Functional Urate-Associated Genetic Variants Influence Expression of lincRNAs LINC01229 and MAFTRR

Megan Leask1,2, Amy Dowdle1, Hamish Salvesen1, Ruth Topless3, Rayza Fadason4, Wenhua Wei5, William Schierding2,4, Judith Marsman1,2, Justin M. O’Sullivan2,4, Tony R. Merriman2,3* and Julia A. Horsfield1,2*

1 Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand, 2 Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Auckland, New Zealand, 3 Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand, 4 Liggins Institute, The University of Auckland, Auckland, New Zealand, 5 Department of Women’s and Children’s Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

Genetic variation in the genomic regulatory landscape likely plays a crucial role in the pathology of disease. Non-coding variants associated with disease can influence the expression of long intergenic non-coding RNAs (lincRNAs), which in turn function in the control of protein-coding gene expression. Here, we investigate the function of two independent serum urate-associated signals (SUA1 and SUA2) in close proximity to lincRNAs and an enhancer that reside ∼60 kb and ∼300 kb upstream of MAF, respectively. Variants within SUA1 are expression quantitative trait loci (eQTL) for LINC01229 and MAFTRR, both co-expressed with MAF. We have also identified that variants within SUA1 are trans-eQTL for genes that are active in kidney- and serum urate-relevant pathways. Serum urate-associated variants rs4077450 and rs4077451 within SUA2 lie within an enhancer that recruits the transcription factor HNF4α and forms long range interactions with LINC01229 and MAFTRR. The urate-raising alleles of rs4077450 and rs4077451 increase enhancer activity and associate with increased expression of LINC01229. We show that the SUA2 enhancer region drives expression in the zebrafish pronephros, recapitulating endogenous MAF expression. Depletion of MAFTRR and LINC01229 in HEK293 cells in turn lead to increased MAF expression. Collectively, our results are consistent with serum urate variants mediating long-range transcriptional regulation of the lincRNAs LINC01229 and MAFTRR and urate relevant genes (e.g., SLC5A8 and EHHADH) in trans.

Keywords: enhancer, eQTL, gout, HNF4A, lincRNA, MAF, non-coding, serum urate

INTRODUCTION

High levels of serum urate are necessary but not sufficient for gout, a debilitating form of inflammatory arthritis (Dalbeth et al., 2016). Hyperuricemia is a predictor of renal disease, cardiovascular disease and components of metabolic disease including obesity, fatty liver, diabetes, and hypertension (Sharaf El Din et al., 2017). Serum urate homeostasis is normally maintained by balancing urate production and excretion, predominantly by the liver and renal system, respectively (Maiuolo et al., 2016). Increased reabsorption of urate in the renal tubules (Hyndman et al., 2016) can cause high serum urate levels. Understanding the molecular mechanisms that control urate
homeostasis is critical to improving the prevention, management, and treatment for individuals affected with gout and possibly other associated metabolic diseases.

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with serum urate levels (Tin et al., 2011; Okada et al., 2012; Köttgen et al., 2013; Kanai et al., 2018). However, the majority of these associated genetic variants lie outside of coding regions and the molecular mechanisms through which they alter urate levels are unknown. Nevertheless, association with serum urate levels indicates that these variants must be linked to or lie within regions of the genome that have biological consequences for serum urate control. To understand how genetic variants might influence disease traits it is important to: (1) identify the function of the DNA regions to which the disease-associated variants map; and (2) identify which gene(s) and pathways are under their control (if the function is regulatory). Previously, we used this approach to assign function to serum urate-associated variants within a conserved non-coding region that regulates expression of PDZK1 (Ketharnathan et al., 2018).

The MAF bZIP transcription factor (MAF) is a strong candidate gene for involvement in serum urate control and gout. MAF is highly expressed in the human and mouse kidney, including the podocytes (Daassi et al., 2016) and proximal tubules (Imaki et al., 2004; Ponten et al., 2008). MAF function is important for the development and differentiation of the proximal tubule cells (Imaki et al., 2004). Additionally MAF has been shown to regulate antioxidant (Dhakshinamoorthy and Jaiswal, 2002; Kikuchi et al., 2018) and apoptotic pathways (Peng et al., 2007) that are required for NLRP3 inflammasome activation implicated in chronic kidney disease and gout (Jhang and Yen, 2017; So and Martini, 2017). Although there are multiple genetically independent signals associated with serum urate levels in a ∼500 kb region upstream of the MAF gene (Figure 1), it is not known if these influence the expression of MAF.

The urate association signals that are located upstream of MAF are in close proximity to multiple long intergenic non-coding (linc) RNAs. Recent evidence has shown that non-coding disease-associated SNPs can regulate the expression of lincRNAs (Kumar et al., 2013; Mirza et al., 2014). As a direct result of changing lincRNA expression, disease-associated SNPs could have indirect tissue-specific effects on protein-coding genes (Tan et al., 2017) that are involved in disease. Here, we investigate how serum urate-associated variants in two prominent urate-association signals (SUA1 and SUA2) that are conserved between GWAS in European and Japanese sample sets could contribute to the regulation of serum urate levels.

RESULTS

In people of European ancestry, two loci located upstream of MAF [Chr16: 79637239–79645062 (build 37.7)] were identified as being associated with serum urate levels (p < 10^-6), hereafter referred to as serum urate association regions 1 (SUA1) and 2 (SUA2) (Figure 1A and Table 1) (Köttgen et al., 2013). SUA1 is located ∼60 kb upstream of the MAF transcriptional start site (TSS) and extends through to the 3′ region of MAF transcriptional regulator RNA (MAFTRR). The lead SUA1 SNP (rs7188445) is associated with gout (Phipps-Green et al., 2016). SUA2 is ∼300 kb upstream of the MAF locus and ∼120 kb upstream of the TSS of long intergenic non-protein coding RNA 1229 (LINC01229) (Figure 1A). SUA1 and SUA2 have also been associated with serum urate levels in East Asian datasets (Okada et al., 2012; Kanai et al., 2018). The lead Okada et al. (2012) SUA3 SNP (rs889472) is associated with gout (Higashino et al., 2018) and is in nearly perfect linkage disequilibrium (LD) (R^2 = 0.97) with the lead SUA3 SNP (rs8050348) in the Kanai et al. (2018) dataset (Figure 1B). A fourth serum urate association signal (SUA4) (Table 1 and Figure 1B) is only evident in the Kanai et al. (2018) Japanese data and is located immediately upstream of LOC1027724084 (Kanai et al., 2018). The maximally associated variants (p = 2.3 x 10^-14) at SUA4 in the Kanai et al. (2018) data (rs4888080, rs4888078, and rs12325508) are in LD (R^2 = 1). The urate-raising alleles for these SNPs have allele frequencies of 0.03 in East Asian individuals from the 1000 Genomes Project and these SNPs are monomorphic in Europeans which explains the absence of the SUA4 signal from the European serum urate datasets. The region upstream of MAF has also been associated with other phenotypic traits (Table 1 and Supplementary Figure S1A). It is unknown whether variants in SUA1–4 affect the expression of MAF, or regulate other genes, including the lincRNAs at this region. In this study, we focussed on assigning function to the two independent regions SUA1 and SUA2 (determined by conditional analysis, Supplementary Figure S1B) that have been identified within both European and East Asian populations and are therefore conserved between population groups. SUA3 and SUA4 were excluded from our study due to their absence in the European population.

SUA1 and SUA2 Colocalize With eQTL for MAFTTR and LINC01229

We used COLOC (Giambartolomei et al., 2014) and expression data from The Genotype Tissue Expression (GTEx) project (Carithers and Moore, 2015) to identify cis-eQTL that colocalize with SUA1 and SUA2. eQTL for the genes MAFTTR and LINC01229 (Figure 2, gene body depicted in black) colocalize with the SUA1 urate signal (Table 2) although the lead eQTL variants are not the lead urate variants at SUA1 (Supplementary Figure S2). SUA1 urate-raising alleles associate with increased expression of MAFTTR but with lowered expression of LINC01229 (e.g., rs7188445_G Supplementary Figure S3 and Supplementary Table S1). An eQTL for LINC01229 in the GTEx tissue Skin - Exposed (Lower leg) (Supplementary Figure S2) colocalises with the SUA2 urate signal (Table 2). Urate-raising variants within SUA2 increase LINC01229 expression (Supplementary Table S2). Colocalization of the SUA1 and SUA2 urate signals with MAFTTR and LINC01229 eQTL suggests that
the causal variant(s) at these regions for serum urate and lincRNA expression are shared. Collectively these results are consistent with expression of MAFTTR and LINCO1229 being functionally important in serum urate control.

MAFTTR eQTL variants (all GTEx tissues) that have the greatest effect on MAFTTR expression lie immediately 3′, or within the 3′ region of the MAFTTR transcript (Supplementary Figure S2). By contrast, the locations of eQTL for LINCO1229 differ in genomic location between tissues (Supplementary Figure S2). eQTL for MAFTTR are not found within SUA1 or SUA2 and instead lie within the WWOX transcript (+375 kb of MAFTTR), downstream and within the MAFTTR transcript, and within the promoter of DYNLRB2 (−1 Mb of MAFTTR) (Supplementary Figure S4).

