



OPEN

GWAS-identified hyperuricemia-associated *IGF1R* variant rs6598541 has a limited role in urate mediated inflammation in human mononuclear cells

Orsolya I. Gaal^{1,2}, Ruiqi Liu², Dragoş Marginean¹, Medeea Badii^{1,2}, Georgiana Cabău¹, Ioana Hotea³, Valentin Nica¹, Doina Colcear⁶, HINT Consortium*, Cristina Pamfil³, Tony R. Merriman^{4,5}, Simona Rednic³, Radu A. Popp¹, Tania O. Crişan^{1,2,7}✉ & Leo A. B. Joosten^{1,2,7}

Gout is a common autoinflammatory joint diseases characterized by deposition of monosodium urate (MSU) crystals which trigger an innate immune response mediated by inflammatory cytokines. *IGF1R* is one of the loci associated with both urate levels and gout susceptibility in GWAS to date, and IGF-1-IGF-1R signaling is implicated in urate control. We investigate the role of IGF-1/IGF1R signaling in the context of gouty inflammation. Also, we test the gout and urate-associated *IGF1R* rs6598541 polymorphism for association with the inflammatory capacity of mononuclear cells. For this, freshly isolated human peripheral blood mononuclear cells (PBMCs) were exposed to recombinant IGF-1 or anti-IGF1R neutralizing antibody in the presence or absence of solubilized urate, stimulated with LPS/MSU crystals. Also, the association of rs6598541 with *IGF1R* and protein expression and with ex vivo cytokine production levels after stimulation with gout specific stimuli was tested. Urate exposure was not associated with *IGF1R* expression in vitro or in vivo. Modulation of IGF1R did not alter urate-induced inflammation. Developing urate-induced trained immunity in vitro was not influenced in cells challenged with IGF-1 recombinant protein. Moreover, the *IGF1R* rs6598541 SNP was not associated with cytokine production. Our results indicate that urate-induced inflammatory priming is not regulated by IGF-1/IGF1R signaling in vitro. *IGF1R* rs6598541 status was not associated with *IGF1R* expression or cytokine production in primary human PBMCs. This study suggests that the role of IGF1R in gout is tissue-specific and may be more relevant in the control of urate levels rather than in inflammatory signaling in gout.

Gout is an important inflammatory disease with high prevalence in developed countries among men and post-menopausal women^{1,2} with prevalence increasing worldwide³. The precondition for developing gout is the deposition of monosodium urate (MSU) crystals in the joint and other tissues as a result of elevated serum urate levels (hyperuricemia)⁴. While most research focuses on inflammation due to MSU crystal deposition⁵⁻⁷, there is evidence that soluble urate also increases pro-inflammatory cytokine production⁸, associating hyperuricemia with a hyper-inflammatory state^{9,10}, highlighting its pro-inflammatory effects within the intracellular setting together with an altered epigenetic landscape¹¹. Additionally, there exist indication on how soluble urate induce epigenetic modifications in myeloid cells, leading to an enhanced inflammatory response^{12,13}. Moreover, hyperuricemia may

¹Department of Medical Genetics, Iuliu Haţieganu University of Medicine and Pharmacy, Str. Pasteur Nr.6, 400349 Cluj-Napoca, Romania. ²Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands. ³Department of Rheumatology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania. ⁴Department of Microbiology, University of Otago, Dunedin, New Zealand. ⁵Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA. ⁶Clinical Infectious Disease Hospital, Cluj-Napoca, Romania. ⁷These authors jointly supervised this work: Tania O. Crişan and Leo A. B. Joosten. *A list of authors and their affiliations appears at the end of the paper. ✉email: tania.crisan@umfcluj.ro

lead to innate immune memory, contributing to a persistently elevated inflammatory status^{11,12,14}. In conditions associated with chronic inflammation, such as metabolic syndrome, obesity and cardiovascular disease, there is an increased prevalence of hyperuricemia, suggesting that the link between hyperuricemia and inflammation is perhaps bidirectional. While inflammation itself may influence urate levels, alternatively elevated urate levels may add to an inflammatory state. The pathogenetic mechanism of gout at molecular level is not well established. Although, several treatment possibilities are already available such as colchicine, nonsteroidal anti-inflammatory drugs, urate lowering therapies, and anti-IL-1 therapies. Nevertheless, potential side effects of therapy are also worth noting, including hypersensitivity, poor tolerance or risk of infection, therefore, finding other effective and better tolerated categories of modulators would be a great benefit for gout patients.

IGF-1 is an important growth factor with signaling roles in numerous cell types, including monocytes¹⁵, macrophages¹⁶ and chondrocytes¹⁷. The protein binds with high affinity to the Insulin Like Growth Factor 1 Receptor (IGF1R)¹⁸. The activated receptor is engaged in cell growth, survival control as well as proliferation and is also known for being involved in metabolic regulation^{19,20}. Previous genome-wide association studies on serum urate concentrations^{21,22}, identified *IGF1R* as a genetic locus for serum urate levels. The index SNP with the lowest *p* value at the *IGF1R* locus in the study performed by Köttgen et al. was rs6598541, of which the minor allele “A” was associated with increased urate levels (0.043 mg/dl increase [CI 0.031–0.055], $p = 5 \times 10^{-15}$)²¹. This variant also associated with urate control in a trans-ancestral meta-analysis study in European and East Asian populations²³. Moreover, this SNP also associated with gout in patients that met the American College of Rheumatology (ACR) classification criteria for gout²⁴.

The IGF-1 pathway was recently linked to innate immune memory and proinflammatory reprogramming induced by metabolic stimuli in human primary monocytes²⁵. Stimulation via the IGF1R by mevalonate or by IGF-1 itself was able to induce trained immunity and epigenetic modifications in human primary monocytes and this contributed to enhanced proinflammatory responses to subsequent stimulation with LPS or Pam3Cys²⁵. SNPs in *IGF1R* were significantly associated to cytokine production in response to training with BCG or β -glucan in vitro²⁵. Additional evidence shows IGF-1 as having pro-inflammatory effects upon 24 h stimulation in primary human PBMCs, albeit these effects were only visible in synergy with TLR ligands such as LPS or Pam3Cys²⁶. These effects were reported to be mediated via the MAPK pathway²⁶. Other evidence shows that IGF-1 enhances chemotactic macrophage migration which leads to tissue inflammation²⁷.

