Functions of 1,25-dihydroxy vitamin D3,vitamin D3 receptor and interleukin-22 involved in pathogenesis of gout arthritis through altering metabolic pattern and inflammatory responses

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Background: Gouty arthritis (GA) is a common type of inflammatory arthritis. Recent studies demonstrated that 1,25-dihydroxy vitamin D3 (1,25(OH) 2 VD3) and vitamin D3 receptor (VD-R) play a protective role in acute inflammation, but interleukin-22(IL-22) promotes inflammation, especially for arthritis. However, our understanding of the responses of 1,25(OH) 2VD3 and IL-22 to gout was still unclear. Presently, in-depth metabolomics, bioinformatics and clinical characteristics analyses were performed to elucidate the pathogenesis and valuable clinical indicators of gouty arthritis. **Methods:** Peripheral venous blood was taken for investigation. The levels of IL-22 and $1,25(OH)_2VD3$ were determined in patient's plasma via ELISA, and the mRNA levels of IL-22 and VD-R were measured via qRT-PCR. The interaction network of VD-R and IL22 were constructed by Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), and the biological function of the related proteins were analyzed by Clusterprofiler Metabolomics were performed to decipher the metabolic variations of GA. **Results:** The levels of VD-R and 1,25(OH) 2 VD3 were identified to be low. What's more, GA patients were reported to have high expression of IL-22. And IL-22 levels positively correlated with C-reactiveprotein (CRP) serum levels in the bivariate correlation analysis, whereas the level of 1,25(OH) 2VD3 negatively correlated with that of CRP. GO and KEGG analyses revealed that IL-22 and 1,25(OH) 2 VD3 were involved in stress immunity and inflammatory responses. These pathways are known to play a role in GA pathogenesis. Meanwhile, the metabolic profiles of GA serum showed that the increase in various amino acids and uric acid are involved in GA pathogenesis. Importantly, VD-R and IL22 closely correlated with the level of key metabolites uric acid, whose increase promoted the occurrence of GA. **Conclusion:** IL-22,

VD-R and 1,25(OH) 2 VD3 play functionally important roles in inflammatory responses and are relevant to gout pathogenesis.

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15   **Key words**: gouty arthritis; uric acid; interleukin-22; 1,25-dihydroxy vitamin D3; vitamin D3 receptor

18 **Abstract**

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21 acute inflammation, but interleukin-22(IL-22) promotes inflammation, especially for arthritis. However, our

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24 pathogenesis and valuable clinical indicators of gouty arthritis.

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26 determined in patient's plasma via ELISA, and the mRNA levels of IL-22 and VD-R were measured via qRT-

27 PCR. The interaction network of VD-R and IL22 were constructed by Search Tool for the Retrieval of

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31 reported to have high expression of IL-22. And IL-22 levels positively correlated with C-reactive protein (CRP)

32 serum levels in the bivariate correlation analysis, whereas the level of 1,25(OH) 2VD3 negatively correlated

33 with that of CRP. GO and KEGG analyses revealed that IL-22 and 1,25(OH) 2 VD3 were involved in stress

34 immunity and inflammatory responses. These pathways are known to play a role in GA pathogenesis.

35 Meanwhile, the metabolic profiles of GA serum showed that the increase in various amino acids and uric acid

36 are involved in GA pathogenesis. Importantly, VD-R and IL22 closely correlated with the level of key

37 metabolites uric acid, whose increase promoted the occurrence of GA.

38   **Conclusion:**IL-22, VD-R and 1,25(OH) 2 VD3 play functionally important roles in inflammatory responses

39   and are relevant to gout pathogenesis.

40   **1. Introduction**

41   Gout is a major public health problem impacting human health(Stack et al. 2019). Currently, the

42   prevalence of gout was higher ranging from 1-4%, and patients with gout suffer from dramatic joint

43   inflammation and excruciating arthritic pain, which profoundly reduced the patients' life quality(Singh &

44   Gaffo 2020); (Yin et al. 2020). Meanwhile, comorbidities such as hypertension, stroke, and heart disease,

45   chronic kidney disease are common in people with gout and complicate its management and disease

46   outcomes (Kuwabara et al. 2017). It is a complex disorder caused by the deposition of monosodium urate

47   (MSU) crystals in joints and soft tissues(Zhu et al. 2018). MSU stimulates the activation of the innate immune

48   system and subsequently releases an array of inflammatory mediators(Nakayama 2018; Yin et al. 2020). These

49   inflammatory factors trigger a strong inflammatory response in joints and periarticular tissues (Richette &

50   Bardin 2010). However, the exact pathogenesis of gout is yet to be elucidated.

51   Elevated levels of specific pro-inflammatory cytokines are known to trigger highly reactive inflammatory

52   infections. Intriguingly, 1,25(OH) 2 VD3 can inhibit inflammation and is commonly found in a range of

53   immune cells. This suggests it plays a key role in the immune system(Ho et al. 2020). Adding to this

54   hypothesis, Wen H indicated that 1,25(OH) 2 VD3 may play as an immune modulating agent to suppress Th17

55   cell cytokines, such as IL-22(Wen et al. 2015). In this study,we hypothesize that IL-22, is correlated with the

56   occurrence and development of gout. We also hypothesized that 1,25(OH) 2 VD3 plays an anti-inflammatory

57   role in gouty arthritis and can even suppress episode of gout.