GTEx currently has no renal eQTL data, therefore we queried the NepheQTL database (Gillies et al., 2018) to assess whether the SUA1 and SUA2 MAFTTR and LINCO1229 eQTL exist in the kidney tubule. MAFTTR has a very strong eQTL signal (FDR adjusted \( p = 8.12 \times 10^{-18} \)) in the tubulointerstitium (Supplementary Figure S2) that coincides with SUA1. Four maximally associated variants (\( R^2 > 0.98 \)) lie immediately 3′ to the MAFTTR transcript consistent with the GTEx data. COLOC indicates that the eQTL signal for MAFTTR and the SUA1 serum urate signal are different (H3 posterior probability = 0.976). Nonetheless, SUA1 urate-raising variants associate with increased expression of MAFTTR in kidney tubule (Supplementary Table S1). No SUA2 variants were eQTL for MAFTTR in the kidney tubules within the NepheQTL database (Gillies et al., 2018) (Supplementary Table S2). Furthermore, there were no significant gene eQTL signals for MAFTTR or LINCO1229 within NepheQTL.
FIGURE 2 | SUA2 has hallmarks of enhancer function. (A) The MAF upstream region marked by SUA variants in Köttgen et al. (2013) and Kanai et al. (2018). LincRNA gene bodies (black) and transcripts (dark blue) are depicted. (B,C) ENCODE screenshots of the SUA1 and SUA2 regions. The presence of enhancer elements; DNaseI hypersensitivity regions (DHS) (Thurman et al., 2012), H3K27ac and H3K4me1 chromatin modifications (Heintzman et al., 2009), transcription factor binding sites (Wang et al., 2012) and predicted ChromHMM enhancers (Ernst and Kellis, 2017) are shown. Yellow lines indicate Köttgen SUA variants ($p < 10^{-5}$). (B) A schematic of the ENCODE annotation of the SUA1 region, which includes the lead SUA1 PAINTOR SNP rs58722186 (red) lead European urate SNP rs7188445 (green) and lead Japanese urate SNP rs144899207 (blue). (C) A schematic of the ENCODE annotation of the SUA2 region, which includes the lead SUA2 PAINTOR SNP rs192158533 (red) lead European urate SNP rs9935686 (green) and lead Japanese urate SNP rs4077450 (blue).

An additional whole kidney-derived eQTL dataset (Ko et al., 2017) and Japanese blood eQTL sample set [monocytes, CD4+, CD8+, natural killer and B cells (Kanai et al., 2018)] were queried. eQTL for MAF were located downstream of MAF (consistent with the GTEx data), however, there were no eQTL data for $MAFTRR$ and $LINC01229$ and no SUA (SUA1-4) variants were significant eQTL in these tissues (Supplementary Figure S5).

SUA1 Serum Urate Variants Are Unlikely to Regulate Transcription

Intergenic disease-associated variants could influence disease traits by regulating gene expression. We used PAINTOR (Kichaev et al., 2014) in conjunction with ENCODE (Encyclopedia of DNA Elements) (ENCODE Project Consortium, 2012) to identify functional variants with regulatory activity at the SUA regions upstream of MAF. Fine mapping with PAINTOR using
kidney cell type annotations (Finucane et al., 2015) as a prior identified the most likely causal variants within SUA1 and SUA2 (Table 3). We interrogated ENCODE to identify putative regulatory elements that overlap the SNPs identified by PAINTOR (Figure 2, Supplementary Tables S1, S2, and Supplementary Figures S6, S7). Functional annotations of the human genome were scanned to identify DNaseI hypersensitivity regions (DHS; characteristic of enriched chromatin accessibility (Thurman et al., 2012)), the coexistence of histone H3 monomethylated on lysine 4 and histone H3 acetylated on lysine 27 (H3K4me1, H3K27ac; hallmarks of active transcription factor binding sites based on chromatin immunoprecipitation (ChIP) studies in HEPG2 and K652 cells, respectively, Supplementary Figure S8A, B) and the urate lowering alleles associate with lowered LINC01229 expression (Gerstein et al., 2012, Wang et al., 2012, 2013).

Our ENCODE analyses indicate that rs58722186 within SUA1 identified by PAINTOR (Table 3, Figures 2A, B, PAINTOR posterior probability = 0.31) is in close proximity to regulatory elements. rs58722186 is adjacent to five predicted transcription factor binding sites based on chromatin immunoprecipitation (ChIP) studies from HEPG2 and K652 cells, falls within a GATA2 and GATA3 binding site and is bound by GATA2 in SH-SY5Y cells (ENCODE: Figure 2B and Supplementary Figure S6). rs58722186 is also flanked by DHS peaks and enriched for histone 3 monomethylation on lysine 4 (H3K4me1) (Figure 2B and Supplementary Figure S6). rs58722186 is one of the maximally associated serum urate variants (p = 7.10 × 10^-13) in the Kanai et al. (2018) dataset. rs58722186 is an eQTL for MAFTRR in multiple tissues with a similar effect on expression as the lead SUA1 urate SNP rs7188445 (p = 1.3 × 10^-36 and p = 9.6 × 10^-37, respectively). Chromatin state segmentation by hidden Markov model (ChromHMM) (Ernst and Kellis, 2017) indicates that rs58722186 is within heterochromatin (Figure 2B and Supplementary Figure S6) but is in proximity to a weak enhancer in K652 cells. We find minimal evidence for regulatory elements that overlap urate-associated SNPs identified in the European dataset in SUA1 (Supplementary Figure S2B and Supplementary Table S1) consistent with our PAINTOR analyses (Supplementary Table 3).

**The SUA2 Region Has Hallmarks of Enhancer Function**

At SUA2 the lead PAINTOR SNP rs192158533 (Table 3, Figures 2A, C, PAINTOR posterior probability = 0.89) does not mark any significant regulatory elements. rs192158533 is monomorphic in East Asian populations yet there is a strong urate association at SUA2 signal in Kanai et al. (2018) data. The second most likely SUA2 PAINTOR SNP (Table 3, Figures 2A, C, PAINTOR posterior probability = 0.27) is rs4077450 and the maximally associated urate SNP in the Kanai et al. (2018) Japanese data (p = 3.9 × 10^-14).

ENCODE identifies that rs4077450 along with SUA2 variant rs4077451 encompass a region that is enriched for H3K4me1 and H3K27ac (Figure 2C and Supplementary Figure S7) (Heintzman et al., 2009). DHS peaks overlap this region in 33 cell lines, including HRECpi renal cortex cells (Figure 2C, Supplementary Figure S7, and Supplementary Table S4) (Thurman et al., 2012). The ENCODE data identifies ~30 predicted transcription factor binding sites within the rs4077450/rs4077451 SNP region in chromatin immunoprecipitation (ChIP) studies in HEPG2 and K652 cells (Figure 2C and Supplementary Figure S7) (Gerstein et al., 2012; Wang et al., 2012, 2013).

ChromHMM (Ernst and Kellis, 2017) predicts that the rs4077450/rs4077451 SNP region marks a strong enhancer in liver carcinoma HEPG2 and leukemia K562 cells (Figure 2C and Supplementary Figure S7). In these cells there is also evidence for strong active transcription signatures at the MAFTRR/LINC01229 TSS but the MAF TSS is marked by repressed chromatin signatures. Our eQTL analyses indicate that SUA2 SNPs rs4077450 and rs4077451 are within the colocalized LINC01229 eQTL (p = 1.2 × 10^-5 and p = 2.0 × 10^-5, respectively, Supplementary Figures S8A, B) and the urate lowering alleles associate with lowered LINC01229 expression (Table 2 and Supplementary Table S2).

Conserved non-coding regions of the genome might indicate developmental enhancers (Polychronopoulos et al., 2017). LINSIGHT (Huang et al., 2017) indicates that the SUA2 SNP region is under sequence constraint (Supplementary Figure S9).

---

**Table 2** | Colocalized GTEx eQTL and SUA upstream of MAF.

| SUA region | eGene | PPC | GTEx eQTL tissue | Effect of urate-raising alleles | GTEx single tissue eGene p-value |
|------------|-------|-----|-----------------|-----------------|-----------------|
| SUA1       | MAFTRR | 0.92 | Pancreas        | +               | 1.34 x 10^-6    |
| SUA1       | MAFTRR | 0.84 | Colon (sigmoid) | +               | 3.00 x 10^-12   |
| SUA1       | LINC01229 | 0.92 | Testis          | –               | 8.53 x 10^-3    |
| SUA2       | LINC01229 | 0.76 | Skin (exposed)  | +               | 1.46 x 10^-97   |

1 Posterior probability of colocalization (PPC) as determined by COLOC.

**Table 3** | PAINTOR analysis of SUA1 and SUA2.

| Variant ID | SUA region | PAINTOR posterior probability | Köttgen p-value | Kanai p-value | EUR MAF | EAS MAF |
|------------|------------|-----------------------------|-----------------|--------------|---------|---------|
| rs192158533 | SUA2       | 0.89                        | 4.6 x 10^-6     | N/A          | 0.01    | 0.00    |
| rs58722186  | SUA1       | 0.31                        | 4.3 x 10^-8     | 7.10 x 10^-13| 0.34    | 0.27    |
| rs4077450   | SUA2       | 0.27                        | 8.29 x 10^-6    | 3.9 x 10^-14 | 0.16    | 0.39    |
| rs7188445   | Lead SUA1 SNP | 0.064                   | 2.08 x 10^-8    | 3.4 x 10^-9  | 0.35    | 0.29    |
| rs9935686   | Lead SUA2 SNP | 0.059                    | 1.14 x 10^-6    | 1.0 x 10^-5  | 0.13    | 0.16    |
SUA2 also encompasses a HAR (Bird et al., 2007) which is located ∼2 kb upstream of the rs4077450 SNP region at Chr16: 79934248–79934397 (Supplementary Figure S9). In combination, our in silico analyses indicate that the SUA2 SNP region is a strong candidate for enhancer function and follow up in functional assays.

The SUA2 Region Functions as a Kidney-Specific Enhancer in Zebrafish

We performed transient enhancer assays in zebrafish embryos to investigate the ability of the SUA2 region to act as an enhancer in vivo. The region encompassing the DHS and TFBS hotspot and spanning rs4077450 and rs4077451 was amplified from 1000 Genome DNA samples heterozygous for each of the allele variants (Figure 2C). Allele fragments containing rs4077450_T-rs4077451_A and rs4077450_G-rs4077451_T are herein named major and minor allele fragments, respectively. The minor alleles associate with increased urate levels and increased LINCO1229 expression.