In the present study we address the hypothesis that signaling via the IGF1R is associated to inflammation in gout. We investigated whether soluble urate modulates *IGF1R* gene expression in human cells and whether the IGF-1 pathway has an effect on the proinflammatory priming elicited by soluble urate in vitro. Moreover, we assessed the association of the *IGF1R* rs6598541 SNP with *IGF1R* expression in circulating mononuclear cells and cytokine production capacity in patients with gout, asymptomatic hyperuricemia and normouricemic controls in an Eastern European population.

Materials and methods

Participants

The participants in this study consisted of patients with gout ($n = 116$), hyperuricemic controls ($n = 78$) and normouricemic volunteers ($n = 174$), followed at the Rheumatology Department of the “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania. Subjects were enrolled after written informed consent. Peripheral blood was drawn from the cubital vein on EDTA tubes under sterile conditions. The patient study was approved by the Ethical Committee of the “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca (approval no. 425/2016) and all participants provided written informed consent. Experiments were conducted according to the principles expressed in the Declaration of Helsinki. All study participants in the gout group were included if they corresponded to the ACR/EULAR 2015 classification criteria with a score of ≥ 8 . The cutoff for the asymptomatic hyperuricemia group was a serum urate concentration of 7 mg/dl and negative history of gout flares. The described groups were similar in age and BMI. The gender distribution shows a higher number of men in the gout study group, consistent with the higher prevalence of gout in males.

PBMC isolation and stimulation

Isolation and stimulation of human peripheral blood mononuclear cells (PBMCs) was assessed as described previously⁸. Briefly, PBMCs were separated using Ficoll-Paque and resuspended in RPMI culture medium with Dutch modification (Gibco), supplemented with human pooled serum. Monocyte isolation was done using hyperosmotic Percoll[®] solution²⁸. Cells were incubated for 24 h with culture medium as negative control, and urate in different concentrations. After priming, culture medium was removed, the remaining adherent cells were washed with warm PBS, then restimulated with medium or LPS with or without MSU crystals. IGF1R was induced by the addition of IGF-1 (R&D Systems, Abingdon, United Kingdom) in concentration of 5 μ g/ml. In separate experiments IGF1R signaling was blocked with anti-IGF1R antibody (R&D Systems).

Cytokine measurements

Cytokine concentrations were determined in cell culture supernatants using specific sandwich ELISA kits for IL-1 β , IL-1Ra, IL-6 (R&D Systems, Minneapolis).

Quantitative PCR for mRNA expression of *IGF1R*

PBMCs were incubated with the stimuli as mentioned above and after 24 h of incubation at 37 °C in 5% CO₂. The plates were centrifuged, the supernatant was collected and the cell pellets were lysed with 300 μ l of TRIzol Reagent (Invitrogen). RNA purification was performed according to manufacturer's instructions. Subsequently, RNA concentrations were determined using NanoDrop software. Isolated RNA was subsequently transcribed

into complementary DNA using High-Capacity cDNA Reverse Transcription Kit (Applied BioSystems) followed by quantitative PCR using the Sybr Green Method. The following primers were used in the reaction: *IGF1R* forward 5'-TCGACATCCGCAACGACTATC-3' and reverse 5'-TCGACATCCGCAACGACTATC-3' and β 2-microglobulin forward 5'-ATGAGTATGCCTGCCGTGTG-3' and reverse 5'-CCAAATGCGGCATCTCAAAC-3'. Results are shown as fold change in mRNA levels in stimulated samples compared to controls.

Genotyping for *IGF1R* rs6598541

Three independent groups were genotyped (gout group N = 116; hyperuricemic group N = 78 and healthy volunteers group N = 174). Genomic DNA was isolated from whole blood (Promega) and genotyping was performed on an Illumina Infinium HD assay platform using The Infinium Global Screening Array-24 v3.0 BeadChip. The quality control protocol for genotyping data was performed Using Illumina's GenomeStudio. The SNPs with < 95% call rate were excluded and all the SNPs with 95–98% call rate were verified and manually re-clustered or removed when necessary. The data was exported to PLINK format and further filters were applied: minor allele frequency > 0.01; hardy–weinberg equilibrium test p value > 10^{-6} ; samples with heterozygosity rate of ± 3 standard deviations and related individuals were excluded. For the final step, the strands were flipped and all the data was verified to align to the GRCh37 hg19 build. The genotypes generated were checked using a predesigned TaqMan SNP genotyping Assay (Applied Biosystems).

Transcriptomics

Freshly isolated PBMCs were frozen in TRIzol Reagent (Invitrogen) and stored at -80°C and were later used for commercial RNA-Seq analysis (Beijing Genomics Institute, BGI, Beijing, China). The integrity of extracted RNA was assessed using Agilent 2100 Bioanalyzer. Oligo dT magnetic beads were used to capture mRNA from total RNA. Purified ligation products were enriched using PCR amplification followed by denaturation and cyclization of ssDNA by splint oligos and DNA ligase generating DNA nanoballs (DNBs). Sequencing of DNBs was performed on DNBseq platform.

Initial quality control was performed with SOAPnuke (v1.5.2). Clean reads were mapped to human transcriptome assembly GRCh37 (hg19) using bowtie2. Read counts were normalized using DESeq2 (Version: DESeq2_1.34.0) median of ratios method using R package (Version: R4.0.4.) and were used for downstream targeted gene expression statistical analysis.

Flow cytometry analysis

50 μl fresh EDTA whole blood and antibody solution were mixed by vortex for 4 s, and followed by 10 min incubation in room temperature avoiding light. Afterwards 1 ml NH_4Cl lysing solution (BD Pharm lyse, BD Biosciences) were added to the stained sample, and vortexed for 5 s. Then the solution was incubated at room temperature for 10 min without light. 300 μl of the lysed sample was used for flow cytometry measurement (Beckman Coulter). The antibody solution contained 5 antibodies, including IGF-1R (APC, Life Technologies), CD14 (FITC, Agilent technologies), CD16 (PE, eBioscience), CD45 (PECy7, Beckman Coulter), HLA-DR (PB, Beckman Coulter). Monocytes were first gated by forward scatter and side scatter, and then by CD45. The geometric mean of IGF-1R was used for t-test analysis.

