Long Non-coding RNAs Genes Polymorphisms and Their Expression Levels in Patients With Rheumatoid Arthritis

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Long non-coding RNAs (lncRNAs) are increasingly recognized to play important roles in multiple autoimmune diseases. This study aimed to evaluate the association of four lncRNAs (ANRIL, lnc-DC, MALAT1, ZFAS1) genes single nucleotide polymorphisms (SNPs) with susceptibility to rheumatoid arthritis (RA) patients, as well as their expression levels. Seventeen SNPs of the four lncRNAs were genotyped in a cohort of 660 RA patients and 710 controls using improved multiple ligase detection reaction (iMLDR). The lncRNAs expressions in peripheral blood mononuclear cells (PBMCs) from 120 RA patients and 120 controls were detected by qRT-PCR. No significant differences were found for the allele and genotype frequencies distribution of ANRIL SNPs (rs1412830, rs944796, rs61271866, rs2518723, rs3217992), lnc-DC SNPs (rs7217280, rs10515177), MALAT1 SNPs (rs619586, rs4102217, rs591291, rs11227209, rs35138901), ZFAS1 SNPs (rs237742, rs73116127, rs6125607, rs6125608) between RA patients and normal controls (all \( P > 0.05 \)). The genotype effects of dominant and recessive models were also evaluated, but no significant association was found. In addition, our results demonstrated that the rs944796 G allele, rs2518723 T allele, rs3217992 T allele frequencies were significantly associated with anti-CCP in RA patients (all \( P < 0.05 \)). The haplotype CGTA frequency for ZFAS1 was significantly higher in RA patients (\( P = 0.036 \)). Compared with normal controls, the expression levels of ANRIL, Inc-DC, MALAT1, ZFAS1 in PBMCs were significantly reduced in RA patients (all \( P < 0.001 \)). Moreover, ZFAS1 expression was negatively associated with CRP in RA patients (\( P = 0.002 \)). In summary, ANRIL, Inc-DC, MALAT1, and ZFAS1 genes SNPs were not associated with RA susceptibility, while altered ANRIL, Inc-DC, MALAT1, ZFAS1 levels in RA patients suggested that these lncRNAs might play a role in RA.

Keywords: ANRIL, Inc-DC, MALAT1, ZFAS1, single nucleotide polymorphisms, rheumatoid arthritis
INTRODUCTION

Rheumatoid arthritis (RA) is known as a common autoimmune, inflammation disease characterized by systemic manifestations of immune and inflammatory response including systemic manifestations of immune and inflammatory response including marginal bone erosion, inflammatory joint fluid, synovitis, and destruction of articular cartilage (1, 2). Several researches have indicated that the incidences of RA in different ethnic groups, geographical areas are different, and the RA prevalence is approximately 1% around the world (3, 4). It has been revealed that genetic susceptibilities, abnormal metabolic enzymes, aberrant immune response are involved in RA development (5, 6). Latterly, a number of single nucleotide polymorphisms (SNPs), the majority of which are located in the non-coding intervals, are gradually identified to be associated with the susceptibility of this disease according to genome-wide association studies (7, 8).

Long non-coding RNAs (lncRNAs), which are defined as RNAs longer than 200 nucleotides in length, have no or little protein-coding capacity (9). LncRNAs are reported to be involved in a variety of autoimmune- and inflammation-related processes, and regulate gene expression in multiple mechanisms including alternative splicing, epigenetics, small RNA sponging (10, 11). Increasing studies have been performed to explore the potential role of lncRNAs on the pathogenesis of autoimmune diseases, such as RA and systemic lupus erythematosus (SLE) (12–14). Our recent study demonstrated that the lnc0640, lnc5150 expression levels were alternated among RA patients, and lnc0640 rs13039216 TT genotype was statistically associated with RA susceptibility (14). Another previous study suggested that aberrant lncRNA expression level in peripheral blood mononuclear cells (PBMCs) could be a potential biomarker for RA diagnosing (13).