The major and minor allele fragments of the SUA2 region are both capable of driving kidney-specific GFP expression in zebrafish larvae (Figure 3). Cells that are destined to form the zebrafish pronephric duct arise from the ventral mesoderm and then populate the intermediate mesoderm where the differentiation of the pronephric duct into the glomerulus, proximal tubule and distal tubule begins (Horsfield et al., 2002; Drummond, 2003). GFP was initially observed in the zebrafish kidney proximal tubule at 24 h post-fertilization (hpf) (Supplementary Figure S10). By 3 days post-fertilization (dpf), the pronephric duct can be clearly divided into four sections; the glomerulus, the proximal convoluted tubule (PCT), the proximal straight tubule (PST) and the distal tubule (DT) (Figure 3A) (Wingert et al., 2007). At 3 dpf, the presence of mosaic GFP showed that the enhancer is active in the PCT, PST and DT (Figure 3B). By 5 dpf GFP kidney expression was confined to the PCT (Figure 3C).

The extent of GFP localization in the PCT, PST, and DT was quantified in 3 dpf embryos. The minor allele fragment drove GFP expression in the cells of the PCT in 34% of embryos, the PST in 26% of embryos and the DT in 13% of embryos at 3 dpf. The major allele fragment drove GFP expression in the PCT in 34% of embryos, PST in 22% of embryos and DT in 5% of embryos at 3 dpf (Figure 3D). The percentage of embryos with GFP in the DT between the major and minor allele fragments was significantly different (Fishers exact test, _p_ = 0.00025) indicating that minor (urate-raising) allele fragment has subtly greater spatial enhancer activity during development.

The Amplitude of SUA2 Enhancer Activity in HEK293 Cells Depends on Allele Identity

The SUA2 major and minor allele fragments were cloned into luciferase reporter constructs and evaluated for differential enhancer activity in the human embryonic kidney cell line HEK293. The major and minor allele fragments both exhibited significant (_p_ = 0.0216 and _p_ = 0.0002, respectively) enhancer activity in HEK293 cells when compared to the vector only control (Figure 3E). Notably, the minor urate-raising allele fragment had elevated enhancer activity when compared to the major allele (_p_ = 0.0032).

Collectively, our results indicate that the genomic region containing the rs4077450-rs4077451 SNPs is a kidney-specific regulatory element (henceforth called the SUA2 enhancer) that shows allele-specific enhancer activity. The minor (urate-raising) alleles increased the range of tissue expression in zebrafish, and also increased the amplitude of expression in HEK293 cells, providing the first indication of a mechanism for influence of SUA2-specific genetic variation on serum urate levels.

The SUA2 Region Physically and Functionally Connects With MAFTRR/LINC01229 and SUA1

CoDeS3D which leverages genome connectivity (Hi-C datasets) and gene expression associations [eQTL data from the GTEx catalog (Carithers and Moore, 2015)] provides evidence that variants within the SUA2 enhancer regulates the LINC01229 locus via long-range interactions. Genome connectivity data from Hi-C datasets for K562, NHKE, IMR90, HMEC, GM12878, and KBM7 cells (Rao et al., 2014) shows that an interaction fragment containing rs4077450 and rs4077451 within SUA2 physically interacts with 45 restriction fragments that fall within a ∼130 kb interaction region [Figure 3F, Chr16: 79712175–79814647 (build 37.7) FDR < 0.02, Supplementary Table S5]. This interaction region includes the transcripts for the lincRNA genes LINC01229, LINC01228, and MAFTRR and a large part of SUA1 (Figure 3F). Hi-C data from renal cells (Caki-2) (Yang et al., 2018) provides further confirmation that a restriction fragment containing rs4077450 interacts with restriction fragments containing rs7188445 (SUAA1) and rs889472 (SUAA3) (Supplementary Figure S11A). Hi-C data also shows that topologically associated domains (TADs) at the MAF locus shift in location depending on cell/tissue type. In NHKE cells MAF is found on the TAD boundary (Supplementary Figure S11B) and in HUVEC, IMR90 and KBM7 cells MAFF shares a TAD with SUA1, MAFTRR, LINC01229, and SUA2 (Supplementary Figure S11B).

The SUA2 Enhancer Binds HNF4α and Its Expression Coincides With maf and hnf4α Expression in Zebrafish Kidney

We used in situ hybridization to examine the spatial location of Danio rerio _maf_ transcripts in zebrafish embryos and assess whether _maf_ expression overlaps with the activity of the kidney specific enhancer. We found that _maf_ is expressed in the developing kidney and proximal tubules at 12 hpf, 24 hpf, and 48 hpf (Figures 4A–C). GFP expression driven by the SUA2 enhancer in the proximal tubules coincides with the developmental expression of zebrafish _maf_ at 48 hpf (Figure 4D).

The HNF4α transcription factor is robustly expressed in the human kidney proximal tubule⁡ (Ponten et al., 2008)

⁡https://www.proteinatlas.org/
FIGURE 3 | SUA2 is a functional enhancer that physically connects to the lincRNA region. An enhancer construct containing the SNP region (e.g., major alleles) injected at the one-cell stage in developing zebrafish embryos drives transient GFP reporter expression in the proximal tubules of 24 hpf, 48 hpf, and 3 dpf embryos. (A) 48 hpf embryo with schematic depicting the zebrafish pronephros: glomerulus (G, red); proximal convoluted tubule (PCT, orange); proximal straight tubule (PST, yellow); distal tubule (DT, green). (B) Representative image from a 3 dpf embryo with mosaic GFP expression in the PCT, PST, and DT. Insets of the PCT, PST, and DT are below. Asterisks indicate GFP positive cells. (C) Representative image from a 5 dpf embryo with mosaic GFP expression found exclusively in the PCT. (D) Percentage of transgene positive embryos that present with GFP-positive cells in the PCT, PST, and DT in enhancer constructs containing the SNP region with either the major or minor alleles for SNPs rs4077450 and rs4077451 at 3 dpf. The total number of embryos that were transgene-positive for the major allele fragment was 349. The total number of embryos that were transgene-positive for the minor allele fragment was 413. Numbers in each group are represented at the bottom of each bar in the graph. (E) Luciferase reporter assay in HEK293 cells with an empty pGL4.23 vector (control), or a pGL4.23 vector containing the SNP region with either the major (urate-lowering) or minor (urate-raising) alleles of the SNP. Luciferase expression is plotted relative to the expression of Renilla and normalized to the expression from the empty vector. A one-way ANOVA test resulted in a significant difference between the means of the control and the SNP region containing both the minor and major allele and a significant difference between the means of the major and minor alleles. Asterisks indicate the set of values that were significantly different from the control; *p < 0.05, **p < 0.005 and ***p < 0.0005. (F) The intergenic region upstream of MAF that encompasses the lincRNAs MAFTFR, LINC01229 and LINC01228. Also shown are the locations of SUA1 and SUA2. The region of interaction for the interaction fragment that encompasses the rs4077450_rs4077451 SNP region is indicated, data are from Rao et al. (2014) using K562 (black), NHK (blue), IMR90 (yellow), HMEC (red), GM12878 (purple) and KBM7 (gray) cells. The interaction region is the combination of 45 interactions with the rs4077450_rs4077451 SNP region.
and regulates the expression of genes encoding proteins that control serum urate (Prestin et al., 2014), including PDZK1 (Ketharnathan et al., 2018). Furthermore, variation at the HNF4A locus is associated with serum urate levels ($P \sim 10^{-6}$) (Köttgen et al., 2013). SUA2 variants rs4077450 and rs4077451 flank a core HNF4α consensus motif (Supplementary Figure S7) (Wang et al., 2012; Huang et al., 2017) and fall inside an HNF4α ChIP-seq peak in HEPG2 cells (Supplementary Figure S7) (Gerstein et al., 2012; Wang et al., 2012, 2013). We conducted ChIP-qPCR using an anti-HNF4α antibody and confirmed that the SUA2 enhancer is physically bound by HNF4α in HEPG2 cells (Figure 4E). HNF4α binding was not assessed in HEK293 cells because HNF4α is not expressed in this cell line (Ketharnathan et al., 2018). GFP expression driven by the SUA2 enhancer also coincides with hnf4α expression in the zebrafish pronephric duct at these stages (Wingert and Davidson, 2011). Combined, our analyses of the SUA2 kidney enhancer indicate that it functionally and physically connects with the MAFTRR/LINC01229 lincRNA region, is likely responsive to HNF4α, and could contribute to MAF expression. However, several other transcription factor consensus motifs, including those for TFAP2A and HNF4G, are also in close proximity to SUA2 variants rs4077450 and rs4077451, therefore it is possible that the variants could influence the binding of one or more transcription factors.

**SUA1 Variants in MAFTRR and LINC01229 Have Targets Independent of MAF That Influence Serum Urate Levels**

CoDeS3D and Hi-C data for K562, NHEK, IMR90, HMEC, GM12878 and KBM7 cells provides spatial data supporting the rs7188445 MAFTRR cis-eQTL (Supplementary Table S6). Hi-C data also provide evidence that the lead MAFTRR eQTL SNPs which lie within the MAFTRR and LINC01229 transcripts physically connect with MAF, however, there are no significant MAF eQTLs associated with these connections (Supplementary Table S7).