58   This study investigated changes in the serum levels of 1,25(OH) 2 VD3 and IL-22 in the peripheral blood

59 of gout arthritis patients. Correlations between serum levels and sedimentation rates of CRP, serum uric acid,

60 creatinine, triglycerides, cholesterol and platelets with 1,25(OH) 2 VD3, and IL-22 were subsequently

61 investigated. Finally, the biological functions of 1,25(OH) 2 VD3 and IL-22 in gout pathogenesis were further

62 explored via bioinformatics analysis, and the detailed variations in metabolic pattern of GA were displayed by

63 metabolomics profiles. These findings provide valuable insight into the mechanisms underpinning gout

64 pathogenesis and provide more ideas for the treatment of gouty arthritis.

65

66 **2. Methods**

67 *2.1. Gout patients recruitment*

68 A total of 58 gout outpatients were recruited from the Rheumatism and Immunology Department

69 of the People's Hospital of Suzhou New District, China. These patients were recruited between

70 March 2019 and September 2019. To be included in the study, patients had to fit into the criteria

71 defined by the American Rheumatism Association (now named the American College of

72 Rheumatology (ACR)) in 1977(Wallace et al. 1977) and the ACR/European League Against

73 Rheumatism (EULAR) in 2015(Neogi et al. 2015). Patients were categorized into two groups:

74 acute (in the last week, with joint swelling and pain, and increased white blood cell or

75 erythrocyte sedimentation rate, n = 30) and non-acute (No joint swelling and pain occurred in the

76 last month, acute joint swelling and pain occurred in the past, and no colchicine, prednisone,

77 non-steroidal anti-inflammatory drugs and urate-lowering therapy was given, n = 28) GA.

78 Patients with secondary gout as a result of drugs, chemoradiotherapy, malignant tumor,

79 endocrine and kidney diseases were excluded from the study. Twenty healthy physical

80 examining persons were recruited as healthy controls who were lacking hyperuricemia and

81 metabolic syndromes as well as other chronic diseases. Participants provided fasting morning

82 venous blood samples and the specific operation was completed by nurses. Participants were

83 matched for age and sex. Written informed consent was obtained from all patients at the time of

84 admission. The People's Hospital of Suzhou New District granted Ethical approval to carry out

85 the study within its facilities (Ethical Application Ref: 2018-006).

86 *2.2. Laboratory indexes*

87 Laboratory indexes were determined in the laboratory of Suzhou High-Tech Zone at the People's Hospital.

88 Blood cell analysis was performed with the blood cell analyzer LH750 (Beckman, US). Analyses including

89 erythrocyte sedimentation rate (ESR), CRP, uric acid, urea, and creatinine were performed with a DxC800

90 automatic biochemical analyzer (Beckman, US).

91

92 *2.3. Quantitative PCR (qPCR)*

93 Whole peripheral blood samples were used for total cellular RNA isolation. Total RNA was extracted

94 using a standard TRIZOL method following Qin et al(Qin et al. 2013) and cDNA was generated using the

95 PrimeScript RT Reagent kit (Takara). IL-22 and VD-R expression was quantified via qPCR using a

96 LightCycler 480 and SYBR Green I Master Mix (Roche). Expression levels were normalized

97 to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Water was used as a negative control lacking

98 template. A standard curve was generated for each assay. Amplification efficiency was calculated based on the

99 mRNA standard curve. Assays were performed in duplicate. The average used in statistical analyses.

100

101   *2.4. Enzyme-linked immunosorbent assay (ELISA)*

102   Peripheral blood was collected in heparin anticoagulant tubes. Plasma was obtained via centrifugation at

103   3000 RPM for 15 minutes. Prior to examination, plasma was stored at – 20 °C. Plasma levels of IL-22 and

104   1,25(OH)2VD3 were determined using a quantitative sandwich technique, for which ELISA kits (eBioscience,

105   US) were used according to the manufacturer's instructions.

106

107   *2.5. Enrichment and protein-protein interaction network analysis:*

108   Gene ontology (GO) enrichment analysis of differentially expressed genes was performed using the

109   ClusterProfiler R package. GO terms with a corrected $p > 0.05$ were considered enriched by differentially

110   expressed genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was subsequently used.

111   This database contains a wealth of understanding of molecular-level information, including large-scale

112   molecular datasets generated via genome sequencing and other high throughput technologies

113   (http://www.genome.jp/kegg/)

114

115   *2.6. Metabolomics analysis:*

116   Detailed steps of metabolomics analysis are as follows: 50 ± 1ml sample from GA patients (N=3) and

117   healthy control (N=3) were extracted with pre-cold extraction mixture (methanol/chloroform (v:v) =3:1) with

118   10 μL internal standard (adonitol), and samples were extracted with ultrasonication for 5 min in ice water.

119   After evaporation in a vacuum concentrator, 40 μL of Methoxyamination hydrochloride (20 mg/mL in pyridine)

120   was added and then incubated at 80 ℃, then derivatized by 60 μL of BSTFA regent (1 % TMCS, v/v) at 70 ℃

121   for 1.5 h. All samples were then analyzed by gas chromatograph using an Agilent 7890 with a time-of-flight

122   mass spectrometer (GC-TOF-MS) and DB-5MS capillary column. 1 μL aliquot of sample was injected in

123   splitless mode. Helium was used as the carrier gas with 1 mL min^{-1} flow rate. The energy was -70 eV in

124   electron impact mode. The mass spectrometry data were acquired in full-scan mode with the m/z range of 50-

125   500 at a rate of 12.5 spectra per second.