Recently, lncRNA ANRIL (antisense non-coding RNA in the INK4 locus) had attracted attention in autoimmune diseases, as it had been implicated in regulation of immune, inflammatory response (15). ANRIL expression was found to be regulated through STAT1 signaling pathway, which participated in immune regulation by induction of the pro-inflammatory cytokine TNF-γ (16). In addition, another study indicated that ANRIL expression level in PBMCs was decreased in RA by lncRNA array (13). Dendritic cell (DC) was a specific antigen presenting cell which link the innate and adaptive immune responses, and was thought to drive the activation of self-peptide-reactive inflammatory T cells, follicular helper T cells and consequently B cells for secreting autoantibodies in RA (17). Lnc-DC was a specialized, highly expressed lncRNA in DCs, and had the ability to regulate Th17 differentiation, DCs to stimulate T cell activation, and the production of interleukin 12 (IL-12) (9). LncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript-1) had been shown to play a role in the development of autoimmune diseases. SLE patients had increased MALAT1 level in PBMCs compared with normal individuals, and knockdown of MALAT-1 significantly suppressed IL-21 level in monocytes (18).

In RA, Pan et al. found that knockdown of MALAT1 could inhibit the apoptosis of fibroblast-like synoviocytes (FLS) and lead to the activation of phosphoinositide 3-kinase (PI3K)/AKT signaling pathway (19). In another study, lncRNA ZFAS1 (zinc finger antisense 1) was shown to participate in RA-FLS migration and invasion by interacting with miR-27a and suppressing miR-27a expression, and ZFAS1 expression level was statistically evaluated in FLS of RA patients (20).

These studies demonstrated that ANRIL, lnc-DC, MALAT1, ZFAS1 might be involved in the occurrence and development of RA. However, no studies regarding the relationship between these lncRNAs genetic variation and RA have been reported. Thus, in the present study, we explored the associations of these lncRNAs genes SNPs with RA risk, as well as these lncRNAs expressions in PBMCs of RA patients and normal controls.

MATERIALS AND METHODS

Patients and Normal Controls

In this study, case-control studies were performed in unrelated ethnic Han Chinese population. A total of 1,370 subjects including 660 RA patients and 710 normal controls were consecutively enrolled to investigate the association between ANRIL, lnc-DC, MALAT1, ZFAS1 genes polymorphisms and RA susceptibility. Then, 120 RA patients and 120 normal controls were included to detect these lncRNAs expression levels, RA patients were selected from the First Affiliated Hospital of University of Science and Technology of China, and the First Affiliated Hospital of Anhui Medical University. The diagnosis of these patients was according to the 1987 American College of Rheumatology revised criteria (21). The normal controls, who were recruited from the healthy blood donors in the same region, did not have no a history of RA, or other inflammatory/autoimmune diseases, cancer. Disease Activity Score 28 (DAS 28) was used to evaluate RA disease activity (22). The demographic data of all subjects were collected, and the following clinical data of RA patients were retrieved from the medical records: complements 3 (C3), complements 4 (C4), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-cyclic citrullinated peptide (anti-CCP), and rheumatoid factor (RF). After informed consent was obtained, peripheral blood samples and data were collected from RA patients and normal controls. This study protocol was approved by the Medical Ethics Committee of Anhui Medical University.

SNP Selection, DNA Extraction, and Genotyping

The genetic and location information were obtained from two public databases, LNCipedia.org (v4.0) and Genome Browser Gateway (UCSC). We selected the tagSNPs with a minor allele frequency (MAF) ≥ 0.05 in CHB capturing all the common SNPs located in the chromosome locus transcribed into these lncRNAs (ANRIL, lnc-DC, MALAT1, ZFAS1) and their flanking 2,000 bp region through using genotype data of Han Chinese in Beijing from Ensembl genome browser 85 and CHBS_1000 g. The selection was conducted through linkage disequilibrium (LD) analysis with $r^2$ threshold > 0.8 by using Haplovieview 4.0 software (Cambridge, MA, USA). In addition, the existing studies about these lncRNA genes polymorphisms were also reviewed. Finally, we selected six tagSNPs (rs1412830, rs7044859, rs944796, rs61271866, rs2518723, rs3217992) in ANRIL, two tagSNPs...
### TABLE 1 | Genotypes and alleles frequencies of lncRNAs genes polymorphisms in RA patients and normal controls.