We also tested whether genes in *trans* have functional genomic regulatory connections at SUA1 (Rinn and Chang, 2012). CoDeS3D identified 12 spatially supported *trans*-eQTL with SUA1 variants located within the MAFTRR and LINC01229 transcripts (FDR < 0.05, Supplementary Table S8) and also with the lead SUA1 SNP rs7188445 (Supplementary Table S6). The lead SNPs for these *trans*-eQTL at SUA1 are different than the lead *cis*-eQTL SNPs for MAFTRR (Supplementary Figures S2, S12 and Figure 5). Importantly COLOC analysis (Giambartolomei et al., 2014) indicates that these *trans*-eQTL signals colocalize with the SUA1 serum urate signal (Table 4) indicating that SUA1 could control serum urate levels via *trans*-eQTL connections. Of the 12 *trans*-eQTL, only 2 did not colocalize (H4 < 0.5) with the SUA1 signal (CCDC79 and TBC1D10B). Of the 10 remaining *trans*-eQTL, four genes (i.e., SLC5A8, CCDC6, EHHAHD, and DLGAP1) are potentially involved in urate metabolism based on our Gene Ontology (GO) analyses and previous urate associations (Köttgen et al., 2013; Kanai et al., 2018) (Figure 5).

CCDC6 is in very close proximity to a urate GWAS signal that sits in the intergenic region between CCDC6 and SLC16A9 (Supplementary Figure S13A). CCDC6 and SLC16A9 are strongly co-regulated ($p = 6.9 \times 10^{-9}$, GeneNetwork.nl) and the lead Köttgen SNP at CCDC6/SLC16A9 (rs1171614) is an eQTL for both SLC16A9 and CCDC6 [GTEx (Carithers and Moore, 2015)]. SUA1 lead urate-raising variant rs7188445_G associates with lowered expression of CCDC6 (Supplementary Figure S13B). At SLC5A8 there is a weak urate GWAS signal in the Japanese population (Kanai et al., 2018) (Supplementary Figure S13A). SLC5A8 is a monocarboxylate transporter that has been strongly implicated in urate transport in the kidney (Merriman, 2015) and associates with the GO biological function term urate transport ($p = 6.3 \times 10^{-3}$) (Supplementary Table S9). The urate-raising allele of rs7188445 associates with increased

---

**Note:**

*www.GeneNetwork.nl*
TABLE 4 | Colocalization of Köttgen SUA1 signal and SUA1 trans-eQTL signals.

| Locus          | PPC | eQTL tissue          |
|----------------|-----|----------------------|
| SLC5A8         | 0.80| Ovary                |
| TNKS           | 0.93| Heart left ventricle |
| EHHADH         | 0.69| Brain frontal cortex |
| ENTPD3-AS1     | 0.85| Stomach              |
| ARHGAP35       | 0.68| Uterus               |
| CCDC6          | 0.63| Brain frontal cortex |
| RP11-386L12.1  | 0.68| Brain caudate basal ganglia |
| SLC03A1        | 0.52| Brain hippocampus    |
| BTLA           | 0.58| Uterus               |
| CCDC79         | 0.25| Breast mammary tissue|
| DLGAP1         | 0.78| Spleen               |
| TBC1D10B       | 0.09| Colon sigmoid        |

1 Posterior probability of colocalization (PPC) as determined by COLOC.

expression of SLC5A8 (Supplementary Figure S13B). The only other genes with a trans-eQTL at SUA1 with evidence for a urate signal was DLGAP1 in the Japanese population (Supplementary Figure S13A). The serum urate-raising and gout risk allele rs7188445_G associates with increased expression of DLGAP1 (Supplementary Figure S13B). The proteins encoded by both DLGAP1 and SLC5A8 contain PDZ3 domains and interact with PDZK1 (Hu et al., 2009), a scaffold protein involved in urate reabsorption within the kidney tubule (Merriman, 2015).

Of the 10 genes with colocalized SUA1 trans-eQTL, EHHADH has the strongest signal in kidney tubule (gene level FDR = 0.078) (Gillies et al., 2018). Expression of EHHADH is enriched in the proximal tubule (Habuka et al., 2014) and associates with the GO biological function term urate transport \((p = 7.7 \times 10^{-3})\) in the GeneNetwork database (Supplementary Table S9). The serum urate-raising and gout risk allele rs7188445_G associates with increased MAFTTR expression and lowered EHHADH expression (Supplementary Figure S13B). These analyses collectively indicate that SUA1 variants associate with the expression of serum urate and kidney relevant genes in trans.

LINC01229, MAFTTR, and Trans-eQTL Are Coexpressed With MAF

Our analyses indicate that LINC01229 and MAFTTR, along with genes in trans, likely have functions in serum urate regulation. In addition, the SUA2 enhancer recruits HNF4α and coincides with the expression of zebrafish hnf4a and maf, implicating MAF in serum urate regulation. Supporting this co-expression analysis the GeneNetwork database (Kumar et al., 2013) indicates that MAFTTR and LINC01229 are strongly co-expressed with MAF \((p = 5.0 \times 10^{-27} \text{ and } p = 2.4 \times 10^{-26}, \text{ respectively})\). However, MAFTTR and LINC01229 are not co-expressed (Supplementary Tables S10, S11). Interestingly, HNF4A is co-expressed with LINC01229 \((p = 0.011)\) but not with MAFTTR (Supplementary Tables S10, S11) consistent with the SUA2 variant effect on
LINC01229 expression. We also assessed whether the SUA1 trans-eQTL genes are co-expressed with MAFFTTR, MAF and/or LINC01229. SLC03A1, TNKS and ENTPD3 (the likely target of ENTPD3-ASI) are co-regulated with MAF \( (p = 0.0088, p = 0.011, \) and \( p = 0.022, \) respectively) (Supplementary Table S12) and rs7188445 G associates with increased SLC03A1, TNKS, and ENTPD3 expression (Supplementary Figure S13B).

**MAFTRR and LINC01229 Regulate MAF and Function in Kidney and Urate Relevant Pathways**

To test if MAFFTTR and LINC01229 can contribute to the regulation of MAF transcription, we depleted MAFFTTR and LINC01229 using siRNA in HEK293 cells. Reduction of MAFFTTR RNA levels (Figure 6A) resulted in upregulation of endogenous MAF mRNA (Figure 6B). Reduction of LINC01229 RNA levels (Figure 6D) also led to upregulation of endogenous MAF mRNA (Figure 6E). Our results indicate that LINC01229 and MAFFTTR normally repress MAF expression in kidney HEK293 cells.

Our analyses indicate that LINC01229 and MAFFTTR have the ability to regulate MAF in kidney cells. We assigned putative phenotypes and biological processes to LINC01229 and MAFFTTR using GeneNetwork (Kumar et al., 2013), which infers functional enrichment from co-expressed genes\(^4\). The top enriched human transcript ontology terms for MAFFTTR and LINC01229 are ‘tubulointerstitial abnormality’ \( (p = 3.9 \times 10^{-4}) \) and ‘accelerated skeletal maturation’ \( (p = 4.3 \times 10^{-5}) \), respectively. The top GO biological process term for MAFFTTR is ‘chondroitin sulfate catabolic process’ \( (p = 8.4 \times 10^{-3}) \). The top GO biological process term for LINC01229 ‘cell development’ \( (p = 7.9 \times 10^{-5}) \) implicates LINC01229 in a pathway with MAF and three other serum urate-associated loci, INHBB, INHBC and INHBE, detected by GWAS (Köttgen et al., 2013) (Supplementary Figures S14A,B). Additionally, HNF4A and LINC01229 share GO enrichment in lipid-related pathways including ‘chylomicron assembly,’ ‘phospholipid efflux,’ and ‘high-density lipoprotein assembly’ (Supplementary Figure S14A). LINC01229 also associates with inflammation- and gout-relevant GO terms including ‘interleukin β secretion,’ ‘T-cell differentiation,’ and ‘interleukin 2 biosynthetic process’ (Supplementary Figure S14A).

**The MAF Upstream Intergenic Region Is Conserved**

The intergenic region upstream of MAF encompassing the lincRNAs is conserved in mammals. Two non-coding RNA loci upstream of MAF in mouse (i.e., Gm30925 and Gm31037) are positionally conserved with respect to MAF, expressed in kidney and homologous with the human lincRNA region (BLAST E-value \( = 3 \times 10^{-59} \) and \( 2 \times 10^{-16} \), respectively) (Supplementary Figure S15). Moreover, sequence homologous (E-value \( = 6 \times 10^{-20} \)) to the SUA2 enhancer in mouse is located 276,670 bp upstream of Maf. Our analyses suggest that the ∼300 kb MAF upstream intergenic region marked by SUA1 and SUA2 represents an evolutionarily conserved regulatory network active in kidney and serum urate regulation.

**DISCUSSION**

Identifying and assigning function to genomic regions encompassing disease-associated variants is crucial for identifying their biological role in disease (Edwards et al., 2013). Here, we provide evidence that the conserved syntenic block encompassing MAF, the lincRNA eQTL SUA1 region, and the enhancer SUA2 region constitute a functional genomic regulatory domain that contributes to serum urate regulation (Figure 7). SUA1 and SUA2 are independent and functionally distinct serum urate-associated regions that physically connect with the lincRNA region and the MAF promoter region (SUA3) (Figure 7). Genetic variation within SUA1 and SUA2 is associated with alterations in the expression of the lincRNAs contained in this genomic block. SUA1 variants physically connect with, and alter the expression of, MAFFTTR, LINC01229 and urate-relevant genes in cis. SUA2 marks an enhancer element that physically and functionally connects with LINC01229 expression, recruits HNF4α, and coincides with the developmental expression of zebrafish maf and hnf4α. Finally, we show that the lincRNAs regulate the expression of MAF in cis. By teasing out the molecular mechanisms that underlie this regulatory network,
urate signal and the MAFTRR (Merriman, 2015). We observed a colocalization of the SUA1 kidney tubules with the remainder managed by the intestine serum urate control.