126   *2.7 Raw Data Preprocessing.*

127   Raw data analysis was finished with Chroma TOF (V 4.3x,LECO) software and LECO-Fiehn Rtx5

128   database was used for metabolite identification with an in-house database. The noise and deviation values were

129   removed by filtering individual peaks and the interquartile range, respectively. Only the peak area data with a

130   hollow value not more than 50% in all groups were retained, then the missing value was simulated by 1/2 of

131   the minimum value. And the data were normalized by an internal standard (IS). The data variation and

132   enrichment analyses were performed by XCMS software and R packages Metaboanalyst 3.0. And the therapy

133   P < 0.05 and FC >2 were used for identification of significantly differential metabolites.

134

135   *2.8. Statistical analysis*

136   Statistical analyses were performed using SPSS Statistics Version 21 (IBM). The groups were tested

137   using two-sided *t* test or ANOVA for continuous variables and the Chi-squared statistic for categorical

138   variables. A two-sided *p* < 0.05 was used to denote statistical significance. Baseline characteristics were

139   presented as mean (with an associated standard deviation [SD]) for continuous variables. For dichotomous

140   variables, proportions (%) were used. Mann-Whitney U tests were used for non-normally distributed variables.

141   A Chi-square test or Fisher's exact test were used for categorical variables. Pearson or Spearman's rank

142   correlation tests were used depending on the data distribution.

143

3. Results

3.1. Patient characteristics

146   The clinical characteristics of recruited gout patients were documented and analyzed. These

147   characteristics included age, body mass index (BMI), uric acid and creatinine levels, platelet levels,

148   erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, cholesterin levels, triglyceride

149   levels, blood glucose levels, and tophi deposits. Other comorbidities were also documented. A summary of

150   the patient characteristics is shown in Table 1. In total, the study recruited 30 AGA patients, 28 NAGA

151   patients, and 20 healthy controls. Participants were age- and sex-matched. It's important to note that the

152   BMIs of patients in the AGA and NAGA groups were significantly higher than those in HCs ($p < 0.05$).

153   This supports the notion that obesity may be a risk factor for gout. Besides, Creatinine levels are higher in

154   patients with gout than in normal subjects, reflecting the fact that gout can impair kidney function. What's

155   more, UA, blood glucose, and ESR were all significantly higher in AG patients than in HC patients. In

156   addition, in AGA patients, CRP levels were found to be highly upregulated in comparison to the NAGA

157   and HC groups. There were no differences in the disease duration, hypertension rates, and diabetes rates in

158   the NAGA patients vs. the AGA patients ($p > 0.05$) (Table 1).

159   AGA: acute gouty; BMI: body mass index; CRP: C-reactive protein;

160   ESR: erythrocyte sedimentation rate; HC: healthy controls; NAGA: non-acute gouty arthritis; UA: uric acid;

161   * $p < 0.05$, between AGA patients vs. HC;# $p < 0.05$,between NAGA patients vs. HC;△ $p < 0.05$,

162   between NAGA patients vs. AGA patients;

3.2. IL-22, VD-R and 1,25(OH) $_2$VD3 levels closely correlated with gout pathogenesis

164   IL-22 is a highly important cytokine and performs various anti-inflammatory and pro-inflammatory

165   functions *in vivo*. In this study, qRT-PCR was performed to investigate the expression levels of IL-22. In

166   comparison to the HC group, IL-22 expression was highly upregulated in the AGA and NAGA groups. On

167   another note, as the receptor for vitamin D, VD-R plays a key role in regulation of the immune system

168   (Medrano et al. 2018). Remarkably, this study reported that VD-R expression levels were dramatically

169   downregulated in the AGA and NAGA groups (Figure1). Subsequent, ELISA analysis and qRT-PCR

170   experimentation revealed a consensus expression level of IL-22 and VD3. What's more, ELISA indicated that

171   IL-22 protein expression was significantly upregulated in both the AGA and NAGA groups. In addition,

172    $1,25(OH)_2VD3$ protein expression was also identified to be significantly decreased in the AGA group by

173   ELISA assay. There was no clear difference between the NAGA and HC group in terms of $1,25(OH)_2VD3$

174   protein expression levels (Figure1).

175   A: Serum 1,25(OH)2VD3 levels in HC, AGA, and NAGA groups (mean ±SD), *p*-value < 0.01; B: Serum IL-

176   22 levels in HC, AGA, and NAGA groups (mean ±SD), *p*-value < 0.01; VD-R(C)and IL-22(D) mRNA levels

177   in serum, Data represent mean ±SD. *p*-value < 0.01;

178

179   ***3.3. Levels of IL-22 and VD-R closely correlated with GA-related metabolites***

180   In order to further investigate the differences between HC and GA patients, untargeted metabolomics was

181   performed. This analysis was performed on the serum from both HC and GA patients. Three replicates were

182   performed for each group. Principal components analysis (PCA) was performed on the metabolic profiles of

183   both the HC and GA patients. This analysis showed that both groups had a distinct profile. This showed that

184   there is measurable variation in metabolic profiles of HC and GA patients (Figure 2C). Furthermore, we