| SNP         | Analyze model | RA (N = 660) n (%) | Control (N = 710) n (%) | Adjustment with sex and age |
|-------------|---------------|--------------------|-------------------------|----------------------------|
|             |               |                    |                         | P value* | OR (95% CI) |
| **ANRIL**   |               |                    |                         |          |             |
| rs1412830   | Genotypes    | TT 13 (1.97)       | 3 (0.42)                | 0.017    | 0.214 (0.060–0.761) |
|             |               | CT 119 (18.03)     | 139 (19.58)             | 0.564    | 1.084 (0.824–1.425) |
|             |               | CC 528 (80.00)     | 568 (80.00)             | Reference|              |
| Alleles     | T             | 145 (10.98)        | 145 (10.21)             | 0.511    | 0.922 (0.722–1.176) |
|             | C             | 1,175 (89.02)      | 1,275 (89.79)           | Reference|              |
|             | Dominant model| CC 528 (80.00)     | 588 (80.00)             | 0.994    | 1.001 (0.766–1.307) |
|             |               | TT+CT 132 (20.00)  | 142 (20.00)             | Reference|              |
|             | Recessive model| TT 13 (1.97)       | 3 (0.42)                | 0.016    | 0.211 (0.059–0.750) |
|             |               | CC+CT 647 (98.03)  | 707 (99.58)             | Reference|              |
| rs944798    | Genotypes    | GG 11 (1.67)       | 31 (4.37)               | 0.013    | 2.452 (1.211–4.962) |
|             |               | GC 238 (36.06)     | 230 (32.39)             | 0.236    | 0.872 (0.695–1.094) |
| Alleles     | G             | 260 (19.70)        | 292 (20.56)             | 0.572    | 1.055 (0.875–1.272) |
|             | C             | 1,060 (80.30)      | 1,128 (79.44)           | Reference|              |
|             | Dominant model| CC 411 (62.27)     | 449 (63.24)             | 0.598    | 1.061 (0.851–1.324) |
|             |               | GG+GC 249 (37.73)  | 261 (36.76)             | Reference|              |
|             | Recessive model| GG 11 (1.67)       | 31 (4.37)               | 0.008    | 2.574 (1.278–5.185) |
|             |               | CC+GC 649 (98.33)  | 679 (95.63)             | Reference|              |
| rs61271866  | Genotypes    | AA 25 (3.79)       | 26 (3.66)               | 0.882    | 0.958 (0.542–1.692) |
|             |               | TA 185 (28.03)     | 214 (30.14)             | 0.437    | 1.099 (0.867–1.392) |
|             |               | TT 450 (68.18)     | 470 (66.20)             | Reference|              |
| Alleles     | A             | 235 (17.80)        | 266 (18.73)             | 0.529    | 0.940 (0.774–1.141) |
|             | T             | 1,085 (82.20)      | 1,154 (81.27)           | Reference|              |
|             | Dominant model| TT 450 (68.18)     | 470 (66.20)             | 0.498    | 0.924 (0.736–1.160) |
|             |               | AA+TA 210 (31.82)  | 240 (33.80)             | Reference|              |
|             | Recessive model| AA 25 (3.79)       | 26 (3.66)               | 0.811    | 0.933 (0.531–1.641) |
|             |               | TT+TA 635 (96.21)  | 684 (96.34)             | Reference|              |
| rs2518723   | Genotypes    | TT 111 (16.82)     | 133 (18.73)             | 0.312    | 1.177 (0.858–1.613) |
|             |               | CT 326 (49.39)     | 353 (49.72)             | 0.535    | 1.079 (0.848–1.372) |
|             |               | CC 223 (33.79)     | 224 (31.55)             | Reference|              |
| Alleles     | T             | 548 (81.42)        | 619 (43.59)             | 0.256    | 1.092 (0.983–1.271) |
|             | C             | 772 (18.58)        | 801 (56.41)             | Reference|              |
|             | Dominant model| CC 223 (33.79)     | 224 (31.55)             | 0.393    | 0.906 (0.721–1.137) |
|             |               | TT+CT 437 (66.21)  | 486 (68.45)             | Reference|              |
|             | Recessive model| TT 111 (16.82)     | 133 (18.73)             | 0.413    | 0.890 (0.673–1.177) |
|             |               | CC+CT 549 (83.18)  | 577 (81.27)             | Reference|              |
| rs3217992   | Genotypes    | TT 160 (24.24)     | 152 (21.41)             | 0.118    | 0.783 (0.576–1.064) |
|             |               | CT 338 (51.21)     | 362 (50.99)             | 0.368    | 0.889 (0.687–1.149) |
|             |               | CC 162 (24.55)     | 196 (27.61)             | Reference|              |
| Alleles     | T             | 658 (49.85)        | 666 (46.90)             | 0.123    | 0.889 (0.765–1.032) |
|             | C             | 662 (50.15)        | 754 (53.10)             | Reference|              |
|             | Dominant model| CC 162 (24.55)     | 196 (27.61)             | 0.206    | 1.170 (0.917–1.483) |
|             |               | TT+CT 498 (75.45)  | 514 (72.39)             | Reference|              |
|             | Recessive model| TT 160 (24.24)     | 152 (21.41)             | 0.199    | 0.846 (0.656–1.092) |
|             |               | CC+CT 500 (75.76)  | 558 (78.59)             | Reference|              |
| **Lnc-DC**  |               |                    |                         |          |             |
| rs7217280   | Genotypes    | AA 3 (0.45)        | 4 (0.56)                | 0.849    | 1.160 (0.253–5.314) |