We have assigned serum urate-associated variants to a distinct biological pathway that is active in the kidney and involved in we have assigned serum urate-associated variants to a distinct biological pathway that is active in the kidney and involved in

Serum urate excretion is predominantly carried out in the kidney tubules with the remainder managed by the intestine (Merriman, 2015). We observed a colocalization of the SUA1 urate signal and the MAFTRR colon cis-eQTL (Table 2 and Supplementary Figure S2) and the partial overlap with the MAFTRR kidney tubule eQTL signal (Supplementary Figure S2). This is consistent with our co-expression analyses which indicate that MAFTRR associates with the kidney-relevant human phenotype ontology term ‘tubulointerstitial abnormality.’ Collectively these data support a biological role for MAFTRR in serum urate regulation in these tissues.

An inverse relationship between MAFTRR levels and MAF expression has previously been identified in patients with multiple sclerosis, whereby higher levels of MAFTRR in CD4+ T lymphocyte cells lowers expression of MAF (Zhang et al., 2017). This relationship shows a strong correlation with annual relapse rates (Zhang et al., 2017). In our study, the urate-raising alleles in SUA1 increase MAFTRR expression (Table 2 and Supplementary Table S1). Chromosome conformation capture data in T-lymphocytes has identified genomic contacts in the region 3’ to MAFTRR that overlap SUA1 and physically connect with the MAF promoter marked by SUA3. In this genomic conformation the MAFTRR transcripts act as a scaffold for regulatory elements including EZH2 that recruit the repressive histone mark, H3K27me3 (Ranzani et al., 2015) which in turn represses MAF transcription. Our analyses of Hi-C data support this physical connection between SUA1 variants and MAF (Supplementary Table S7). We also observed that depleting MAFTRR or LINC01229 increases MAF transcription in kidney cells (Figure 6), consistent with this chromatin interaction data from T-cells. We propose that the SUA1 variant effect on MAFTRR and/or LINC01229 expression may also contribute to the 3D structure at this locus in a tissue-dependent manner, altering the expression of MAF in cis (Figure 7).

We also observed that SUA1 variants form functional trans chromatin contacts that colocalize with the SUA1 urate signal (Table 4 and Figures 5, 7). We identified SLC5A8 which encodes sodium-coupled monocarboxylate transporter 1 (SMCT1). SLC5A8/SMCT1 transports monocarboxylates into the kidney tubule, which are exchanged for urate by urate reuptake transporters such as SLC22A12/URAT1 (Merriman, 2015). The urate-raising allele of rs7188445 is associated with increased expression of SLC5A8, which would be predicted to increase the monocarboxylate pool for reabsorption of urate. Any serum urate signal at the SLC5A8 locus, if real, is weak (Supplementary Figure S13); nevertheless, our data suggest that the expression of SLC5A8 is controlled from the MAF SUA1 locus. EHHADH (associated with the GO term ‘urate transport’) is involved in fatty acid oxidation, the main energy source for kidney tubule cells (Balaban and Mandel, 1988) and EHHADH mutations have been implicated in Fanconi’s syndrome which is characterized by renal proximal tubule dysfunction (Klootwijk et al., 2014). Furthermore Fanconi’s syndrome can result in hypouricemia due to high urate clearance (Meisel and Diamond, 1977). A reduction in EHHADH expression has been observed in mouse models for chronic kidney disease (Trudu et al., 2017), hyperlipidemia (Mirzoyan et al., 2017), obesity and fatty liver disease (Vandanmagsar et al., 2011). This is consistent with the effect seen for serum urate-raising variant rs7188445_G at SUA1, which associates with lowered EHHADH expression.

We also find that a subset of genes with spatial trans-eQTL at SUA1 are co-regulated with MAF. TNKS and SLCO3A1 are co-regulated with MAF, and expressed in kidney (Tamai et al., 2000; Karner et al., 2010). SLCO3A1 mediates the transport of prostaglandins, thyroxin and vasopressin (Huber et al., 2007) and TNKS is essential for kidney development (Karner et al., 2010), ENTPD3-AS1, likely regulates ENTPD3 (co-regulated with MAF), which is involved in purine metabolism (Kanehisa et al., 2017) of which urate is a by-product.

SUA2, the second independent region of association upstream of MAF, has previously been validated as a functional enhancer by high-throughput massively parallel reporter assays in HEPG2 cells (Kheradpour et al., 2013). Our study confirms that this region represents a conserved enhancer for tissue specific expression in the kidney tubule (Figure 3). A 562-bp DNA fragment containing the rs4077450_rs4077451 SNP region within SUA2 is sufficient to confer kidney tubule expression that coincides with expression of zebrafish maf (Figure 4), matches the tissue expression profile of MAF in human and is consistent with the role MAF plays in kidney tubule development (Imaki et al., 2004). We show that the urate-raising minor alleles also increase enhancer activity in kidney cell lines (Figure 3). Our results indicate that the serum urate-associated SNPs at this
region are functional and alter the function of the SUA2 enhancer, consistent with our PAINTOR analyses (Table 3).

The underlying mechanism for enhancer function has been suggested to involve the formation of long-range chromatin loops (Denker and de Laat, 2016) that bring enhancers and promoters into spatial proximity (Dixon et al., 2012). Our analysis of publicly available Hi-C data (Rao et al., 2014) indicates that the SUA2 kidney enhancer element physically interacts with the neighboring lincRNA region, including LINC01229, MAFTRR and part of SUA1 (Figure 3). Hi-C data from renal cells supports this interaction, in addition to a further connection between SUA2 and the MAF promoter (SUA3) (Supplementary Figure S11). Our eQTL analyses indicate that the SUA2 genomic contacts with LINC01229 are functional (Fadason et al., 2017), colocalize with the SUA2 urate signal (Table 2) and that the serum urate-raising alleles of rs4077450 and rs4077451 increase the expression of LINC01229 but not of MAFTRR (Supplementary Figure S8).

Because the SUA2 human enhancer element can drive gene expression in zebrafish kidney, this genomic region must contain highly conserved kidney-responsive regulatory elements. rs4077450 and rs4077451 flank an HNF4α site which has previously been shown to be required for the enhancer activity of the region (Kheradpour et al., 2013). We confirmed that HNF4α binds to this locus in HEpG2 cells (Figure 4E). Previous observations in zebrafish found that hnf4a is expressed in the zebrafish proximal tubule in the same cell types and stages in which the SUA2 enhancer is active (Wingert and Davidson, 2011). Therefore, HNF4α is a likely candidate for driving kidney-specific activity of this enhancer. This is consistent with our understanding of (1) the role HNF4α plays in the development of the human kidney tubule (Martovetsky et al., 2013); and (2) the association of HNF4A with serum urate (Köttgen et al., 2013; Ketharnathan et al., 2018). Our co-expression analysis also indicates that HNF4A, is co-expressed with LINC01229 but not MAFTRR, consistent with the SUA2 LINC01229 eQTL and the coincidental expression of the SUA2 enhancer and hnf4a. LINC01229 and HNF4A also share enriched GO terms in lipid relevant pathways that involve genes with (albeit unreplicated) reports of association with urate (e.g., APOE) (Sun et al., 2015) and gout (e.g., ABCG1 and ABCA1) (Lai et al., 2012) (Supplementary Figure S14).

While the eQTL signals for MAFTRR in all GTEx tissues are confined to the same region that generally overlaps SUA1, the genomic locations of the eQTL signals for LINC01229 are diverse and tissue dependent (Supplementary Figure S2). We propose that while MAFTRR expression is controlled in a similar manner in a range of tissues, LINC01229 expression is under the control of tissue-specific and perhaps development-specific regulatory elements that are distributed throughout the lincRNA locus. The SUA2 region is evidence of one such region: (1) it contains an enhancer element that drives expression exclusively in the developing zebrafish kidney tubule; and (2) it contains SUA genetic variants that have the ability to alter LINC01229 expression. We propose that in kidney, long-range chromatin interactions bring the HNF4α-responsive SUA2 enhancer into close spatial proximity with the SUA1 lincRNA region and the SUA3 MAF promoter region, implicating LINC01229 as a likely regulatory target of the SUA2 enhancer. We predict that this chromosome conformation (Figure 7) would have consequences for MAF expression, as evidenced by the loss of MAF repression following siRNA knockdown of LINC01229 in kidney cells (Figure 6), and consistent with the upregulation of maf observed in hnf4a mutant zebrafish (Davison et al., 2017). Our GO analyses indicate that LINC01229 could function in a cell development pathway with MAF and the inhibin genes INHBC, INHBA and INHHB previously associated with urate (Köttgen et al., 2013). LINC01229 and MAF could mediate serum urate homeostasis through known inhibin pathways such as apoptosis (Denkova et al., 2004) and inflammation (Okuma et al., 2005) consistent with our GO analyses, which identified ‘negative regulation of cell apoptotic process’ and ‘interleukin 1 β secretion’ as enriched terms for LINC01229. In addition to this ‘interleukin 1 β secretion’ is strongly implicated in gout (So and Martinon, 2017).

The multiplicity of phenotype associations at the lincRNA locus suggests that variants at this region and their effect on MAFTRR, MAF and/or LINC01229 could also be biologically important in other disease pathways (Table 1 and Supplementary Figure S1). Serum urate levels have previously been correlated with multiple sclerosis (Wang et al., 2016), hyperthyroidism (Giordano et al., 2001; See et al., 2014), obesity (Tanaka et al., 2015), fatty liver disease and chronic kidney disease and the SUA regions upstream of MAF coincide with GWAS signals of these conditions. SUA1 coincides with liver- and thyroid-related traits whereas SUA2 coincides with kidney related traits (Supplementary Figure S1). MAF expression has also been associated with multiple sclerosis (Zhang et al., 2017), a range of cancers including renal cancer (Ponten et al., 2008), glucagon expression and biosynthesis (Planque et al., 2001; Kataoka et al., 2004; Gosmain et al., 2007) and autoimmune diabetes (Pauza et al., 2001). Thus, the shared association of these diseases at the lincRNA region may provide a causal link to serum urate and/or the expression of the lincRNAs and MAF.

