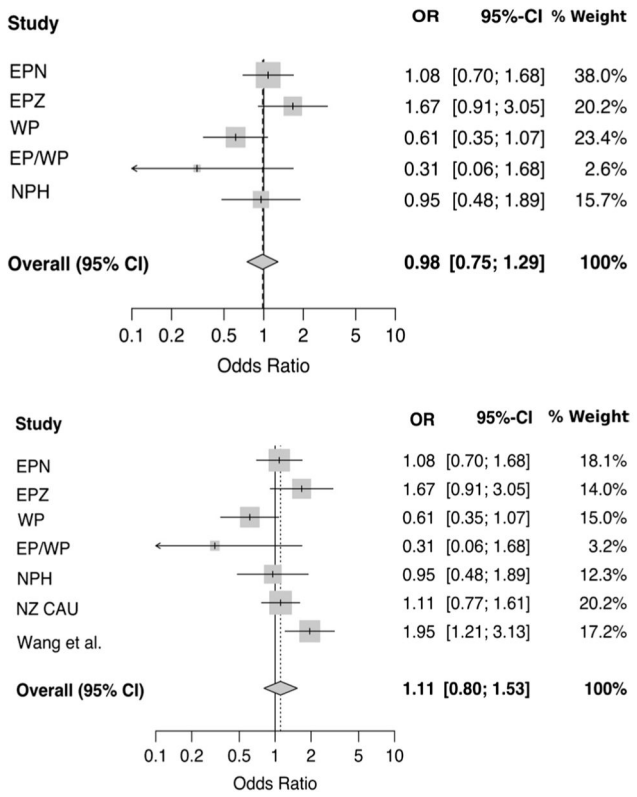

Fig. 1.

Meta-analysis of NZ Polynesian sample sets for association of *rs4994* with gout (*top;* P_{OR} = 0.88 and $P_{Het.}$ = 0.10) and meta-analysis of all available sample sets for association of *rs4994* with gout (*bottom;* P_{OR}= 0.52 and $P_{Het.}$= 0.03)