(Continued)
| SNP          | Analyze model | RA (N = 660) n (%) | Control (N = 710) n (%) | Adjustment with sex and age |
|--------------|---------------|--------------------|-------------------------|----------------------------|
|              |               |                    |                         | P value*                   |
|              |               |                    |                         | OR (95% CI)                |
| GA          | 52 (7.88)     | 77 (10.85)         | 0.084                   | 1.388 (0.957–2.014)        |
| GG          | 605 (91.67)   | 629 (88.59)        | Reference               |
| Alleles A   | 58 (4.39)     | 85 (5.99)          | 0.062                   | 1.385 (0.984–1.951)        |
| Alleles G   | 1,262 (95.61)| 1,335 (94.01)      | Reference               |
| Dominant    | GG 605 (91.67)| 629 (88.59)        | 0.085                   | 0.727 (0.506–1.045)        |
| Recessive   | AA 55 (8.33)  | 81 (11.41)         | Reference               |
| rs10515177  | Genotypes    |                    |                         |                            |
| GG          | 4 (0.61)      | 5 (0.70)           | 0.870                   | 1.118 (0.294–4.249)        |
| AA          | 94 (14.24)    | 117 (16.48)        | 0.330                   | 1.159 (0.861–1.560)        |
| Alleles G   | 102 (7.73)    | 127 (8.94)         | 0.251                   | 1.173 (0.893–1.540)        |
| Alleles A   | 1,218 (92.27)| 1,293 (91.06)      | Reference               |
| Dominant    | AA 562 (85.15)| 588 (82.82)        | 0.327                   | 0.864 (0.645–1.157)        |
| Recessive   | GG 657 (99.55)| 706 (99.44)        | Reference               |
| rs619586    | Genotypes    |                    |                         |                            |
| GG          | 6 (0.91)      | 4 (0.56)           | 0.350                   | 0.544 (0.151–1.951)        |
| AA          | 111 (16.82)   | 113 (15.92)        | 0.628                   | 0.931 (0.698–1.243)        |
| Alleles G   | 123 (9.32)    | 121 (8.52)         | 0.464                   | 0.906 (0.697–1.179)        |
| Alleles A   | 1,197 (90.68)| 1,209 (91.48)      | Reference               |
| Dominant    | AA 543 (82.27)| 593 (83.52)        | 0.517                   | 1.098 (0.827–1.458)        |
| Recessive   | GG 117 (17.73)| 117 (16.48)        | Reference               |
| rs4102217   | Genotypes    |                    |                         |                            |
| CC          | 20 (3.03)     | 13 (1.83)          | 0.306                   | 0.688 (0.337–1.408)        |
| CG          | 154 (23.33)   | 205 (28.87)        | 0.020                   | 1.340 (1.047–1.713)        |
| GG          | 486 (73.64)   | 492 (69.30)        | Reference               |
| Alleles C   | 194 (14.70)   | 231 (16.27)        | 0.257                   | 1.128 (0.916–1.388)        |
| Alleles G   | 1,126 (85.30)| 1,189 (83.73)      | Reference               |
| Dominant    | CC 486 (73.64)| 492 (69.30)        | 0.053                   | 0.791 (0.624–1.003)        |
| Recessive   | CC 174 (26.36)| 218 (30.70)        | Reference               |
| rs591291    | Genotypes    |                    |                         |                            |
| TT          | 124 (18.79)   | 132 (18.59)        | 0.496                   | 1.113 (0.818–1.513)        |
| CT          | 298 (45.15)   | 347 (48.87)        | 0.125                   | 1.207 (0.949–1.534)        |
| CC          | 238 (36.06)   | 231 (32.53)        | Reference               |
| Alleles T   | 546 (84.16)   | 611 (83.03)        | 0.378                   | 1.071 (0.920–1.246)        |
| Alleles C   | 774 (58.64)   | 809 (56.97)        | Reference               |
| Dominant    | CC 238 (36.06)| 231 (32.53)        | 0.153                   | 0.848 (0.677–1.063)        |
| Recessive   | TT 124 (18.79)| 132 (18.59)        | 0.979                   | 0.996 (0.757–1.310)        |
| rs11227209  | Genotypes    |                    |                         |                            |
| GG          | 3 (0.45)      | 3 (0.42)           | 0.880                   | 0.883 (0.176–4.420)        |
| CG          | 71 (10.76)    | 79 (11.13)         | 0.773                   | 1.052 (0.747–1.480)        |
| CC          | 586 (88.79)   | 628 (88.45)        | Reference               |