In our analyses, we have identified a program of genes that can be modulated by variants within SUA1 and SUA2 and are likely important in kidney tubule development and function and serum urate regulation. We conclude that tissue-specific and developmental control of the lincRNA region appears to be important for the modulation of MAF and other urate relevant genes pathways in trans.

MATERIALS AND METHODS

Genetic Analyses

Publicly available summary level statistics from the Köttgen et al. (2013) GWAS for serum urate were used. Conditional association analysis on rs7188445 was performed using the Genome-Wide Complex Trait Analysis (GCTA) software (Yang et al., 2011). 6,654 HapMap2 imputed genotypes from European participants of the Atherosclerosis Risk in Communities study were used as a reference. LD R² values were calculated using LDLink (Machiela and Chanock, 2015) with the 1000 Genomes European and East Asian reference panels.
PAINTOR Analysis

ImpG (v1.0) (Pasaniuc et al., 2014) was used to impute Z-scores into the European urate GWAS (Köttgen et al., 2013) summary statistics. For the reference haplotypes, the latest release of the 1000 Genomes project was used, and only bi-allelic SNP markers having a minor allele frequency greater than 0.01 in the relevant population were included. All imputed markers with a predicted LD $R^2$ of less than 0.8 were removed. PAINTOR leverages functional genomic annotation data, in addition to genetic association strength, to prioritize functional variants. The LD matrix used for fine mapping of the Köttgen GWAS data in PAINTOR was calculated based on European data from the 1000 Genomes Project Phase 1. The annotation matrix used in PAINTOR was kidney cell-type DHS (Finucane et al., 2015). PAINTOR (v3.0) (Kichaev et al., 2014) was carried out using a LD matrix matched locus file for SUA1 and SUA2.

COLOC Analysis

We used COLOC (Giambaritoloemei et al., 2014) to assess the similarity between the urate-associated loci SUA1 and SUA2 and publicly available eQTL data from the Genotype Tissue Expression Project (GTEx). COLOC is a Bayesian method that compares four different statistical models at a locus and gives a posterior probability for each test. The four tests are: H0: no causal variant in the GWAS or an eQTL region; H1: a causal variant in either the GWAS or the eQTL region but not both; H3: a causal variant in the GWAS and the eQTL region that is different; H4: a causal variant in the GWAS and the eQTL region that is shared.

Integration of GWAS and eQTL Results With CoDeS3D

The Contextualize Developmental SNPs using 3D Information (CoDeS3D) algorithm (GitHub\textsuperscript{1}) (Fadason et al., 2017) was used to identify long-distance regulatory relationships for rs4077450 and rs4077451 (SUAA), rs7188445 (SUAA) and SUA1 SNPs that overlap the MAFTTR transcript. This analysis leverages known spatial associations from Hi-C databases (Rao et al., 2014) and gene expression associations [eQTL data from the GTEx catalog (GTEx 2013)] to assess regulatory connections. Briefly, SNPs were mapped onto Hi-C restriction fragments, the genes that physically interact with these restriction fragments identified and collated (SNP-gene spatial pairs). SNP-gene pairs were screened through GTEx to identify eQTLs. The false discovery rate (FDR) was calculated using a stepwise Benjamini-Hochberg correction procedure and incorporated the number of tests and eQTL value list. A FDR value of $<0.05$ was accepted as statistically significant (Fadason et al., 2017).

Vectors and Constructs

A DNA fragment (562 bp) harboring rs4077450_G-rs4077451_T (Chr16: 79931421–79931980) was amplified from the genomic DNA of individuals who were heterozygous for the major alleles (rs4077450_T-rs4077451_A) and the minor alleles (rs4077450_G-rs4077451_T), respectively. Primers F: 5′-CTCCATACAGTTCCAGCA-3′ and R: 5′-TGAGGCTTTTGAGTTTAC-3′ were used for amplification. Amplicons were cloned into the pCR®/8/GW/TOPO (Invitrogen) entry vector and gateway-cloned into the destination vectors pGL4.23 (addgene 60323) and ZED (Bessa et al., 2009) using LR Clonase\textsuperscript{®} enzyme (Invitrogen) for the enhancer assays in human cell lines and zebrafish, respectively.

Cell Culture and Transfections

Human embryonic kidney HEK293 cells (ATCC) and human hepatocellular carcinoma HEPG2 cells (ATCC) were grown (37°C in a 5% CO\textsubscript{2}) and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) and Eagle’s Minimum Essential Media (EMEM), respectively, (Life Technologies), supplemented with 10% fetal bovine serum.

For luciferase assays, HEK293 cells were transiently transfected with pGL4.23 constructs and Renilla, using Lipofectamine 3000 (Invitrogen). Luminescence was measured 48 h post-transfection using the Dual Glo Luciferase Assay (Promega), on the Perkin Elmer Victor X4 plate reader. Values were normalized to the expression of Renilla luciferase.

For the siRNA knockdown experiments, HEK293 cells were reverse-transfected with ON-TARGETplus Non-targeting Control Pool siRNA (5 nM) (Dharmacon Cat# D-001810-10), Lincode SMARTpool LINCO1229 siRNA (5 nM) (Dharmacon Cat# R-191862-00-0005), and Lincode SMARTpool MAFTTR (5 nM) (Dharmacon Cat# R-192817-00-0005) using RNAiMax (Invitrogen) for gene expression analysis.

Quantitative PCR

Total RNA was isolated from control and siRNA-treated HEK293 cells at 48 h post-treatment using the Nucleospin RNA kit (Macherey-Nagel). cDNA was synthesized with qScript cDNA SuperMix (Quanta Biosciences). MAE, MAFTTR and LINCO1229 expression were measured using TaKaRa SYBR Premix Ex Taq\textsuperscript{TM} (Clontech) on a LightCycler400 (Roche Diagnostics). Primers MAE F: 5′-AGGAATGACACCCACAGA-3′ and R: 5′-CGAGTGAGGCTTGATATG-3′, MAFTTR F: 5′-CCTTGACAATGTGGTTTTT-3′ and R: 5′-CACGTTCCTTCATTTTGCTT-3′ and LINCO1229 F: 5′-ATGGAGACTCACCAGCAGG-3′ and R: 5′-TGGGTGCTTTAAACAGAGA-3′ were used for amplification. Gene expression analyses were carried out on qBase Plus (Biogazelle) and were normalized relative to the mean of reference genes encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and ribosomal protein L13a (RPL13A).

Quantitative Chromatin Immunoprecipitation

Chromatin was extracted from 10\textsuperscript{-7} HEPG2 cells, sonicated (Vibra Cell VXC130 Sonicator, Sonics) to approximately 500 bp (determined experimentally) and diluted 10 times with immunoprecipitation (IP) buffer as described previously (Veto et al., 2017). Chromatin immunoprecipitation (ChiP) was carried out according to Vaisanen et al. (2005) with minor

\textsuperscript{1}https://github.com/Genome3d/codes3d-v1
Crosslinking was reversed with Proteinase K (final concentration EDTA, 1% SDS) at room temperature for 30 min with rotation. eluted in ChIP elution buffer (50 mM Tris, pH 8, 10 mM EDTA). Finally, the beads were washed twice with 1 ml of (v/v) Nonidet P-40, 1% (w/v) sodium deoxycholate, 1 mM wash buffer [10 mM Tris–HCl (pH 8.1), 0.25 mM LiCl, 1% (v/v) Nonidet P-40, 1% (w/v) sodium deoxycholate, 1 mM EDTA].

Dynabeads G. The immunocomplexes were then pelleted by centrifugation at 4°C for 1 min at 10 000 g and washed sequentially for 5 min by rotation with 1 ml of the following buffers: low-salt wash buffer [20 mM Tris–HCl (pH 8), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl], high-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl] and LiCl high-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl], buffers: low-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl].

Dynabeads protein G (Thermo Fisher). Immunoprecipitations were performed overnight at 4°C with 10 µl of a ChIP grade HNF4α (ab41898, Abcam) antibody conjugated to 50 µl of Dynabeads G. The immunocomplexes were then pelleted by centrifugation at 4°C for 1 min at 100 × g and washed sequentially for 5 min by rotation with 1 ml of the following buffers: low-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl], high-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl] and LiCl high-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl], buffers: low-salt wash buffer [20 mM Tris–HCl (pH 8.1), 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl].

immunoassays, 1 µl of pre-cleared or immunoprecipitated chromatin was used for each reaction. HNF4α binding at the SNP region, negative binding site B-globin and positive binding site ABCC6 (Veto et al., 2017) was expressed relative to the pre-cleared input chromatin after subtraction from no antibody control. (Veto et al., 2017) was expressed relative to the pre-cleared input chromatin after subtraction from no antibody control.

Primers SNP region F: 5′-CCTCCATACAGTGTCACAGCA-3′ and R: 5′-TGGACCCTGTGTGCTTTAC-3′, B-globin F: 5′-AGGACAGGTACGGCTGTCATC-3′ and R: 5′-TTTATGCCAGCCCTTGCGTCATC-3′ and ABCC6 F: 5′-AGGCATGGCTTGCTCATC-3′ and R: 5′-ATGGAGACCGCGTCACAG-3′ were used for amplification.