185   constructed a coexpression network on IL22, VDR and metabolites from metabolomics. We found various

186   metabolites involved in Aminoacyl-tRNA biosynthesis, Pantothenate and CoA biosynthesis, Purine

187   metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis, Arginine biosynthesis, Valine, leucine and

188   isoleucine biosynthesis, beta-Alanine metabolism and Phenylalanine metabolism were closely correlated with

189   IL22 and VDR (Figure 2A; Table 2). We noted that six metabolites including 3-Hydroxyanthranilic acid,

190   Palatinose, sulfuric acid, serine, 2,6-Diaminopimelic acid, Phytol and uric acid were positively correlated with

191   IL22, whereas their correlation with VDR were negative (Figure 2A). The level of these metabolites were

192   higher in GA patients than that in HC (Figure 2B). Of note, metabolic alterations performed various functions

193   in the pathogenesis of GA(Hoque et al. 2020). Subsequently, 446 metabolites were identified in the serum. Out

194   of these metabolites, the levels of 147 metabolites were highly increased in serum of GA patients. The levels

195   of 33 metabolites were decreased in the serum of GA patients (Figure 2D). Subsequent pathway enrichment

196   analysis, using the KEGG database, was performed on the 147 metabolites upregulated in GA patients. This

197   analysis identified 45 pathways, 9 pathways of which were significantly enriched (Figure 2E). This included

198   the following pathways: aminoacyl-tRNA biosynthesis; arginine biosynthesis; pantothenate and CoA

199   biosynthesis; alanine, aspartate and glutamate metabolism; phenylalanine, tyrosine and tryptophan biosynthesis;

200   glycine, serine and threonine metabolism; galactose metabolism; glutathione metabolism and valine; leucine

201   and isoleucine biosynthesis, revealing the influence of these metabolic alterations which correlated with IL22

202   and VDR in the pathogenesis of GA. In this study, a variety of amino acid-related pathways were significantly

203   activated in GA patients. This indicates that a high level of amino acids are involved in GA pathogenesis.

204   Notably, research has shown that elevated levels of proteinuria is indicative of arthritis(Luo et al. 2018;

205   Teumer et al. 2019). In agreement, this study identified that serum uric acid levels of GA patients were 2.8-

206   fold higher than that in the HC group (Figure 2B). Uric acid is known for its inductive function in pathogenesis

207   of GA(Huang et al. 2020). Remarkably, levels of IL-22 and VD-R closely correlated with the uric acid levels

208   in the serum of both HC and GA patients. Thus, We concluded that IL22 and VD-R were closely correlated

209   with the GA-related metabolic alterations, supporting their functions in the pathogenesis of GA.

210   **A:** Coexpression network between IL22, VDR and metabolic signature; Negative and positive relationship

211   were shown represented by red and blue line, respectively; **B:** Relative level of metabolites which were closely

212   associated with both IL22 and VDR; **C:** PCA plots of the metabolomics patterns of the GA and HC groups**; D:**

213   Volcano plots displayed differential metabolites in the metabolomics data of the GA and HC groups**; E:**

214   Pathway enrichment analysis of the upregulated metabolites of GA patients.

215

216   *3.4. IL-22 and 1,25 (OH) $_2$VD3 levels in serum closely correlated with CRP levels*

217   As shown in Figure 3, the level of IL-22 in the serum positively correlated with CRP levels (r = 0.32, *p* <

218   0.05). In addition, 1,25(OH)$_2$VD3 levels negatively correlated with CRP levels (r = -0.40, *p* < 0.01) (Figure 3).

219   CRP is a non-specific inflammatory marker. Interestingly, IL-22 levels in the serum positively correlated with

220   CRP levels. However, the serum levels of 1,25(OH)$_2$D3 negatively correlated with the serum CRP levels.

221   Correlation analysis was performed to confirm the correlations between the levels of IL-22, 1,25(OH)$_2$D3 and

222   other measurements such as platelets, ESR. No significant correlations were identified between these

223   indicators. Similarly, the correlation between CRP and IL-22 and VD3 are not similar for all 3 groups of

224   participants. These findings suggested that 1,25(OH)$_2$VD3 and IL-22 are associated with inflammatory

225   expression in gout arthritis.

226

227 **3.5. IL-22 and VD-R are imperative in the inflammatory response network**

228   In order to further investigate the function of IL-22 and VD-R in the pathogenesis of gout, a complex

229 protein-protein interaction network was constructed using Search Tool for the STRING. The created network

230 showed that various genes were involved in inflammatory response. These genes included STA3, MED25,

231 MED22, STAT1, CREB1, HIF1, and IL-10 (Figure 4D). These interactions indicated that IL-22 and VD-R

232 could in fact further influence protein functions associated with the inflammatory response. The topology of

233 this complex network was subsequently analyzed. This analysis showed that IL-22 and VD-R were at the core

234 of the network. This suggests important roles for both IL-22 and VD-R in the regulation inflammatory

235 responses (Figure 4D).

236   Furthermore, GO enrichment analysis was performed on the identified regulatory network. This analysis

237 showed that these proteins were involved in various terms associated with the cellular component category.

