Correspondence on ‘Variants in urate transporters, ADH1B, GCKR and MEPE genes associated with transition from asymptomatic hyperuricaemia to gout: results of the first gout versus asymptomatic hyperuricaemia GWAS in Caucasians using data from the UK Biobank’

Sandoval-Plata et al\(^1\) report 13 independently associated genetic variants in the ABCG2, SLC2A9, SLC22A11, GCKR, MEPE, PPM1K-DT, LOC105377323 and ADH1B genes in a genome-wide association study (GWAS) of gout using people with asymptomatic hyperuricaemia as the comparison group. Eight of the genetic variants (representing four of the genes: ABCG2, MEPE, PPM1K-DT, LOC105377323) mapped to a 658 kb region on Chr4. This locus is the strongest for gout with the missense ABCG2 variant (rs2231142, p.Gln141Lys), an extremely likely causal variant.\(^2\)\(^3\) This variant was the lead-associated variant at the ABCG2 locus (OR=1.65 (95% CI 1.58 to 1.73), \(p=3.3\times10^{-10}\)) in the Sandoval-Plata et al\(^1\) study.

The reporting of eight independent genetic effects at the ABCG2 locus stood out. Given the extremely strong statistical evidence for association of rs2231142, it is necessary to exclude the possibility that residual linkage disequilibrium (LD) is responsible for the apparently independent association of the seven additional variants. In the GWAS setting, a filtering step can be applied using ‘LD clumping’ in the PLINK software,\(^4\) whereby an LD threshold within a defined region is set to determine the genetic variant(s) representing the genetic association. If independent genetic effects were observed, the LD clumping is followed by testing putative independent genetic variants for association conditioned on (adjusted for) genotype at the lead genetic variant. In GWAS, putative independent genetic variants should retain statistically significant evidence for association (\(p<5\times10^{-8}\)) after conditioning. Sandoval-Plata et al used LD clumping with an \(r^2<0.1\) threshold to conclude independent effects at ABCG2 without follow-up testing by conditional analysis. Selection of the \(r^2\) threshold is important when effect size of a locus is strong and/or when sample size is large, as weak residual LD from a lead variant can cause apparently independent genome-wide significant associations using the LD clumping method if the clumping threshold is set too high.

We performed a GWAS in individuals of European ancestry comparing gout (\(n=7131\)) with people with asymptomatic hyperuricaemia (\(\geq7\text{mg/dl. serum urate; } n=27018\)) in the UK Biobank using an LD clumping cut-off of \(r^2<0.1\), a window size of 500 kb and adjusting by age, sex and principle components 1–40. This was followed by conditional analysis. Our analysis differed from Sandoval-Plata et al\(^1\) as follows: (1) we used the entire UK Biobank sample set rather than divided for discovery and replication; (2) we used imputed genotypes rather than directly genotyped single-nucleotide polymorphisms (SNPs); and (3) we used a threshold for asymptomatic hyperuricaemia consistent with the definition of pathological hyperuricaemia of serum urate concentration over the saturation threshold\(^5\) rather than >6 mg/dl. as used by Sandoval-Plata et al. Intermarker LD was quantitated for LD clumping using the same UK Biobank samples as used in our GWAS. Sandoval-Plata et al\(^1\) did not specify the reference panel that they used. LD clumping was performed in PLINKv1.9b4.\(^6\) with a significance threshold \(p<5\times10^{-8}\).

There were 13 separate SNP effects (three at ABCG2, three at SLC2A9, two at SLC22A11/A12 and one at each of ADH1B, GCKR, MLXIPL, CNBD1, PDX1). Conditional analysis at each of the ABCG2 and SLC2A9 loci, adjusting the logistic regression analysis by the lead SNPs, rs2231142 and rs16890979, respectively (figure 1), revealed evidence for an independent signal at SLC2A9 approaching genome-wide significance, but not at ABCG2. At ABCG2, of the seven variants reported as independent signals by Sandoval-Plata et al, only rs13120400 retained nominal significance (\(p<0.01\)) after conditioning on rs2231142, with \(p\) value reducing from \(1.3\times10^{-12}\) to \(8.8\times10^{-4}\). This variant maps within ABCG2 ~19 kb from rs2231142. Conversely, rs2231142 retained strong evidence for association with gout (\(p<1\times10^{-46}\)) after conditioning by each of the seven other ABCG2 locus variants reported by Sandoval-Plata et al (not shown). We therefore conclude that there are no genome-wide significant independent effects associated with gout using asymptomatic controls with hyperuricaemia at the genes MEPE, PPM1K-DT and LOC105377323 within the ABCG2 locus.

Repeating the LD clumping using a more stringent threshold of \(r^2<0.01\), we detected 11 separate SNP effects: two at ABCG2 (rs2231142 and rs148356273), three at SLC2A9 (rs16890979, rs3796834—\(r^2=0.78\) with rs2078267, rs7805504—\(r^2=0.93\) with rs7800932, rs87943154—\(r^2=0.93\) with rs2078267) reported by Sandoval-Plata et al\(^1\) (not shown). We therefore conclude that there are no genome-wide significant independent effects associated with gout using asymptomatic controls with hyperuricaemia at the genes MEPE, PPM1K-DT and LOC105377323 within the ABCG2 locus.\(^6\)
We detected all genetic loci detected by Sandoval-Plata et al and three additional loci (PDX1, MLXIPL, CNBD1).

Finally, we note that the Sandoval-Plata et al study reports three apparently novel genes from their GWAS comparing gout with normouricaemic controls (<6.0 mg/dL)—MTX1, PRSS16 and AP5B1. However, both MTX1 and AP5B1 are within established serum urate GWAS loci (‘TRIM46’ and ‘OVOL1’, respectively). It is important also when determining candidate causal genes not to assume that the closest gene is necessarily causal. At these loci, colocalisation of GWAS signals with expression quantitative trait loci implicates MUC1, GBAP1 and FAM189B genes as candidate causal genes at the TRIM46 locus and OVOL1-AS1 at the OVOL1 locus.

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REFERENCES