Whole Mount in situ Hybridization

Full length maf riboprobe synthesis and whole mount in situ hybridization was performed as described previously (Monnich et al., 2009). The cDNA of MAF on a pcMV SPORT 6.1 plasmid was acquired from Life Biosciences as the Danio rerio maf cDNA DR.81288. The plasmid was linearized with Apal and the antisense DIG-labeled riboprobe was generated by in vitro transcription using SP6 RNA polymerase (Roche).

Zebrafish Enhancer Assays

Wild type (WIK) fish lines were maintained according to established protocols (Westerfield, 2000). The University of Otago Animal Ethics Committee approved all zebrafish research.

An enhancer test vector ZED (Bessa et al., 2009) was used to investigate the enhancer capacity of the genomic regulatory regions in zebrafish. MAF et al., 2009). The cDNA of Danio rerio maf was acquired from Life Biosciences as the Danio rerio maf cDNA DR.81288. The plasmid was linearized with Apal and the antisense DIG-labeled riboprobe was generated by in vitro transcription using SP6 RNA polymerase (Roche).

GraphPad PRISM 7 (GraphPad software, San Diego, CA, United States) was used for performing all statistical analysis. One-way ANOVA, (Tukey’s multiple comparisons tests), Fisher exact tests and unpaired t-tests were used for estimating the statistical significance of the luciferase, enhancer assays and quantitative PCR data, respectively. All data are presented as mean and error bars represent standard error of the mean (SEM).

Zebrafish Enhancer Assays

Wild type (WIK) fish lines were maintained according to established protocols (Westerfield, 2000). The University of Otago Animal Ethics Committee approved all zebrafish research.

An enhancer test vector ZED (Bessa et al., 2009) was used to investigate the enhancer capacity of the genomic regulatory regions in zebrafish. MAF et al., 2009). The cDNA of Danio rerio maf was acquired from Life Biosciences as the Danio rerio maf cDNA DR.81288. The plasmid was linearized with Apal and the antisense DIG-labeled riboprobe was generated by in vitro transcription using SP6 RNA polymerase (Roche).

GraphPad PRISM 7 (GraphPad software, San Diego, CA, United States) was used for performing all statistical analysis. One-way ANOVA, (Tukey’s multiple comparisons tests), Fisher exact tests and unpaired t-tests were used for estimating the statistical significance of the luciferase, enhancer assays and quantitative PCR data, respectively. All data are presented as mean and error bars represent standard error of the mean (SEM).

AUTHOR CONTRIBUTIONS

ML, JH, JO’S, and TM designed the study. ML performed the functional annotations, the eQTL analyses, the enhancer assays, the in situ hybridization, and the siRNA assays. HS isolated and cloned the rs4077450_rs4077451 minor and major allele fragments into the plasmid constructs and was supervised by JM. AD carried out the luciferase assay. RT carried out the conditional analysis. WS and TF carried out the CoDeS3D analyses. ML, JH, and TM wrote the manuscript with input from JO’S and WW.

FUNDING

This research was supported by Health Research Council of New Zealand grant #15/623 to TM, JO’S, and JH. TF was supported by a scholarship from a High Value Nutrition National grant (MBIE/HVN grant #3710040). ML was supported by a Lotteries Health Research Postdoctoral Fellowship.

ACKNOWLEDGMENTS

We thank Noel Jhinku for management of the Otago Zebrafish Facility.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00733/full#supplementary-material

REFERENCES

Balaban, R. S., and Mandel, L. J. (1988). Metabolic substrate utilization by rabbit proximal tubule. Am. J. Physiol. 254(3 Pt 2), F407–F416.
Bird, C. P., Stranger, B. E., Liu, M., Thomas, D. J., Ingle, C. E., Beazley, C., et al. (2007). Fast-evolving noncoding sequences in the human genome. *Genome Biol.* 8:R118. doi: 10.1186/gb-2007-8-6-r118

Carithers, L. J., and Moore, H. M. (2015). The genotype-tissue expression (GTEx) project. *Biopreserv. Biobank.* 13, 307–308. doi: 10.1177/2055378614560463

Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., et al. (2012). ANNOVAR: functional annotation of genetic variation from high-throughput sequencing data. *Nucleic Acids Res.* 40, 1–16. doi: 10.1093/nar/gkr472

Denker, A., and de Laat, W. (2016). The second decade of 3C technologies: detailed chromatin interactions. *Biochem. Biophys. Res. Commun.* 473, 118–124. doi: 10.1016/j.bbr.2016.03.063

Dalbeth, N., Merriman, T. R., and Stamp, L. K. (2016). Gout. *Am. J. Physiol. Cell Physiol.* 229, C351–C358. doi: 10.1152/ajpcell.00259.2015

Drummond, I. (2003). Making a zebrafish kidney: a tale of two tubes. *Dev. Biol.* 254, 219–232. doi: 10.1016/S0012-1606(03)00056-9

Doen, R. N., Bae, B. I., Cubelos, B., Chang, C., Hossain, A. A., Al-Saad, S., et al. (2016). Identifying regulatory connections for obesity and type 2 diabetes associated SNPs. *Genet. Med.* 18, 99–108. doi: 10.1038/gim.2015.131

Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., et al. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485, 376–380. doi: 10.1038/nature11082

Doan, R. N., Bae, B. I., Cubelos, B., Chang, C., Hossain, A. A., Al-Saad, S., et al. (2016). Mutations in human accelerated regions disrupt cognition and social behavior. *Cell* 167, 341–354.e12. doi: 10.1016/j.cell.2016.08.071

Drummond, I. (2003). Making a zebrafish kidney: a tale of two tubes. *Trends Cell Biol.* 13, 357–365. doi: 10.1016/S1471-4914(03)00124-7

Edwards, S. L., Beesley, J., French, J. D., and Dunning, A. M. (2013). Beyond GWAS: illuminating the dark road from association to function. *Am. J. Hum. Genet.* 93, 779–797. doi: 10.1016/j.ajhg.2013.10.012

Ernst, J., and Kellis, M. (2017). Chromatin-state discovery and genome annotation with ChromHMM. *Nat. Protoc.* 12, 2478–2492. doi: 10.1038/nprot.2017.124

Fadason, T., Ekblad, C., Ingram, J. R., Schierding, W. S., and O’Sullivan, J. M. (2017). Physical interactions and expression quantitative traits loci identify regulatory connections for obesity and type 2 diabetes associated SNPs. *Front. Cell Dev. Biol.* 8:150. doi: 10.3389/fcell.2017.00150

Finucane, H. K., Bulik-Sullivan, B., Grusey, A., Trynka, G., Reshef, Y., Loh, P. R., et al. (2015). Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat. Genet.* 47, 1228–1235. doi: 10.1038/ng.3404

Gerstein, M. B., Kundaje, A., Hariharan, M., Landt, S. G., Yan, K. K., Cheng, C., et al. (2012). Architecture of the human regulatory network derived from ENCODE data. *Nature* 489, 91–100. doi: 10.1038/nature11245

Giambartolomei, C., Vukcevic, D., Schadt, E. E., Franke, L., Hingorani, A. D., et al. (2012). Architecture of the human regulatory network derived from ENCODE data. *Hum. Cell Biol.* 37, 2039–2052. doi: 10.1016/j.humce.2011.10.006

Gosmain, Y., Avril, I., Mamin, A., and Philippe, J. (2007). Pax-6 and c-Maf mediated detoxifying enzyme genes expression and anti-oxidant induction. *Hum. Cell Biol.* 28, 3964–3973. doi: 10.1016/j.humce.2007.10.002

Huang, Y. F., Gulkos, B., and Siepel, A. (2017). Fast, scalable prediction of deleterious noncoding variants from functional and population genomic data. *Hum. Mol. Genet.* 26, 1323–1327. doi: 10.1093/hmg/ddw322

Jhang, J. J., and Yen, G. C. (2017). The role of Nrf2 in NLRP3 inflammasome activation. *Cell. Mol. Immunol.* 14, 1011–1012. doi: 10.1016/j.cmi.2017.11.014

Kano, M., Akiyama, M., Takahashi, A., Matoba, N., Momozawa, Y., Ikeda, M., et al. (2018). Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.* 50, 390–400. doi: 10.1038/s41588-018-0047-6

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., and Morishima, K. (2017). KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* 45, D353–D361. doi: 10.1093/nar/gkw1092

Karner, C. M., Merkel, C. E., Dodge, M., Ma, Z., Lu, J., Chen, C., et al. (2010). Tankyrase is necessary for canonical Wnt signaling during kidney development. *Dev. Dyn.* 239, 2014–2023. doi: 10.1002/dvdy.22340

Kataoka, K., Shioda, S., Ando, K., Sakagami, K., Handa, H., and Yasuda, K. (2004). Differentially expressed Maf family transcription factors, c-Maf and Mafa, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. *J. Mol. Endocrinol.* 32, 9–20. doi: 10.1677/jme.0.032009

Kawakami, K. (2005). Transposon tools and methods in zebrafish. *Dev. Dyn.* 234, 244–254. doi: 10.1002/dvdy.20516

Keathnathan, S., Leask, M., Boocock, J., Phipps-Green, A. J., Antony, J., O’Sullivan, J. M., et al. (2018). A non-coding genetic variant maximally associated with serum urate levels is functionally linked to HNF4A-dependent PDZK1 expression. *Hum. Mol. Genet.* 27, 3964–3973. doi: 10.1093/hmg/ddy295

Kheradpour, P., Ernst, J., Melnikov, A., Rogov, P., Wang, L., Zhang, X., et al. (2013). Systematic dissection of regulatory motifs in 2000 predicted human enhancers using a massively parallel reporter assay. *Genome Res.* 23, 800–811. doi: 10.1101/gr.144899.112