Results

The effect of urate on the expression of *IGF1R*

To assess the effect of urate on the expression of *IGF1R*, mRNA was measured after stimulation of PBMCs of eight healthy donors with different concentrations of urate for 24 h. No differences were observed in the expression of the receptor itself (Fig. 1A). In addition, we assessed the mRNA level of the receptor in PBMCs from gout patients in the experimental setup of 24 h urate priming followed by stimulation with LPS 10 ng/ml (TLR4 ligand) for another 24 h. In line with the previous results, no differences regarding expression of *IGF1R* were observed after stimulation of the cells (Fig. 1B). In contrast, we could observe an increased steady-state mRNA expression of *IGF1R* in unstimulated monocytes originating from gout patients compared to normouricemic controls (Fig. 1C).

Activation or inhibition of *IGF1R* does not modify cytokine production in urate priming or trained immunity experiments

Next, we studied the possible contribution of *IGF1R* to inflammation in an experimental setup consisting of 24 h exposure to recombinant IGF-1 protein (Fig. 2A) (R&D Systems) or anti-*IGF1R* neutralizing antibody (Fig. 2B) in the presence or absence of solubilized urate, followed by 24 h stimulation with LPS and MSU crystals. Cells treated with solubilized urate (50 mg/dl) together with the recombinant IGF-1 protein (5 ng) produced more IL-1 β , but not IL-6 or IL-1Ra compared to control. The cytokine production was not modified by anti-*IGF1R* neutralizing antibody. Further, we tested if IGF-1 could influence cytokine production in a trained immunity experimental design^{29,30}, consisting of stimulation for 24 h with IGF-1 or β -glucan (BG) as positive control, followed by washout and rest for 5 days and subsequent second stimulation with LPS (10 ng/ml). We did not observe an enhanced training effect by IGF1 (Fig. 2C). Bekkering et al. reported SNPs in *IGF1R* (rs150571637, rs9672558, rs34428109, rs1573891) associated with trained immunity in response to Bacille Camette-Guerin (BCG) and beta-glucan isolated from *C. albicans*³¹. Thereafter we assessed *IGF1R* rs1573891 in our study and found no association of this SNP to priming of PBMCs by soluble urate (Fig. 2D).

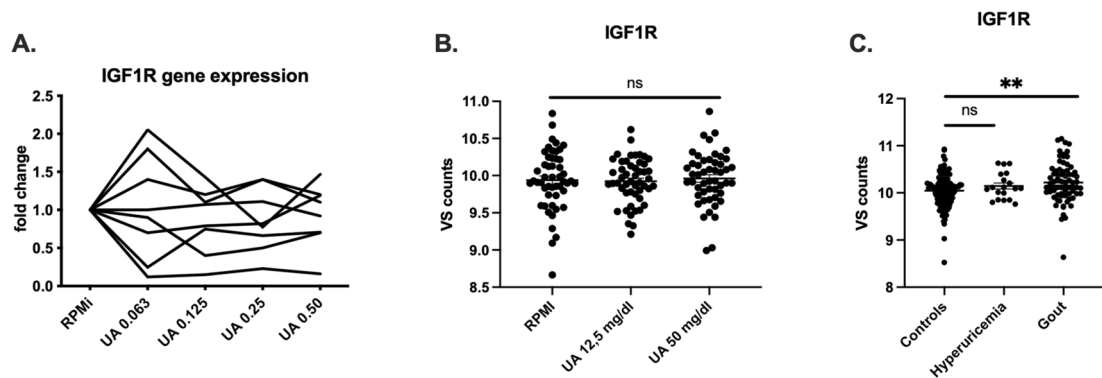


Figure 1. Urate effects on mRNA expression in vitro and basal expression of *IGF1R* of the studied groups. **(A)** mRNA expression of freshly isolated PBMCs originating from healthy donors ($n=8$) treated with different concentrations of uric acid (UA). **(B)** mRNA expression of freshly isolated PBMCs originating from gout patients ($n=50$) treated with UA for 24 h and restimulated with LPS 10 ng/ml. Repeated measurements one-way ANOVA and Tukey's multiple comparisons test, $*p<0.05$. **(C)** mRNA expression of freshly isolated PBMCs originating from healthy controls ($n=113$), hyperuricemic patients ($n=19$) and gout patients ($n=72$). The data is represented as normalized counts. Brown-Forsythe and Welch ANOVA, $*p<0.05$.

IGF1R rs6598541 SNP and *IGF1R* expression levels in freshly isolated PBMCs

We further examined the *IGF1R* SNP rs6598541, previously associated with serum urate levels^{21,23} and gout²⁴, for association with *IGF1R* expression in patients with gout, hyperuricemia and normouricemic controls. The basal expression level of *IGF1R* in freshly isolated PBMCs from the three groups (Fig. 3A–C) was not associated with rs6598541. Additionally, flow cytometric assessment of *IGF1R* surface expression on unstimulated PBMCs from healthy donors was also not association with *IGF1R* rs6598541 (Fig. 3D).

IGF1R rs6598541 SNP and cytokine production in stimulated PBMCs

To further study the possible implication of rs6598541 on inflammation, we assessed the ex vivo cytokine secretion by freshly isolated PBMCs challenged with various stimuli in association with the *IGF1R* rs6598541 genotypes: cytokine production in response to 24 h stimulation with MSU, palmitate (C16.0) or the combination of MSU crystal with C16.0 (Fig. 4A); urate priming for 24 h and stimulation with LPS for 24 h (Fig. 4B); or 24 h stimulation with LPS 100 ng (Fig. 4C). No significant association was found between the SNP and ex vivo cytokine production (IL-6, IL-1 β or IL-1Ra). The same analysis of data obtained following the same experiment carried out for the hyperuricemia individuals and for patients with gout (both active and non-active form) also showed no significant differences for these conditions between genotypes (supplementary Figs. 1, 2, 3).

