No evidence for association of Chr 9p21 variant rs1333049 with gout in New Zealand case-control sample sets

Sir, Gout is characterized by inflammatory attacks in joints, triggered by the deposition of crystallized monosodium urate as a result of hyperuricaemia. It is multifactorial with genetic and environmental predisposition [1]. Two loci encoding urate transporters have been confirmed as associated with gout: SLC2A9 and ABCG2 [2–4]. Gout is associated with ischaemic heart disease and heart failure, and with a number of cardiovascular risk factors such as hypertension, dyslipidaemia, insulin resistance and type 2 diabetes [5]. However, it is unclear whether or not the cardiovascular disease association is independent of traditional cardiovascular risk factors [5]. The strongest association signal for coronary artery disease and myocardial infarction in genome-wide association studies was from single nucleotide polymorphism (SNP) rs1333049 at chromosome 9p21 [6, 7]. This risk locus was found to affect the expression of CDKN2BAS and have long-range interactions with MTAP and INFA21 [8]. The physiological effect of rs1333049 on cardiovascular function is unknown, although recent evidence suggested vascular dysfunction in homozygotes for the risk allele C when compared with GG homozygotes, as measured by a lowered vasodilation response to acetylcholine and glycerol trinitrite [9].

Recently it was reported that the C allele of rs1333049 conferred risk for gout in a Han Chinese population [461 cases and 439 controls; odds ratio (OR) (95% CI) = 1.26 (1.06, 1.54), P = 0.01, risk allele frequency in cases and controls was 0.564 and 0.503, respectively] [10]. Therefore we tested this SNP for association with gout in New Zealand (NZ) gout case and control sample sets; East Polynesian (NZ Māori, Cook Islands; 241 cases and 438 controls), West Polynesian (Samoa, Tonga, Tokelau, Niue; 252 cases and 143 controls) and Caucasian (421 cases and 638 controls). Gout cases fulfilled the ACR criteria for gout by clinical examination and controls self-reported as not having gouty arthritis. Serum urate levels at recruitment were different between cases (0.41, 0.45, 0.49 mmol/l for Caucasian, Eastern Polynesian and Western Polynesian, respectively) and controls (0.29, 0.34, 0.37 mmol/l; P < 1 × 10⁻²¹ for ancestral-specific comparisons by t-test). Ethical approval was given by the Lower South Ethics Committee (OTA/99/11/098) and the NZ Multi-region Ethics Committee (MEC/05/10/130). All subjects provided written informed consent according to the Declaration of Helsinki. Taqman SNP genotyping was conducted using a Lightcycler 480 Real-Time PCR System (Roche Applied Science, IN, USA) with the assay C_1754666_10. Caucasian ancestry admixture was adjusted for in the Polynesian sample sets using STRUCTURE and STRAT (http://pritch.bsd.uchicago.edu/software.html). Twenty-six biallelic markers were used as genomic controls [4], with the following markers also included: rs10025373, rs1143634, rs11536879, rs1205, rs2812378, rs3014875, rs344542, rs40401, rs452204, rs4780884, rs4781011, rs4804221, rs4845622, rs4889640, rs507879, rs6005863, rs6815987, rs6835636, rs7811892, rs7842, rs795467, rs8075846, rs8122, rs9639436 and rs9882205. Publicly available genotype data from the Framingham Heart Study (FHS) were accessed (67 cases and 4712 controls), with case ascertainment being self-reported gout on two or more study visits.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Genotype, n (frequency)</th>
<th>Allele C, n (frequency)</th>
<th>OR (95% CI)</th>
<th>Allelic Pa</th>
<th>Allelic Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAUSIAN</td>
<td>GG 124 (0.295) 214 (0.510) 82 (0.195)</td>
<td>378 (0.450) 617 (0.484)</td>
<td>0.87 (0.73, 1.04)</td>
<td>0.13</td>
<td>–</td>
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<tr>
<td></td>
<td>GC 0.270 315 (0.494) 151 (0.237)</td>
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<tr>
<td></td>
<td>CC 25 (0.104) 94 (0.390) 122 (0.506)</td>
<td>338 (0.701) 576 (0.659)</td>
<td>1.21 (0.96, 1.54)</td>
<td>0.11</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Control 60 (0.137) 178 (0.407) 199 (0.455)</td>
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</tr>
<tr>
<td>EAST POLYNESIAN</td>
<td>GG 49 (0.195) 110 (0.438) 92 (0.367)</td>
<td>294 (0.586) 166 (0.581)</td>
<td>1.02 (0.76, 1.37)</td>
<td>0.86</td>
<td>0.84</td>
</tr>
<tr>
<td>WEST POLYNESIAN</td>
<td>GG 19 (0.134) 81 (0.570) 42 (0.296)</td>
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</tr>
<tr>
<td></td>
<td>Control 18 (0.269) 32 (0.478) 17 (0.254)</td>
<td>66 (0.493) 4837 (0.513)</td>
<td>0.92 (0.65, 1.29)</td>
<td>0.63</td>
<td>–</td>
</tr>
<tr>
<td>FHS</td>
<td>GG 1127 (0.239) 2333 (0.495) 1252 (0.2657)</td>
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</tr>
</tbody>
</table>

Pa Adjusted for BMI in logistic regression analysis; NZ Caucasian, Eastern and Western Polynesian P-values were 0.14, 0.62 and 0.93, respectively. Pb Adjusted for Caucasian ancestry (excluding 90 East Polynesian controls without genomic control data).
in addition to the use of gout medication, with exclusion of familiarly related individuals.

There was no significant association between rs1333049 and gout in any of the sample sets— ORs ranged from 0.87 to 1.21 and P-values from 0.13 to 0.97 (Table 1). To increase power, the two Polynesian and the two Caucasian sample sets were separately combined using Mantel-Haenszel meta-analysis, with no significant association found in either analysis [C allele: OR (95% CI) = 1.13 (0.94, 1.36), P = 0.19 and OR (95% CI) = 0.88 (0.76, 1.03), P = 0.12, respectively].

The replication of results is important for genetic associations in complex phenotypes. In this case, however, we were unable to replicate the association of rs1333049 with gout reported in a Han Chinese sample set [10]. Power to detect association in the combined sample sets (Polynesian and Caucasian) was 76 and 93%, respectively (OR = 1.26, Polynesian C allele frequency 0.503 and Caucasian 0.458, z = 0.05). Hence there was adequate power to detect association at an effect equivalent to that reported by Wang et al. [10].

Other reasons may exist for the lack of replication of rs1333049 association with gout. First, the initial report could represent a false positive association. Secondly, the association may be present in Caucasians and Polynesians but with a weaker effect, with our power to detect such an effect being low. Thirdly, the association may be present exclusively in the Han Chinese ethnic group. Finally, there were differences in the sample sets used. For example BMI was considerably higher in our sample sets (available in 903 cases and 513 controls of Caucasian, East and West Polynesian sample sets); values in kg/m² were 33.5 in cases and 31.4 in controls compared with 27.1 in cases and 23.3 in controls in the Han Chinese samples [10]. However, accounting for BMI did not substantially alter P-values in NZ Caucasians and Western Polynesians, with an increase observed in Eastern Polynesians (Table 1). In conclusion, we were unable to detect any evidence for association of the CDKN2BAS SNP rs1333049 with gout.

Rheumatology key message

- Association of rs1333049 with gout in Han Chinese was not replicated in New Zealand.

Acknowledgements

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