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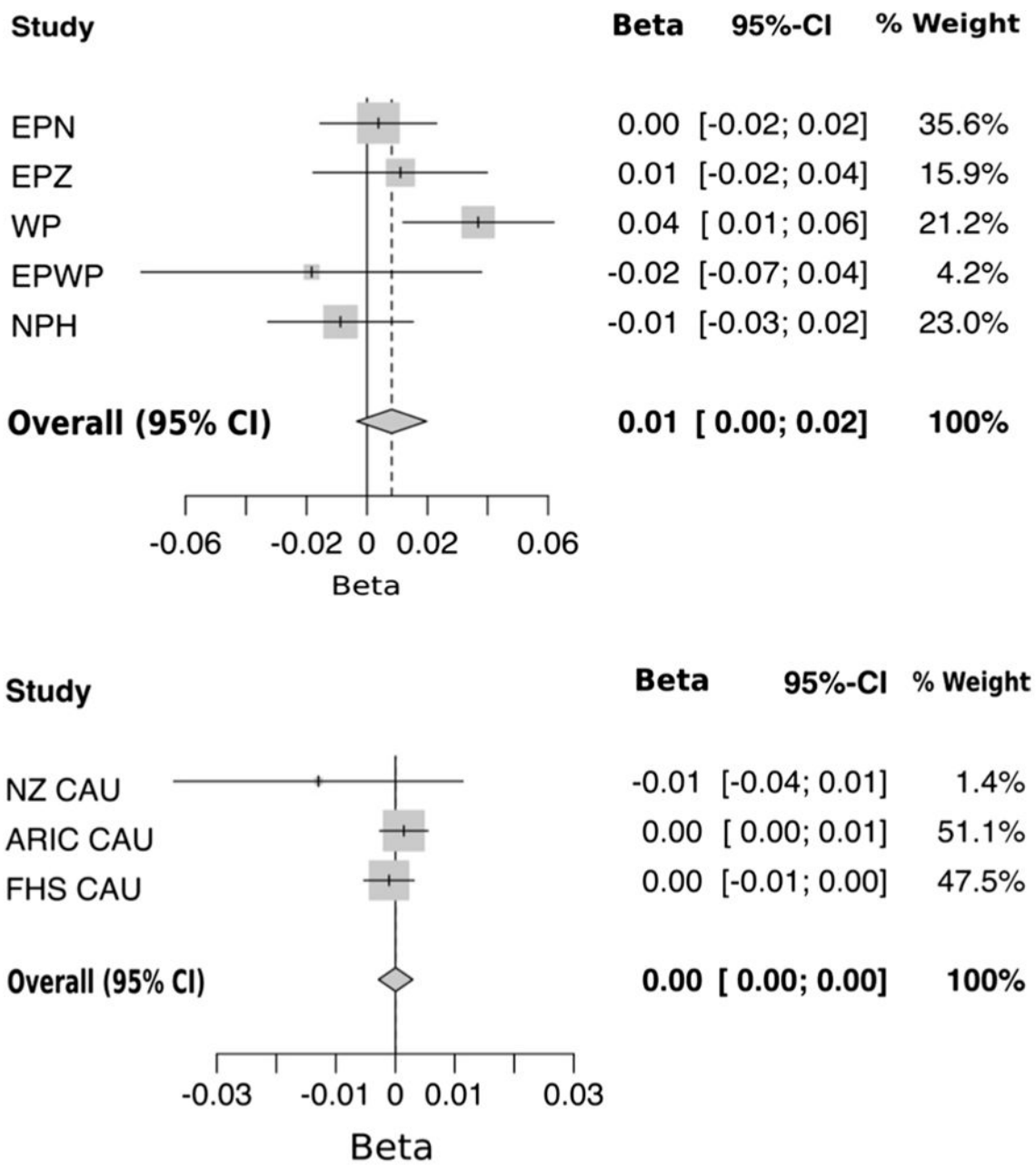

Fig. 2.

Meta-analysis of NZ Polynesian sample sets for association of *rs4994* with serum urate (*top;* P_{Beta} = 0.16 and $P_{\text{Het.}}$ = 0.08) and meta-analysis of European sample sets for association of *rs4994* with serum urate (*bottom;* P_{Beta}= 0.98 and $P_{\text{Het.}}$= 0.41)

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Table 1

Association analysis of *rs4994* with gout

	AA	AG	GG	G	OR (95 % CI) (unadjusted)	$P_{unadjusted}$	OR (95 % CI) (adjusted)[a]	$P_{adjusted}$ [a]	HWE
EPN									
Cases	266 (0.796)	66 (0.197)	2 (0.006)	70 (0.105)	0.89 (0.64–1.25)	0.53	1.08 (0.69–1.68)	0.72	0.33
Control	306 (0.780)	82 (0.209)	4 (0.011)	90 (0.114)					0.56
EPZ									
Cases	122 (0.777)	33 (0.210)	2 (0.012)	37 (0.117)	1.49 (0.95–2.35)	0.07	1.66 (0.91–3.08)	0.09	0.89
Control	262 (0.842)	47 (0.151)	2 (0.006)	51 (0.081)					0.94
WP									
Cases	336 (0.915)	31 (0.084)	0 (0.000)	31 (0.042)	0.62 (0.38–0.99)	0.04	0.61 (0.34–1.06)	0.08	0.39
Control	272 (0.877)	34 (0.109)	4 (0.012)	42 (0.067)					0.02
EP/WP									
Cases	27 (0.931)	2 (0.069)	0 (0.000)	2 (0.034)	0.31 (0.04–1.21)	0.13	0.31 (0.04–1.44)	0.17	0.84
Control	57 (0.814)	13 (0.185)	0 (0.000)	13 (0.092)					0.39
NPH									
Cases	156 (0.800)	39 (0.200)	0 (0.000)	39 (0.100)	1.07 (0.66–1.73)	0.77	0.95 (0.47–1.90)	0.89	0.11
Control	160 (0.833)	28 (0.144)	4 (0.020)	36 (0.093)					0.04
NZ European									
Cases	543 (0.838)	102 (0.157)	3 (0.004)	108 (0.083)	1.00 (0.76–1.30)	0.99	1.11 (0.77–1.61)	0.57	0.44
Control	735 (0.838)	138 (0.157)	4 (0.004)	146 (0.083)					0.35
Han Chinese [18]									
Cases	298 (0.793)	104 (0.252)	10 (0.024)	124 (0.150)	1.50 (1.09–2.06)	0.01	1.95 (1.22–3.13)	0.02	0.79
Control	248 (0.795)	62 (0.199)	2 (0.006)	66 (0.106)					0.37