Discussion

IGF-1 is known to have modulatory roles in human immune responses and a regulatory role in the activation of the peripheral monocytes^{26,32}. Interestingly, IGF-1 has dual roles, being involved in inflammation in a context-dependent mode, presenting both pro- and anti-inflammatory properties. After myocardial infarction, IGF-1 acted like an anti-inflammatory cytokine on myeloid cells in vitro while negating the pro-inflammatory phenotype of neutrophils and macrophages in vivo³³. Also, several in vitro studies revealed anti-inflammatory properties of IGF-1 on astrocytes and microglia^{34,35}, whereas another study focused on its role in decreasing the release of IL-1Ra and increasing IL-1 β ³⁶.

The IGF-1 pathway has recently been shown to be involved in the induction of trained immunity. Since innate immune memory is increasingly shown to be important for urate-induced inflammation, in the present study, we were interested in assessing IGF-1/*IGF1R* signaling in urate priming using primary PBMCs.

Our data indicate that IGF-1 does not contribute to urate-induced inflammation and blocking *IGF1R* did not influence the inflammatory responses triggered by urate. We show that urate does not modulate the expression of *IGF1R* itself in vitro in PBMCs from healthy donors nor in vivo in PBMCs from gout patients.

However, we noticed an increased steady-state mRNA expression of *IGF1R* in unstimulated PBMCs from gout patients. Increased expression of *IGF1R* was also seen in leukocytes of rheumatoid arthritis patients, which was associated with systemic inflammation and pain³⁷. Interestingly, RA patients with high *IGF1R* gene expression values were found to have low IGF-1 serum concentrations³⁷. In contrast, elevated circulating IGF-1 concentrations were reported in patients with gout and insulin resistance³⁸.

Bekkering et al. describe four SNPs in *IGF1R* associated with differential cytokine production in monocytes trained with β -glucan or BCG vaccine (*Bacillus Calmette–Guérin*)²⁵. We have also assessed one of these four SNPs with respect to urate priming and found no association of these variants to changes in cytokine profiles after urate exposure (Fig. 2D). Therefore, our data provide no evidence for a role of *IGF1R* in urate-mediated inflammatory priming.

We explored association of the *IGF1R* rs6598541 SNP, identified as a genetic susceptibility variant for gout and serum urate levels^{21,23,24}, with cytokine production in human PBMCs. *IGF1R* genetic variants are potentially functionally relevant in gout and hyperuricemia since the genetic control of urate levels and risk of gout at the