(Continued)
| SNP | Analyze model | RA (N = 660) n (%) | Control (N = 710) n (%) | Adjustment with sex and age |
|-----|---------------|---------------------|-------------------------|-----------------------------|
|     |               |                     |                         |                             |
|     | G             | 77 (5.83)           | 85 (5.99)               |                              |
|     | C             | 1,243 (94.17)       | 1,335 (94.01)           | Reference                    |
| Dominant model | CC | 586 (88.79) | 628 (88.45) | 0.799 | 0.957 (0.684–1.339) |
|     | CG+GG         | 74 (11.21)          | 82 (11.55)              | Reference                    |
| Recessive model | GG | 3 (0.45) | 3 (0.42) | 0.874 | 0.878 (0.176–4.393) |
|     | CG+CC         | 657 (99.55)         | 707 (99.58)             | Reference                    |
| rs35138901 Genotypes | CC | 4 (0.61) | 2 (0.28) | 0.469 | 0.532 (0.097–2.933) |
|     | CT            | 93 (14.09)          | 115 (16.20)             | 0.252 | 1.191 (0.883–1.606) |
|     | TT            | 563 (85.30)         | 593 (83.52)             | Reference                    |
| Alleles | C | 101 (7.65) | 119 (8.38) | 0.483 | 0.518 (0.094–2.862) |
|     | T             | 1,219 (92.35)       | 1,301 (91.62)           | Reference                    |
| Dominant model | TT | 563 (85.30) | 593 (83.52) | 0.312 | 0.859 (0.639–1.154) |
|     | CT+CC         | 97 (14.70)          | 117 (16.48)             | Reference                    |
| Recessive model | TT | 91 (13.79) | 104 (14.65) | 0.611 | 1.083 (0.797–1.470) |
|     | CT+TT         | 656 (99.39)         | 708 (99.72)             | Reference                    |
| ZFAS1 rs237742 Genotypes | TT | 91 (13.79) | 104 (14.65) | 0.994 | 0.999 (0.717–1.391) |
|     | CT            | 322 (48.79)         | 320 (45.07)             | 0.212 | 0.863 (0.685–1.088) |
|     | CC            | 247 (37.42)         | 286 (40.29)             | Reference                    |
| Alleles | T | 504 (76.16) | 529 (76.20) | 0.590 | 0.958 (0.821–1.119) |
|     | C             | 816 (61.82)         | 892 (62.82)             | Reference                    |
| Dominant model | CC | 247 (37.42) | 286 (40.29) | 0.309 | 1.121 (0.900–1.395) |
|     | CT+TT         | 413 (62.58)         | 424 (59.72)             | Reference                    |
| Recessive model | TT | 91 (13.79) | 104 (14.65) | 0.611 | 1.083 (0.797–1.470) |
|     | CT+CC         | 569 (86.21)         | 606 (85.35)             | Reference                    |
| rs73116127 Genotypes | AA | 1 (0.15) | 3 (0.42) | 0.384 | 2.739 (0.283–26.506) |
|     | GA            | 109 (16.52)         | 133 (18.73)             | 0.294 | 1.162 (0.878–1.538) |
|     | GG            | 550 (83.33)         | 574 (80.85)             | Reference                    |
| Alleles | A | 111 (8.41) | 139 (9.79) | 0.211 | 1.182 (0.910–1.535) |
|     | G             | 1,209 (91.59)       | 1,281 (90.21)           | Reference                    |
| Dominant model | GG | 550 (83.33) | 574 (80.85) | 0.253 | 0.850 (0.643–1.123) |
|     | AA+GA         | 110 (16.67)         | 136 (19.15)             | Reference                    |
| Recessive model | AA | 1 (0.15) | 3 (0.42) | 0.398 | 2.661 (0.275–25.738) |
|     | GG+GA         | 659 (99.85)         | 707 (99.58)             | Reference                    |
| rs6125607 Genotypes | TT | 74 (11.21) | 48 (6.76) | 0.007 | 0.576 (0.387–0.857) |
|     | CT            | 277 (41.97)         | 310 (43.66)             | 0.978 | 1.003 (0.801–1.256) |
|     | CC            | 309 (46.82)         | 352 (49.58)             | Reference                    |
| Alleles | T | 425 (63.20) | 406 (52.50) | 0.040 | 0.843 (0.716–0.992) |
|     | C             | 895 (67.80)         | 1,014 (71.41)           | Reference                    |
| Dominant model | CC | 309 (46.82) | 352 (49.58) | 0.407 | 1.095 (0.884–1.356) |
|     | TT+CT         | 351 (53.18)         | 398 (50.42)             | Reference                    |
| Recessive model | TT | 74 (11.21) | 48 (6.76) | 0.005 | 0.576 (0.393–0.844) |
|     | CC+TC         | 598 (88.78)         | 662 (93.23)             | Reference                    |
| rs6125608 Genotypes | GG | 9 (1.36) | 11 (1.55) | 0.716 | 1.181 (0.483–2.890) |
|     | GA            | 125 (18.94)         | 158 (22.25)             | 0.153 | 1.213 (0.931–1.582) |
|     | AA            | 526 (79.70)         | 541 (75.20)             | Reference                    |
| Alleles | G | 143 (10.83) | 180 (12.68) | 0.135 | 1.195 (0.946–1.509) |
|     | A             | 1,177 (89.17)       | 1,240 (87.32)           | Reference                    |
| Dominant model | AA | 526 (79.70) | 541 (75.20) | 0.147 | 0.826 (0.638–1.069) |
|     | GG+GA         | 134 (20.30)         | 169 (23.80)             | Reference                    |
| Recessive model | GG | 9 (1.36) | 11 (1.55) | 0.780 | 1.136 (0.465–2.775) |
|     | AA+GA         | 651 (98.64)         | 699 (98.45)             | Reference                    |