238 This included various components: transcription factor complex, RNA polymerase II transcription factor

239 complex, protein-containing complex, nucleus, nucleoplasm part, and nuclear transcription factor complex

240 (Figure 4A). For the molecular function category, protein functions were mainly involved in vitamin D

241 receptor binding, transcription regulator activity, transcription factor binding, thyroid hormone receptor

242 binding, and RNA polymerase II-specific DNA-binding transcription factor binding (Figure 4B). It was also

243 noted that various terms associated with biological progress were overrepresented. This included transcription,

244 DNA-templates, transcription initiation from the RNA polymerase II promoter, RNA metabolic process,

245 regulation of transcription, and positive regulation of nucleic acid-templated transcription (Figure 4C). All

246 these terms closely correlated with the transcription activity *in vivo* (Figure 4).

247   Subsequently, pathway enrichment analysis was performed on these target genes using the KEGG

248   database. Pathway enrichment analysis revealed that various pathways associated with inflammatory responses

249   system were significantly enriched. This included: viral carcinogenesis, tuberculosis, rhyroid hormone

250   signaling pathway, prostate cancer, pathways in cancer, JAK/STAT signaling pathway, inflammatory bowel

251   disease (IBD), HIF-1 signaling pathway, herpes simplex infection, hepatitis B, and Epstein-Barr virus infection

252   (Figure 4E). In summary, these results agreed that IL-22 and VD-R play functionally important roles in the

253   inflammatory response and are therefore relevant to gout pathogenesis (Figure 4).

254   A: Cellular Component analysis on the regulatory network of IL-22 and VD-R; B: Molecular Function

255   analysis on the regulatory network of IL-22 and VD-R; C: Biological Progress analysis on the regulatory

256   network of IL-22 and VD-R; D: The regulatory network of IL-22 and VD-R; E:Pathway enrichment analysis

257   on VD-R and IL-22.

258

259   **4. Discussion**

260   Gout arthritis is an auto-inflammatory disease(Salehzadeh et al. 2019). The acute onset of gout is an

261   inflammatory process induced by urate crystals. These crystals manifest as inflammatory arthritis and cause

262   painful disability. Various researchers have proposed that the gout arthritis is closely linked with various

263   inflammatory reactions, including NLRP3, IL-1 and IL-22(Cabau et al. 2020; Luo et al. 2017). However, the

264   role of IL-22 and VD3 in gout pathogenesis is yet to be elucidated. In this study, GO and KEGG pathway

265   analyses identified important roles of both IL-22 and $1,25(OH)_2VD3$ in stress immunity as well as

266   inflammatory responses. These processes are known to be associated with gout arthritis pathogenesis.

267   Remarkably, the levels of IL-22 were shown to be higher in patients with gouty arthritis in either the

268   acute phase or non-acute phase in comparison to healthy controls. What's more, decreased levels of VD-R

269   mRNA and 1,25(OH) $_2$VD3 in both AGA and NAGA groups were verified via ELISA and qRT-PCR.

270   Furthermore, the levels of IL-22 and VD3 correlated with that of CRP in gout patients. Importantly, we found

271   that uric acid highly accumulated in GA patients, and the levels of VD-R and IL22 closely correlated with the

272   level of uric acid, supporting the imperative roles of VD-R and IL22 in the pathogenesis of GA. In summary,

273   this study reported the involvement of IL-22 and VD3 in inflammation associated with gout pathogenesis.

274   These results suggest a pivotal role of IL-22 and VD3 in mediating gout inflammation. These findings provide

275   a novel treatment strategy for gout.

276   IL-22 is the source of increasing attention in a wide range of inflammatory and autoimmune diseases due

277   to its specific function *in vivo*. Remarkably, this study showed that mRNA expression levels of IL-22 were

278   higher in the acute and remission phases of gout. This indicates that IL-22 was involved in the pathogenesis of

279   gouty arthritis. Previous research has proven the role of IL-22 in the regulation of inflammatory responses

280   associated with inflammatory diseases by binding to the IL-22 receptor. This receptor is transmembrane in

281   nature, and is composed of two subunits (IL-22R1 and IL-10R2)(Lu et al. 2015; Perusina Lanfranca et al.

282   2016). Notably, IL-22 is characterized as a two-faced cytokine. This means it performs not only deleterious

283   roles but also protective roles(Huan et al. 2016). It is also an important driver of autoimmune diseases and is

284   involved in the pathogenesis of arthritis(Yang & Zheng 2014). A range of studies have shown that patients

285   with rheumatoid arthritis and psoriatic arthritis frequently have abnormal IL-22 expression(Ezeonyeji et al.

286   2017; Miyazaki et al. 2018). IL-22 promoted inflammatory response and osteoclast formation in rheumatoid

287   arthritis via the promotion of fibroblast-like synoviocytes proliferation and monocyte chemoattractant protein

288   (MCP) expression(Li et al. 2019). Marijnissen et al. reported IL-22 and IL-22 receptor levels to be increased in

289   synovial tissue during inflammation(Marijnissen et al. 2011). On another note, IL-22 promoted the expression

290   of IFN-γ, thereby inhibiting intestinal coronavirus replication(Xue et al. 2017). IL-22 and IFN-γ have been

291   shown to suppress pulmonary inflammation in pneumonia caused by *Pseudomonas aeruginosa* (Broquet et al.

292   2020). The therapeutic benefits of IL-22 pathway activation have been demonstrated in experimental

293   colitis(Lamas et al. 2016). In this study, levels of IL-22 in plasma were increased in both acute and chronic

294   gout patients. These levels correlated positively with CRP levels. This suggests the imperative role of IL-22 in

295   the pathogenesis of gout arthritis.