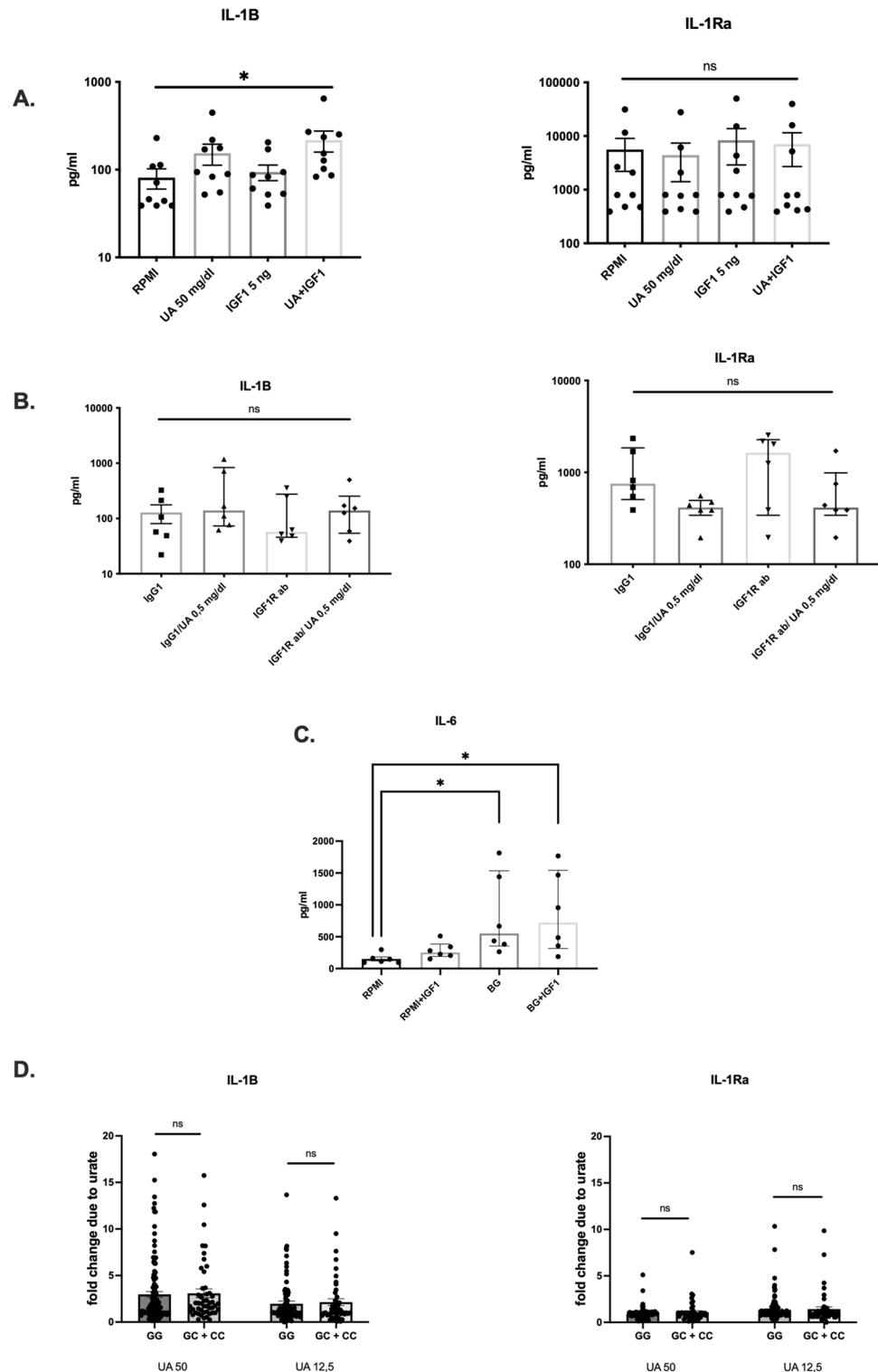


Figure 2. Role of IGF1R in urate induced inflammation in vitro. **(A)** Freshly isolated PBMCs isolated from healthy controls ($n=9$) were stimulated with RPMI, uric acid 50 mg/dl, IGF-1 recombinant protein (R&D Systems) 5 ng for 24 h. After 24 h the cells were restimulated with LPS 10 ng/ml together with MSU 300 mg/dl. **(B)** Freshly isolated Percoll enriched Monocytes ($n=6$) were treated with RPMI, IGF1R antibody (R&D Systems) or IgG1 isotype control and uric acid 50 mg/dl for 24 h. After 24 h monocytes were restimulated with LPS 10 ng/ml with MSU 300 mg/dl. **(C)** Freshly isolated PBMCs ($n=6$) were trained in vitro with IGF-1 5 ng and beta-glucan (BG) 1 μ g/ml for 24 h, subsequently washed, rested for 5 days, and at day 6 restimulated for 24 h with 10 ng/ml LPS. **(D)** Freshly isolated PBMCs originating from healthy controls ($n=174$). Concentration IL-1 β and IL-6 measured in the supernatants of PBMCs after stimulation with urate of conc. 50 mg/dl and 12.5 mg/dl for 24 h, followed by restimulation with LPS 10 ng/ml. IL-1 β , and IL-1Ra (R&D Systems, Minneapolis) was measured in supernatant by ELISA. Graphs depict means \pm SEM. Friedman test, Dunn's multiple comparisons test, $p^* < 0.05$.

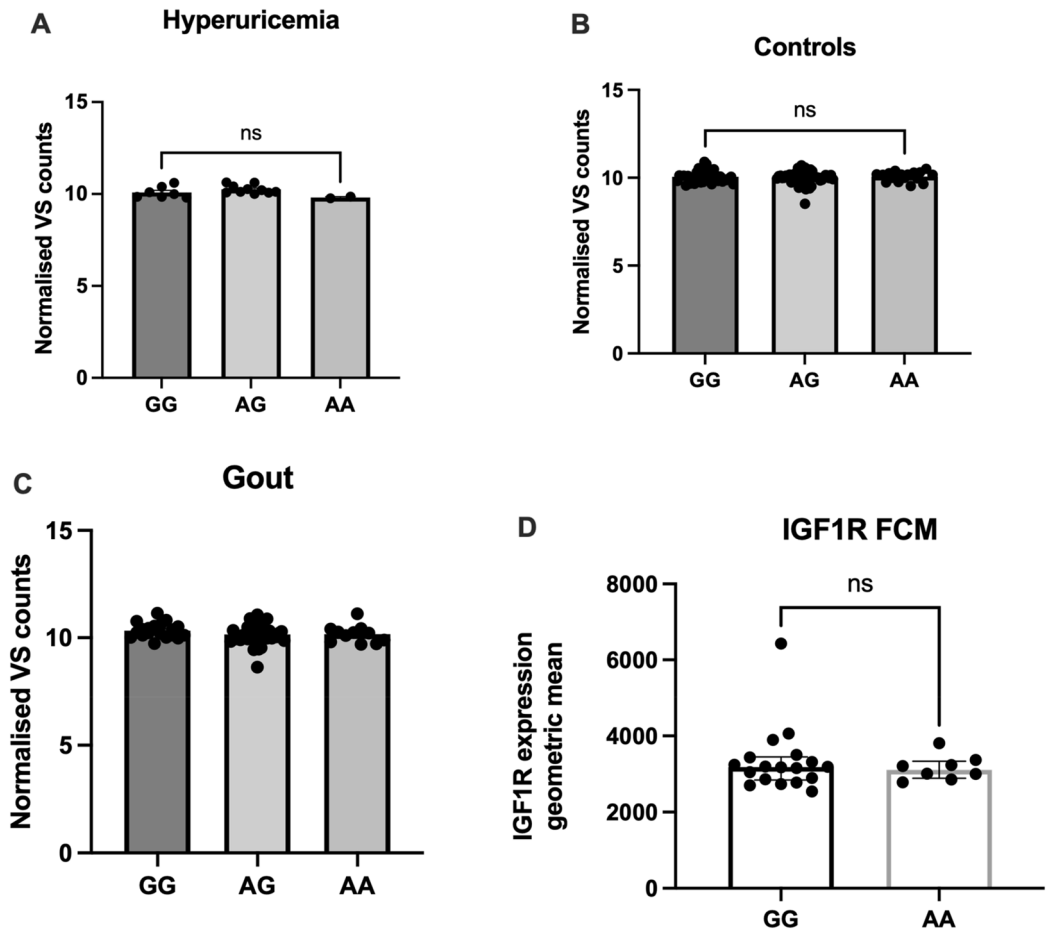


Figure 3. Corellation of the GWAS SNP rs6598541 with IGF1R expression levels. mRNA expression of freshly isolated PBMCs originating from (A) hyperuricemic controls (n = 19) (B) normouricemic controls (n = 105) and (C) gout patients (n = 68). The data is represented as normalized counts. (D) IGF1R protein surface expression measurement by flow cytometric assay of unstimulated PBMCs originating from healthy donors (n = 26). Brown-Forsythe and Welch ANOVA, * $p < 0.05$.