[a]All values are adjusted against sex, age and BMI and self-reported grandparental ancestry for Polynesian data sets

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Table 2

Association analysis of *rs4994* with gout adjusted for comorbidities

	Baseline adjustment[a]		Diabetes (T2D)[a]		Hypertension[a]		Renal dysfunction[a]	
	OR (95 % CI)	***P***	**OR (95 % CI)**	***P***	**OR (95 % CI)**	***P***	**OR (95 % CI)**	***P***
EPN	1.08 (0.69–1.68)	0.72	1.03 (0.66–1.62)	0.86	1.08 (0.69–1.70)	0.72	0.93 (0.58–1.49)	0.77
EPZ	1.66 (0.91–3.08)	0.09	1.78 (0.96–3.34)	0.06	1.56 (0.80–3.05)	0.18	1.86 (0.98–3.60)	0.05
WP	0.61 (0.34–1.06)	0.08	0.57 (0.32–1.01)	0.05	0.56 (0.31–1.01)	0.05	0.53 (0.29–0.95)	0.03
EP/WP	0.31 (0.04–1.44)	0.17	0.31 (0.04–1.45)	0.17	0.35 (0.04–1.76)	0.24	0.41 (0.05–2.02)	0.31
NPH	0.95 (0.47–1.90)	0.89	0.93 (0.46–1.89)	0.85	0.92 (0.45–1.90)	0.83	0.98 (0.48–1.98)	0.96
NZ European	1.11 (0.77–1.61)	0.57	1.12 (0.77–1.63)	0.55	1.12 (0.76–1.65)	0.56	1.11 (0.76–1.63)	0.58

[a]All values are adjusted against sex, age and BMI and self-reported grandparental ancestry for Polynesian data sets

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Table 3

Association analysis of *rs4994* with serum urate (mmol L^{-1})

	Unadjusted		Adjusted[a]	
	β-Coef. (95 % CI)	***P***	**β-Coef. (95 % CI)**	***P***
EPN	0.004 (−0.016–0.026)	0.66	0.003 (−0.015–0.023)	0.70
EPZ	0.017 (−0.018–0.054)	0.33	0.011 (−0.018–0.040)	0.45
WP	0.036 (0.009–0.063)	0.01	0.036 (0.011–0.062)	0.01
EP/WP	−0.021 (−0.088–0.044)	0.51	−0.018 (−0.076–0.039)	0.53
NPH	−0.006 (−0.041–0.026)	0.68	−0.021 (−0.051–0.008)	0.16
NZ CAU	−0.025 (−0.054–0.004)	0.09	−0.012 (−0.037–0.012)	0.29
ARIC CAU	0.001 (−0.003–0.006)	0.56	0.001 (−0.002–0.005)	0.49
FHS CAU	0.001 (−0.005–0.006)	0.90	−0.001 (−0.005–0.003)	0.61

[a]All values are adjusted against sex, age and BMI and self-reported grandparental ancestry for Polynesian data sets