* After FDR correction, no P value was statistically significant (all P > 0.05).
TABLE 2 | The positive findings of associations between ANRIL gene polymorphisms and anti-CCP of RA patients.

| SNP       | Allele | Clinical features | Group     | Alleles n (%) | P value |
|-----------|--------|-------------------|-----------|---------------|---------|
| rs944796  | C/G    | Anti-CCP          | Positive  | 853 (79.28)   | 223 (20.72) | 0.039   |
| rs2518723 | C/T    | Anti-CCP          | Negative  | 143 (86.14)   | 23 (13.86)  | 0.039   |
| rs3217992 | C/T    | Anti-CCP          | Positive  | 615 (57.16)   | 461 (42.84) | 0.039   |
|           |        |                   | Negative  | 109 (65.66)   | 57 (34.34)  |         |

Bold value means P < 0.05.

TABLE 3 | Haplotype analysis of IncRNA genes in RA patients and controls.

| Haplotype | RA patients [n(%)] | Controls [n(%)] | P value | OR (95% CI) |
|-----------|--------------------|----------------|---------|-------------|
| ANRIL     |                    |                |         |             |
| rs1412830 | 91.88 (7.0)        | 125.21 (8.8)   | 0.077   | 0.776 (0.586–1.028) |
| rs61271866| 105.84 (8.0)       | 108.60 (7.6)   | 0.692   | 1.058 (0.800–1.400) |
| rs2518723 | 674.30 (66.5)      | 621.58 (48.6)  | 0.109   | 1.135 (0.972–1.324) |
| rs3217992 | 116.57 (8.8)       | 128.95 (9.1)   | 0.847   | 0.974 (0.749–1.268) |
| Lnc-DC    |                    |                |         |             |
| rs7217280 | 119.02 (14.4)      | 220.98 (15.6)  | 0.419   | 0.916 (0.742–1.132) |
| rs10515177| 122.50 (9.3)       | 135.90 (9.6)   | 0.824   | 0.971 (0.751–1.256) |
| MALAT1    |                    |                |         |             |
| rs619586  | 58.00 (4.4)        | 85.00 (6.0)    | 0.061   | 0.722 (0.512–1.017) |
| rs4102217 | 1,218.00 (92.3)    | 1,293.00 (91.1)| 0.250   | 1.173 (0.893–1.540) |
| rs591291  | 1,287.00 (90.9)    | 1,332.00 (89.9)| 0.573   | 1.131 (0.736–1.738) |
| rs11227209| 44.00 (3.3)        | 42.00 (3.0)    | 0.573   | 1.131 (0.736–1.738) |
| rs35138901| 75.01 (6.7)        | 83.88 (5.9)    | 0.799   | 0.959 (0.696–1.322) |

Frequency < 0.03 in both controls and RA patients has been dropped. Bold value means P < 0.05.

The genomic DNA was extracted from the peripheral blood leukocytes by the Flexi Gene-DNA Kit (Qiagen, Valencia, CA). Improved multiple ligase detection reaction (iMLDR) genotyping assay, with technical support from the Center for Genetic & Genomic Analysis, Genesky Biotechnologies (Inc., Shanghai), was used for genotyping. Those individuals with 100% genotyping success rate for the above SNPs were included for final analysis.

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