IGF1R locus also colocalizes with genetic control of *IGF1R* expression data²³. For *IGF1R* rs6598541, the gout risk and elevated serum urate associated allele A is associated with lower *IGF1R* expression in heart tissue (left ventricle)³⁹. In our study, when assessing freshly isolated PBMCs from patients with gout or controls we did not observe association of *IGF1R* gene expression with rs6598541. In addition, IGF1R surface expression evaluated by flow cytometry in monocytes of healthy donors also did not show modified IGF1R expression associated with rs6598541 genotype. In line with this, ex vivo cytokine secretion by freshly isolated PBMCs challenged with certain stimuli was also not associated with rs6598541. This indicates that this SNP may exert relevant functional roles in gouty inflammation, but they may be tissue specific and mononuclear cells do not show *IGF1R* expression patterns nor inflammatory cytokine production capacity in relationship to this SNP.

However, data on the role of IGF-1 on urate control give more mechanistic insight. A genetic variant associated with elevated IGF-1 levels (rs35767) correlated to diminished serum urate levels and higher uricosuria⁴⁰. Exposure of HEK293 human embryonic kidney cells to IGF1 resulted in a dose-dependent increase of secretory urate transporters MRP4, NPT1 and ABCG2 and a simultaneous reduction of *GLUT9* expression at both the mRNA and protein levels⁴⁰. *GLUT9a* is the chief transporter for basolateral exit of reabsorbed urate into blood⁴¹. As reported by Kottgen et al., the urate-associated *IGF1R* rs6598541 variant is also associated to lower fractional excretion of urate, supporting a role of the IGF1-IGF1R axis on urate transport. This is supported by data showing that, in *Xenopus laevis* oocytes expressing human IGF1R and urate transporters, IGF-1 promotes urate uptake via IGF1R³⁹. Therefore *IGF1R* association to gout is most probably exerted via urate control rather than inflammatory mechanisms, via IGF1R, *GLUT9* expression and activation, leading to urate reabsorption⁴².

Another mechanism linking *IGF1R* rs6598541 to urate levels is insulin resistance. *IGF1R* rs6598541 was reported to be genome-wide associated with fasting glucose: people without diabetes carrying the A-allele have increased fasting glucose levels, suggesting an association with insulin resistance^{43,44}. It is known that hyperuricemia might be contributed to the effect of insulin on decreasing renal urate clearance and sodium excretion in individuals^{42,45,46}.

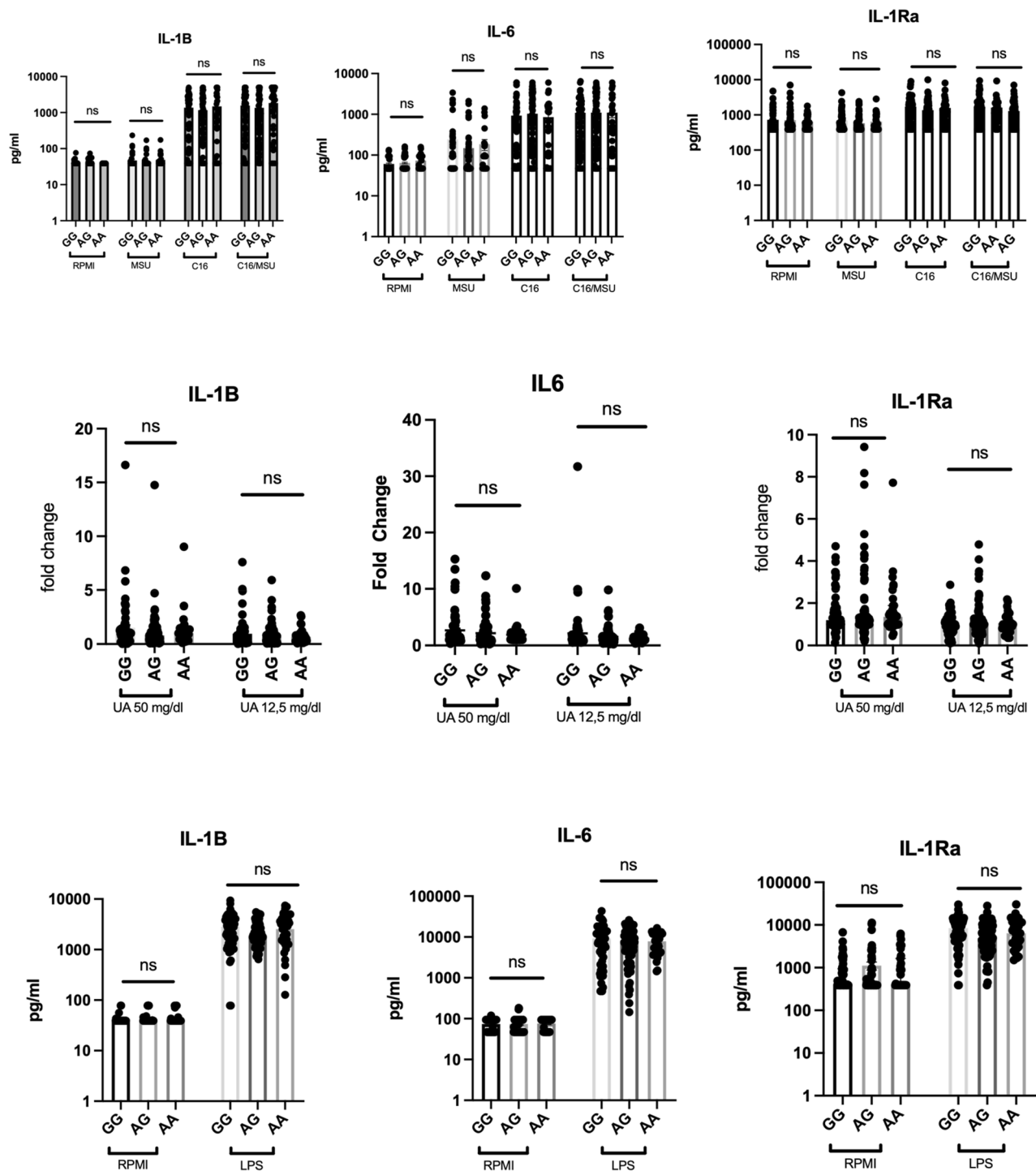


Figure 4. Correlation of the rs6598541 SNP with ex vivo cytokine production. (A) Freshly isolated PBMCs originating from healthy controls ($n = 187$) stimulated with RPMI, MSU 300 mg/dl, C16, C16/MSU 300 mg/dl for 24 h. After 24 h the supernatants were collected and IL-1 β , IL-6 and IL-1Ra (R&D Systems, Minneapolis) was measured. (B) Concentration IL-1 β , IL-6 and IL-1Ra measured in the supernatants of PBMCs after stimulation with uric acid of conc. 50 mg/dl and 12.5 mg/dl for 24 h, followed by restimulation with LPS 10 ng/ml. (C) IL-1 β , IL-6 and IL-1Ra levels measured in the supernatants of PBMCs after stimulation with LPS 100 ng for 24 h. Graphs depict means \pm SEM.