Kichaev, G., Yang, Y., Lindstorm, S., Hnookzdri, F., Eskin, E., Price, A. L., et al. (2014). Integrating functional data to prioritize causal variants in statistical fine-mapping studies. *PLoS Genet.* 10:e1004722. doi: 10.1371/journal.pgen.1004722

Kikuchi, K., Iida, M., Ikeda, N., Moriyama, S., Hamada, M., Takahashi, S., et al. (2014). Integrating functional data to prioritize causal variants in statistical fine-mapping studies. *PLoS Genet.* 10:e1004722. doi: 10.1371/journal.pgen.1004722

Kikuchi, K., Iida, M., Ikeda, N., Moriyama, S., Hamada, M., Takahashi, S., et al. (2014). Macrophages switch their phenotype by regulating Maf expression during different phases of inflammation. *J. Immunol.* 201, 631–635. doi: 10.4049/jimmunol.1800040

Klootwijk, E. D., Reichold, M., Helip-Wooley, A., Tolaymat, A., Broeker, C., Robinson, S. L., et al. (2014). Mis-targeting of peroxisomal EHHADH and deleterious noncoding variants from functional and population genomic data. *Hum. Mol. Genet.* 26, 1323–1327. doi: 10.1093/hmg/ddw322

Ko, Y. A., Yi, H., Qiu, C., Huang, S., Park, J., Ledo, N., et al. (2017). Genetic-variation-driven gene-expression changes highlight genes with important function.
functions for kidney disease. *Am. J. Hum. Genet.* 100, 940–953. doi: 10.1016/j.ajhg.2017.05.004

Köttgen, A., Albrecht, E., Teumer, A., Vitart, V., Krumdieck, J., Huedenmark, C., et al. (2013). Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat. Genet.* 45, 145–154. doi: 10.1038/ng.2500

Kumar, V., Westra, H. J., Karjalainen, J., Zhnarzakova, D. V., Esko, T., Hrdlickova, B., et al. (2013). Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. *PLoS Genet.* 9:e1003201. doi: 10.1371/journal.pgen.1003201

Lai, H. M., Chen, C. J., Su, B. Y., Chen, Y. C., Yu, S. F., Yen, J. H., et al. (2012). Gout and type 2 diabetes have a mutual inter-dependent effect on genetic risk factors and higher incidences. *Rheumatology* 51, 715–720. doi: 10.1093/rheumatology/ker573

Machiela, M. J., and Chanock, S. J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 31, 3555–3557. doi: 10.1093/bioinformatics/btv402

Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C., and Mollace, V. (2016). *J. Biol. Chem.* 281, 27393–27399. doi: 10.1074/jbc.M116.732521

Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C., and Mollace, V. (2016). *Ann. Rheum. Dis.* 75, 124–130. doi: 10.1136/annrheumdis-2014-205877

Polychronopoulos, D., King, J. W. D., Nash, A. J., Tan, G., and Lenhard, B. (2017). Conserved non-coding elements: developmental gene regulation meets genome organization. *Nucleic Acids Res.* 45, 12611–12624. doi: 10.1093/nar/gkx1074

Ponten, F., Jirstrom, K., and Uhlen, M. (2008). The human protein atlas—a tool for pathologists. *J. Pathol.* 216, 387–391. doi: 10.1002/path.2440

Porcu, E., Medici, M., Pistis, G., Volpato, C. B., Wilson, S. G., Cappola, A. R., et al. (2013). A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *PLoS Genet.* 9:e1003266. doi: 10.1371/journal.pgen.1003266

Prestin, K., Wolf, S., Feldmann, R., Husser, J., Geissler, I., Rimmbach, C., et al. (2014). Transcriptional regulation of urate transportersome member SLC2A9 by nuclear receptor HNF-6alpha. *Am. J. Physiol. Renal Physiol.* 307, F1041–F1051. doi: 10.1152/ajprenal.00640.2013

Pruiu, R. J., Welch, R. P., Sanna, S., Teslovich, T. M., Chines, P. S., Gliedt, T. P., et al. (2010). *LocusZoom: regional visualization of genome-wide association scan results.* Bioinformatics 26, 2336–2337. doi: 10.1093/bioinformatics/btq419

Ranzani, V., Rossetti, G., Panzeri, L., Arrigoni, A., Bonnal, R. J., Curtis, S., et al. (2015). Long-term intergenic noncoding RNA landscape of human lymphocytes highlights the regulation of T cell differentiation by linc-MAF-4. *Nat. Immunol.* 16, 318–325. doi: 10.1038/ni.3093

Rao, S. S., Hunfeld, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., et al. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159, 1665–1680. doi: 10.1016/j.cell.2014.11.021

Rimm, J. L., and Chang, H. Y. (2012). Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem.* 81, 145–166. doi: 10.1146/annurev-biochem-051410-092902

See, L. C., Kuo, C. F., Yu, K. H., Luo, S. F., Chou, I. J., Ko, Y. S., et al. (2014). Hyperuricemia and hypertension status was strongly associated with gout and weakly associated with hyperuricemia. *PLoS One* 9:e114579. doi: 10.1371/journal.pone.0114579

Sharaf El Din, U. A. A., Salem, M. M., and Abdulazim, D. O. (2017). Uric acid in the pathogenesis of metabolic, renal, and cardiovascular diseases: a review. *J. Adv. Res.* 8, 537–548. doi: 10.1016/j.jare.2016.11.004

So, A. K., and Martinon, F. (2017). Inflammation in gout: mechanisms and therapeutic targets. *Nat. Rev. Rheumatol.* 13, 639–647. doi: 10.1038/nrrheum.2017.155

Sun, Y. P., Zhang, B., Miao, L., Wang, X. M., Yu, J. H., Luo, L., et al. (2015). Association of apolipoprotein E (ApoE) polymorphisms with risk of primary hyperuricemia in Uygur men, Xinjiang, China. *Lipids Health Dis.* 14:25. doi: 10.1186/s12944-015-0025-2

Tamaï, L., Nezu, J., Uchino, H., Sai, Y., Oka, A., Shimane, M., et al. (2000). Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem. Biophys. Res. Commun.* 273, 251–260. doi: 10.1006/bbrc.2000.2922

Tanj, J. Y., Smith, A. A. T., Ferreira da Silva, M., Matthey-Doret, C., Rueedi, R., Sonmez, R., et al. (2017). cis-Acting complex-trait-associated lincRNA expression correlates with modulation of chromosomal architecture. *Cell Rep.* 18, 2280–2288. doi: 10.1016/j.celrep.2017.02.009

Tanaka, K., Ogata, S., Tanaka, H., Omura, K., Honda, C., Osaka Twin Research Group, et al. (2015). The relationship between body mass index and uric acid: a study on Japanese adult twins. *Environ. Health Prev. Med.* 20, 347–353. doi: 10.1007/s12541-015-0473-3

Teumer, A., Rawal, R., Homuth, G., Ernst, F., Heier, M., Evert, M., et al. (2011). Genome-wide association study identifies four genetic loci associated with thyroid volume and goiter risk. *Am. J. Hum. Genet.* 88, 664–673. doi: 10.1016/j.ajhg.2011.04.015

Thurman, R. E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M. T., Haugen, E., et al. (2012). The accessible chromatin landscape of the human genome. *Nature* 489, 75–82. doi: 10.1038/nature11232

Tin, A., Woodward, O. M., Kao, W. H., Liu, C. T., Lu, X., Nalls, M. A., et al. (2012). Genome-wide association study for serum urate concentrations and gout among African Americans identifies genomic risk loci and a novel URA1 loss-of-function allele. *Hum. Mol. Genet.* 20, 4056–4068. doi: 10.1093/hmg/ddr307
Leask, M., Schaeffer, C., Riba, M., Ikehata, M., Brambilla, P., Messa, P., et al. (2017). Early involvement of cellular stress and inflammatory signals in the pathogenesis of tubulointerstitial kidney disease due to UMOD mutations. Sci. Rep. 7:7383. doi: 10.1038/s41598-017-07804-6

Vaisanen, S., Dunlop, T. W., Sinkkonen, L., Frank, C., and Carlberg, C. (2005). Spatio-temporal activation of chromatin on the human CYP24 gene promoter in the presence of 1alpha,25-Dihydroxyvitamin D3. J. Mol. Biol. 350, 65–77. doi: 10.1016/j.jmb.2005.04.057

Vandanmagsar, B., Youm, Y. H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., et al. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat. Med. 17, 179–188. doi: 10.1038/nm.2279

Wingert, R. A., Selleck, R., Yu, J., Song, H. D., Chen, Z., Song, A., et al. (2007). The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros. PLoS Genet. 3:e189. doi: 10.1371/journal.pgen.0030189

Yang, D., Jang, I., Choi, J., Kim, M. S., Lee, A. J., Kim, H., et al. (2018). 3DIV: a 3D-genome Interaction Viewer and database. Nucleic Acids Res. 46, D52–D57. doi: 10.1093/nar/gkx1017

Yang, J., Lee, S. H., Goddard, M. E., and Visscher, P. M. (2011). GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88, 76–82. doi: 10.1016/j.ajhg.2010.11.011

Zhan, M., Chen, G., Pan, C. M., Gu, Z. H., Zhao, S. X., Liu, W., et al. (2014). Genome-wide association study identifies a novel susceptibility gene for serum TSH levels in Chinese populations. Hum. Mol. Genet. 23, 5505–5517. doi: 10.1093/hmg/ddu250

Zhang, F., Liu, G., Wei, C., Gao, C., and Hao, J. (2017). Linc-MAF-4 regulates Th1/Th2 differentiation and is associated with the pathogenesis of multiple sclerosis by targeting MAF. FASEB J. 31, 519–525. doi: 10.1096/fj.201600838R

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Leask, Dowdle, Salvesen, Topless, Fadason, Wei, Schierding, Marsman, O’Sullivan, Merriman and Horsfield. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.