296   As an active vitamin D *in vivo*, $1,25(OH)_2VD3$ plays role in maintaining the balance of calcium and

297   phosphorus various biological processes by binding to VD-R. Detailed studies have shown that $1,25(OH)_2VD3$

298   plays an important role in immune regulation, anti-inflammation, and anti-infection(Holick 2014). In this study,

299   we reported that the levels of VD-R mRNA and protein were significantly decreased in both the AGA and

300   NAGA group. This suggests that VD-R may play a negative regulatory role in the inflammatory process of

301   gout arthritis. The decrease in $1,25(OH)_2VD3$ levels in serum are potentially caused by high uric acid

302   levels(Chen et al. 2014). A study has described that $1,25(OH)_2VD3$ effectively inhibited Th17 cell

303   differentiation *in vitro* and regulated the immune response of T cells. The study reported that $1,25(OH)_2VD3$

304   treatment could cause decreases in the levels of IL-17A, IL-22 and IFN-γ(Gu et al. 2017). What's more,

305     $1,25(OH)_2VD3$ promoted the formation of STAT1-VD-R complexes in monocytes. Interestingly,

306     $1,25(OH)_2VD3$ significantly reduced the transcriptional activity of VD-R, but enhanced the transcription of

307   STAT1. Thus, it can be hypothesized that correlations between the VD-R and STAT signaling pathways exist,

308   and that the anti-inflammatory effects of $1,25(OH)_2VD3$ may be related to the effects of vitamin D on the

309   JAK/STAT pathway. Another study found that $1,25(OH)_2VD3$ can in fact control the expression of VD-R,

310   signal transducers, and transcriptional activator 5 (STAT5) via the regulation of signal transduction

311   pathways(Yang et al. 2015). KEGG pathway analyses showed that VD-R and IL-22 mRNA were mainly

312   concentrated in multiple signal transduction pathways such as the JAK/STAT signaling pathway in

313   tuberculosis , prostate cancer, and inflammatory bowel disease. VD-R can combine with STAT3 to inhibit the

314   JAK/STAT signaling transduction pathway(Zhang et al. 2020). Research has also proven that $1,25(OH)_2 D3$

315   can be expressed via the promotion of STAT5 and its phosphorylation(Gu et al. 2017). This can in turn

316   promote Th17 cells specific cytokine IL-17 as well as the expression and secretion of IL-22. Andoh et al.

317   reported that IL-22 raised IL-6, IL-8, IL-11, and other inflammatory mediators and chemokine expression by

318   activating SEMF nf-kappa B cells, JAK-2/STAT and AP-1-3 signaling pathways(Andoh et al. 2005). Thus

319   exerting a pro-inflammatory effect.

320   As a non-specific inflammatory marker, CRP can bind to a variety of eukaryotic and prokaryotic

321   pathogens, facilitating complement activation through the classical pathway(Pepys & Hirschfield 2003). This

322   leads to immune activation, lymphocyte infiltration, immune molecules consumption, and widespread

323   inflammation. Levels of IL-22 positively correlated with serum CRP levels, which confirmed that IL-22 was

324   involved in the inflammatory response in the pathogenesis of gout arthritis. Serum 1,25(OH)2D3 levels

325   negatively correlated with serum CRP levels. This indicated that VD3 played a protective role in gout. In

326   addition, functional enrichment and KEGG signaling pathway analyses of target genes predicted the molecular

327   regulatory networks related to disease occurrence. In parallel, we detailedly depicted the landscape of

328   metabolic alterations between gout patients and normal human that the levels of various amino acids and uric

329   acid associated with the gout pathogenesis were higher in gout patients. What's more, serum VD-R and IL-22

330   were increased in patients and may be involved in the pathogenesis of gout arthritis via influencing the uric

331   acid level of gout patients that both VD-R and IL-22 closely correlated with the level of uric acid of GA

patients. This goes some way to elucidate the molecular regulatory network controlling gout arthritis

pathogenesis.

However, it is important to note that there are limitations to the interpretation of these results due to the

small sample size. In this study the levels of 1,25(OH)$_2$VD3 and IL-22 were reported to be abnormal in the

serum of patients with gout arthritis, but the specific mechanism underpinning this remain unclear. Therefore,

further studies are required to confirm the mechanisms via which 1,25 (OH)$_2$VD$_3$ affects the secretion of IL-22

in gout.

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469

Figure 1

mRNA and protein levels of IL-22, VD-R and 1,25 (OH)$_2$VD3 in the plasma from gouty arthritis patients