In conclusion, our data do not support a role of IGF-1/IGF1R signaling in soluble urate-induced inflammation in primary PBMCs. Consistent with this, functional data associated to genetic variants in *IGF1R* shows no association with ex vivo cytokine production.

Therefore, these results suggest that a role of IGF1R in gout may be more relevant for the control of urate levels rather than on the inflammatory process.

Data availability

All the datasets generated and analysed during the current study are available in the Supplementary Table 1.

Received: 29 June 2023; Accepted: 29 January 2024

Published online: 12 February 2024

References

- Bardin, T. & Richette, P. The epidemiology and genetic of gout. *Presse Med.* <https://doi.org/10.1016/j.lpm.2011.04.012> (2011).
- Roddy, E., Zhang, W. & Doherty, M. The changing epidemiology of gout. *Nat. Clin. Pract. Rheumatol.* <https://doi.org/10.1038/npcrheum0556> (2007).
- Mattiuzzi, C. & Lippi, G. Recent updates on worldwide gout epidemiology. 10–12 (2019).
- Dalbeth, N. *et al.* Gout. *Nat. Rev. Dis. Prim.* **5**, 25 (2019).
- Renaudin, F. *et al.* Gout and pseudo-gout-related crystals promote GLUT1-mediated glycolysis that governs NLRP3 and interleukin-1 β activation on macrophages. *Ann. Rheum. Dis.* **79**, 1506–1514 (2020).
- Huang, Q. *et al.* HSP60 regulates monosodium urate crystal-induced inflammation by activating the TLR4-NF- κ B-MyD88 signaling pathway and disrupting mitochondrial function. *Oxid. Med. Longev.* **20**, 20 (2020).
- Vazirpanah, N. *et al.* MTOR inhibition by metformin impacts monosodium urate crystal-induced inflammation and cell death in gout: A prelude to a new add-on therapy?. *Ann. Rheum. Dis.* **78**, 663–671 (2019).
- Crisan, T. O. *et al.* Soluble uric acid primes TLR-induced proinflammatory cytokine production by human primary cells via inhibition of IL-1Ra. *Ann. Rheum. Dis.* <https://doi.org/10.1136/annrheumdis-2014-206564> (2016).
- Sah, O. S. P. & Qing, Y. X. Associations between hyperuricemia and chronic kidney disease: A review. *Nephrourol. Mon.* **7**, 25 (2015).
- Stewart, D. J., Langlois, V. & Noone, D. Hyperuricemia and hypertension: Links and risks. *Integr. Blood Press. Control* **12**, 43–62 (2019).
- Badii, M. *et al.* Urate-induced epigenetic modifications in myeloid cells. *Arthritis Res. Ther.* **23**, 1–11 (2021).
- Cabău, G., Crișan, T. O., Klück, V., Popp, R. A. & Joosten, L. A. B. Urate-induced immune programming: Consequences for gouty arthritis and hyperuricemia. *Immunol. Rev.* **294**, 92–105 (2020).
- Badii, M., Gaal, O., Popp, R. A., Crișan, T. O. & Joosten, L. A. B. Trained immunity and inflammation in rheumatic diseases. *Jt. Bone Spine* **89**, 25 (2022).
- Joosten, L. A. B., Crișan, T. O., Bjornstad, P. & Johnson, R. J. Asymptomatic hyperuricaemia: A silent activator of the innate immune system. *Nat. Rev. Rheumatol.* **16**, 75–86 (2020).
- Ge, R. T. *et al.* Insulin-like growth factor-1 endues monocytes with immune suppressive ability to inhibit inflammation in the intestine. *Sci. Rep.* **5**, 1–7 (2015).
- Gow, D. J., Sester, D. P. & Hume, D. A. CSF-1, IGF-1, and the control of postnatal growth and development. *J. Leukoc. Biol.* **88**, 475–481 (2010).
- Schalkwijk, J., Joosten, L. A. B., Van Den Berg, W. B., Van Wyk, J. J. & Van Putte, L. A. D. Insulin-like growth factor stimulation of chondrocyte proteoglycan synthesis by human synovial fluid. *Arthritis Rheum.* **32**, 66–71 (1989).
- Rosenzweig, S. A., Pharmacology, M. & Therapeutics, E. The continuing evolution of insulin-like growth factor signaling. *F1000Research* **9**, 1–10 (2020).
- Kineman, R. D., del Rio-Moreno, M. & Sarmiento-Cabral, A. 40 years of IGF1: Understanding the tissue-specific roles of IGF1/IGF1R in regulating metabolism using the Cre/loxP system. *J. Mol. Endocrinol.* <https://doi.org/10.1530/JME-18-0076> (2018).
- Xu, Y. *et al.* How ligand binds to the type 1 insulin-like growth factor receptor. *Nat. Commun.* **9**, (2018).
- Köttgen, A. *et al.* Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat. Genet.* **45**, 145–154 (2013).
- Tin, A. *et al.* Target genes, variants, tissues and transcriptional pathways influencing human serum urate levels. *Nat. Genet.* <https://doi.org/10.1038/s41588-019-0504-x> (2019).
- Boocock, J. *et al.* Genomic dissection of 43 serum urate-associated loci provides multiple insights into molecular mechanisms of urate control. *Hum. Mol. Genet.* <https://doi.org/10.1093/hmg/ddaa013> (2020).
- Phipps-Green, A. J. *et al.* Twenty-eight loci that influence serum urate levels: Analysis of association with gout. *Ann. Rheum. Dis.* **75**, 124–130 (2016).
- Bekkering, S. *et al.* Metabolic induction of trained immunity through the mevalonate pathway. *Cell* **172**, 135–146.e9 (2018).
- Wolters, T. L. C., Netea, M. G., Hermus, A. R. M. M., Smit, J. W. A. & Netea-Maier, R. T. IGF1 potentiates the pro-inflammatory response in human peripheral blood mononuclear cells via MAPK. *J. Mol. Endocrinol.* <https://doi.org/10.1530/JME-17-0062> (2017).
- Furundzija, V. *et al.* IGF-1 increases macrophage motility via PKC/p38-dependent α v β 3-integrin inside-out signaling. *Biochem. Biophys. Res. Commun.* **394**, 786–791 (2010).
- Meital, L. T. *et al.* A simple and effective method for the isolation and culture of human monocytes from small volumes of peripheral blood. *J. Immunol. Methods* **472**, 75–78 (2019).
- Bekkering, S. *et al.* In Vitro experimental model of trained innate immunity in human primary monocytes. *Clin. Vaccine Immunol.* **23**, 926–933 (2016).
- Domínguez-Andrés, J. *et al.* In vitro induction of trained immunity in adherent human monocytes. *STAR Protoc.* **2**, 25 (2021).
- Bekkering, S. *et al.* Metabolic induction of trained immunity through the mevalonate pathway. *Cell* <https://doi.org/10.1016/j.cell.2017.11.025> (2018).
- Higashi, Y. *et al.* Interaction between insulin-like growth factor-1 and atherosclerosis and vascular aging. *Cardiovasc. Issues Endocrinol.* <https://doi.org/10.1159/000360571> (2014).
- Nederlof, R. *et al.* Insulin-like growth factor 1 attenuates the pro-inflammatory phenotype of neutrophils in myocardial infarction. *Front. Immunol.* **13**, 1–16 (2022).
- Bluthé, R. M., Kelley, K. W. & Dantzer, R. Effects of insulin-like growth factor-I on cytokine-induced sickness behavior in mice. *Brain. Behav. Immun.* **20**, 57–63 (2006).
- Palin, K. *et al.* TNF α -induced sickness behavior in mice with functional 55 kD TNF receptors is blocked by central IGF-I. *J. Neuroimmunol.* **187**, 55–60 (2007).
- Ueland, T. *et al.* Associations between body composition, circulating interleukin-1 receptor antagonist, osteocalcin, and insulin metabolism in active acromegaly. *J. Clin. Endocrinol. Metab.* **95**, 361–368 (2010).
- Erlandsson, M. C. *et al.* IGF-1R signalling contributes to IL-6 production and T cell dependent inflammation in rheumatoid arthritis. *Biochim. Biophys. Acta Mol. Basis Dis.* <https://doi.org/10.1016/j.bbadis.2017.06.002> (2017).
- Kushnarenko, N., Medvedeva, T., Govorin, A. & Kushnarenko, K. Significance of growth factors (IGF-1, FGF-b, PDGF-AA), pro-inflammatory cytokines and cytokine soluble receptors in gouty patients with insulin resistance. *Atherosclerosis* **263**, e111 (2017).

39. Mandal, A. K. *et al.* Genetic and physiological effects of insulin-like growth factor-1 (IGF-1) on human urate homeostasis. *J. Am. Soc. Nephrol.* <https://doi.org/10.1681/ASN.0000000000000054> (2023).
40. Mannino, G. C. *et al.* The polymorphism rs35767 at IGF1 locus is associated with serum urate levels. *Sci. Rep.* **8**, 1–9 (2018).
41. Caulfield, M. J. *et al.* SLC2A9 is a high-capacity urate transporter in humans. *PLoS Med.* **5**, 1509–1523 (2008).
42. Mandal, A. K. *et al.* Genetic and physiological effects of insulin on human urate homeostasis. *Front. Physiol.* **12**, 1–17 (2021).
43. Lagou, V. *et al.* Sex-dimorphic genetic effects and novel loci for fasting glucose and insulin variability. *Nat. Commun.* **12**, 1–18 (2021).
44. Chen, J. *et al.* The trans-ancestral genomic architecture of glycemic traits. *Nat. Genet.* **53**, 840–860 (2021).
45. Ter Maaten, J. C. *et al.* Renal handling of urate and sodium during acute physiological hyperinsulinaemia in healthy subjects. *Clin. Sci.* **92**, 51–58 (1997).
46. McCormick, N. *et al.* Assessing the causal relationships between insulin resistance and hyperuricemia and gout using bidirectional mendelian randomization. *Arthritis. Rheumatol.* **73**, 2096–2104 (2021).

Acknowledgements

This work was supported by a Competitiveness Operational Programme grant of the Romanian Ministry of European Funds (P_37_762, MySMIS 103587). I.O.Gaal was supported by a Grant for doctoral research projects (2462/22) of the University of Medicine and Pharmacy “Iuliu Hațieganu”, Cluj-Napoca, Romania.

Author contributions

Author contributions O.I.G. conceived and performed experiments, analyzed the data and wrote the manuscript—original draft. T.O.C., L.A.B., T.R.M. conceived experiments, wrote the manuscript—review and editing. T.O.C., L.A.B. and secured funding. Investigation and resources: R.L., D.M., M.B, G.C., D.C., V.N., I.H., C.P., S.R., R.A.P. coordinate patient recruitment and sample acquisition. All authors contributed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-53209-7>.

Correspondence and requests for materials should be addressed to T.O.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024

HINT Consortium

Leo A. B. Joosten¹, Ioan V. Pop¹, Radu A. Popp¹, Simona Rednic³, Cristina Pamfil³, Tania O. Crișan¹, Marius Farcaș¹, Dragoș H. Marginean¹, Orsolya I. Gaal^{1,2}, Medeea O. Badii¹, Ioana Hotea³, Loredana Peca¹, Andreea-Manuela Mirea¹, Georgiana Cabău¹, Valentin Nica¹, Doina Colcear⁶, Mariana S. Pop¹ & Ancuta Rus¹