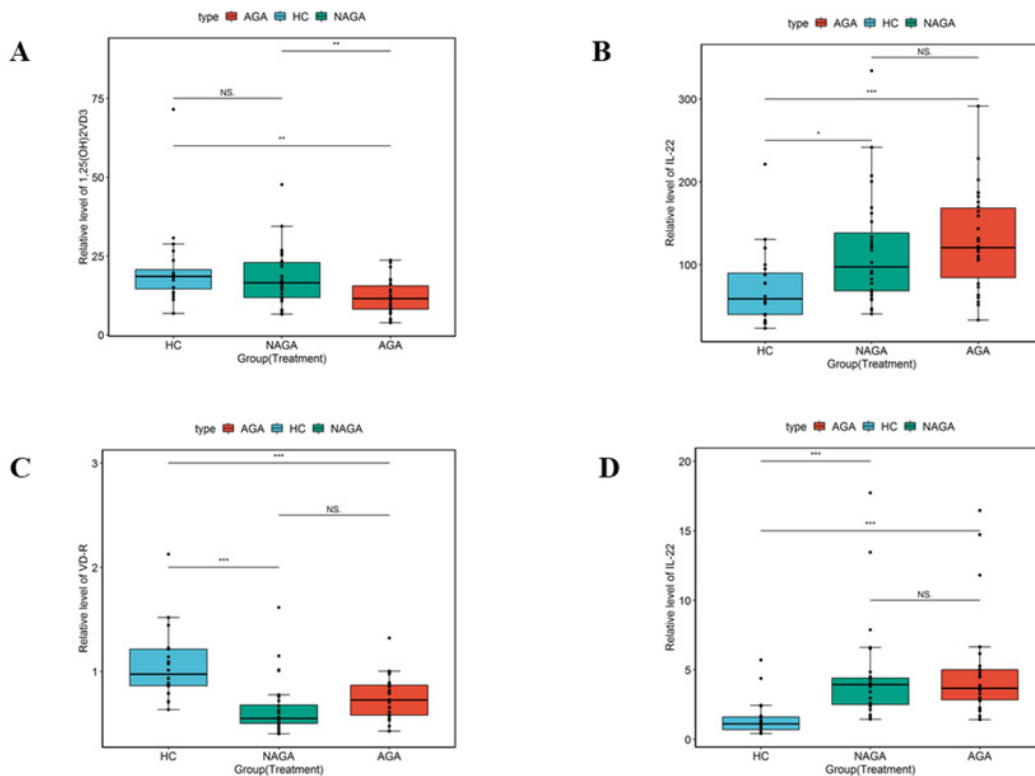



Figure 2

Metabolomic landscape of the GA patients

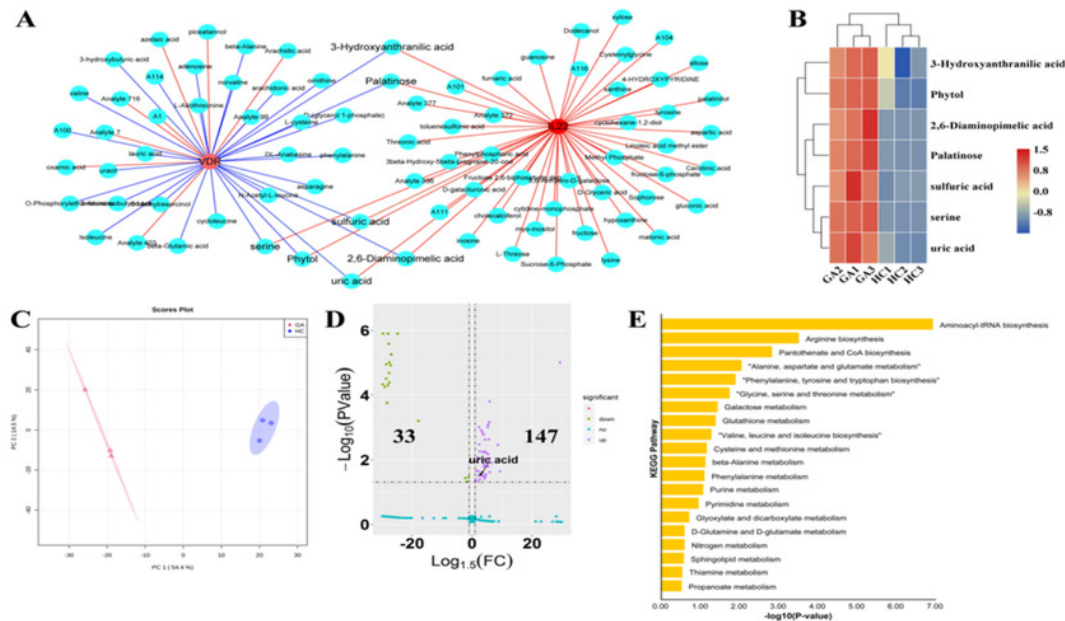



Figure 3

Correlations in the serum levels of LogCRP and IL-22 or 1,25(OH)$_2$VD3

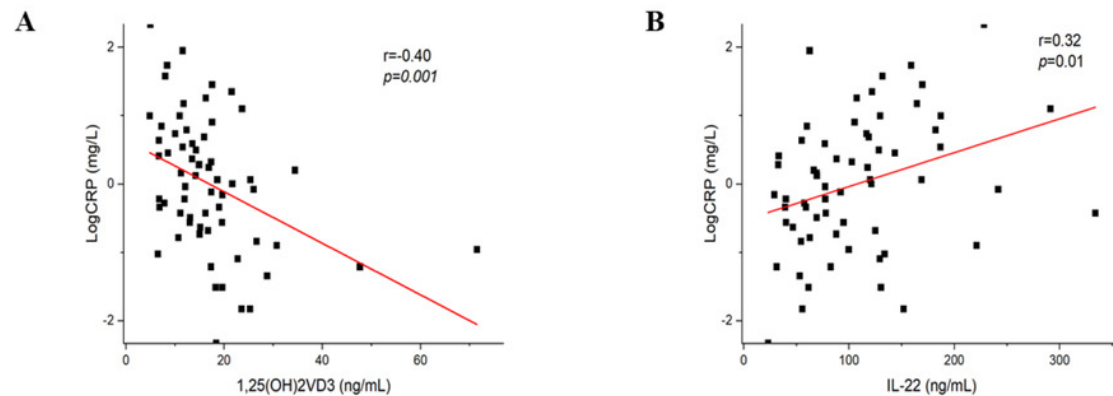
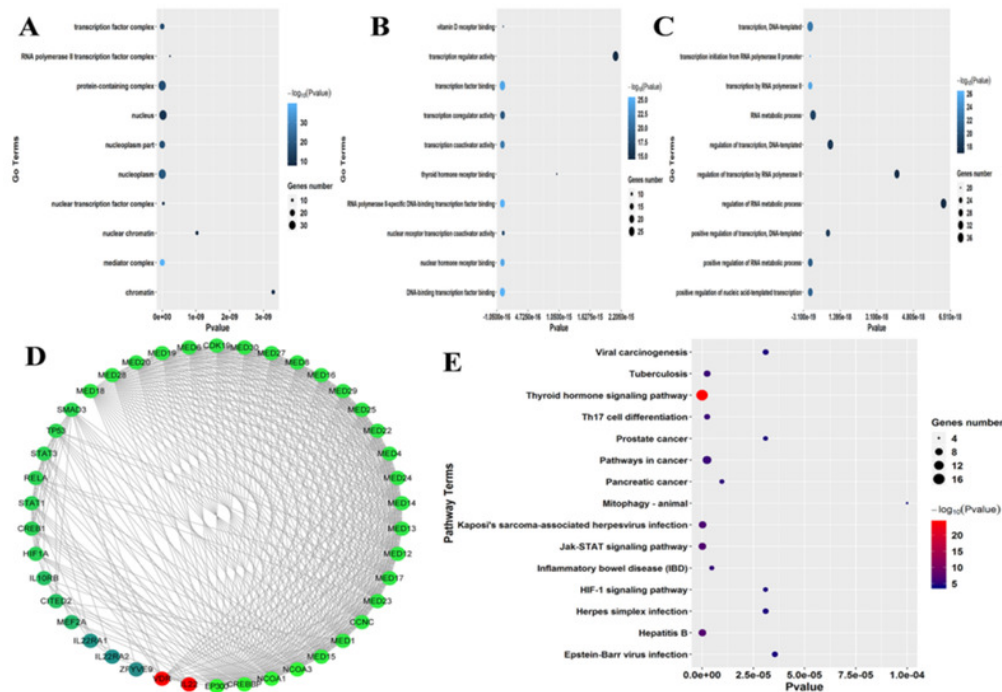



Figure 4

Complex regulation network and imperative roles of IL-22 and VD-R were involved in GA-related inflammatory responses

Patient characteristics

1 **Table 1 Patient characteristics**

Variable	AGA Patients N=30	NAGA Patients N=28	HC N=20	*p*
Age,y	43±15.68	36.7±11.4	43.0±8.6	
BMI kg/m^2*#	26.6±3.5	27.4±4.5	24.23±1.3	*=0.022, #=0.003
UA umol/L*	446.63±150.95	419.14±149.20	341.45±66.99	*=0.08
Cr umol/L*#	100.72±26.33	92.58±19.34	79.72±13.19	*=0.001, #=0.041
Platelet 10$^{\wedge 9}$/L	218.83±63.97	250.43±71.45	227.15±54.68	
ESR mm/h	20.2±5.2	13.60±4.8	10.8±3.5	
CRP mg/L*△	24.42±13.42	2.45±1.50	1.85±1.68	*< 0.001, △< 0.001
Cholesterin mmol/L	4.30±0.83	4.57±0.98	4.23±0.6	
Triglyceride mmol/L	1.97±0.96	2.45±1.5	1.85±1.68	
Blood glucose mmol/L*	5.64±1.06	5.35±0.48	5.03±0.51	*< 0.011
Tophi n	5 (16.7%)	3 (10.7%)	Not available	
Disease duration m	27.8±34.5	41.9±52.5	Not available	
Comorbidities				
Hypertension	7(24.1%)	7 (25.0%)	1 (5%)	
Diabetes	4(13.3%)	5 (17.9%)	0 (0.0%)	

2 AGA: acute gouty; BMI: body mass index; CRP: C-reactive protein;

3 ESR: erythrocyte sedimentation rate; HC: healthy controls; NAGA: non-acute gouty arthritis; UA: uric acid;

4   * $p < 0.05$, between AGA patients vs. HC. # $p < 0.05$, between NAGA patients vs. HC. $\triangle$ $p < 0.05$, between

5   NAGA patients vs. AGA patients;

Pathway analysis on metabolites closely associated with IL22 and VDR

name	Hits	Raw p	Impact
Aminoacyl-tRNA biosynthesis	9	1.64E-05	0.16667
Pantothenate and CoA biosynthesis	5	0.000285	0.02143
Purine metabolism	7	0.004921	0.03226
Phenylalanine, tyrosine and tryptophan biosynthesis	2	0.006345	1
Arginine biosynthesis	3	0.009984	0.06091
Valine, leucine and isoleucine biosynthesis	2	0.027162	0
beta-Alanine metabolism	3	0.030917	0.39925
Phenylalanine metabolism	2	0.04182	0.35714