PBMCs were isolated from 5 ml anticoagulated peripheral blood, and stored at −80°C until processed. Total RNA in PBMCs was extracted with TRIZol Reagent (Invitrogen, Carlsbad, CA, USA) and the concentration of RNA was quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Then, the PrimeScript™ TM RT reagent Kit (Takara Bio Inc., Japan) was used to reverse-transcribed total RNA into cDNA.

The expression levels of ANRIL, Inc-DC, MALAT1, ZFAS1 in PBMCs were detected by qRT-PCR with SYBR Green (SYBR Premix Ex Taq II, Takara Bio Inc., Japan). This experiment was performed on QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and according to the following cycle conditions: 95°C for 10 s, 60°C for 1 min, followed by 42 cycles at 95°C for 10 s, 55°C for 30 s and 72°C for 1 min. The relative expression levels of IncRNAs were calculated by housekeeping gene β-actin in the same sample as internal control, and expressed using 2−ΔΔCt method normalized to endogenous control (23).

Statistical Analysis

Statistical analysis was performed with the SPSS 23.0 (SPSS Inc., IL, USA). We performed Hardy-Weinberg equilibrium test by Chi-square (χ²) among normal controls. For the associations of genotype, allele distribution frequencies of each SNP with RA were estimated by logistic regression analyses. The IncRNAs levels were shown as median value and interquartile range, and the differences in IncRNAs levels between two groups, three groups were analyzed by Mann-Whitney U-test, Kruskal-Wallis H-test, respectively. The correlations between IncRNAs levels and several experimental indexes of RA patients were analyzed by Spearman rank correlation coefficient test. Dominant model, recessive mode was used for statistical analysis, and haplotype...

(rs7217280, rs10515177) in Inc-DC, five tagSNPs (rs619586, rs4102217, rs591291, rs11227209, rs35138901) in MALAT1, four tagSNPs (rs237742, rs73116127, rs6125607, rs6125608) in ZFAS1 for genotyping in the present study.
analysis was conducted with the SHEsis software (24). P value (two-sided) < 0.05 was considered as statistically significant. False discovery rate (FDR) was used for multiple testing in SNP analysis.

RESULTS

Association of IncRNAs Genes Polymorphisms With RA Susceptibility

We included 546 females and 114 males in RA patients for genotyping with a median age of 51, while there were 574 females and 136 males with a median age of 49 in normal controls. The observed genotype frequencies of rs7044859 was not conform to Hardy-Weinberg equilibrium, thus we excluded this SNP in finally analysis. The results of allele and genotype frequencies of all SNPs were summarized in Table 1.

In ANRIL gene, the rs1412830 TT genotype frequency was significantly increased in RA patients in comparison to normal controls, while the rs944796 GG genotype frequency was significantly decreased (TT vs. CC: P = 0.017; GC vs. CC: P = 0.013, respectively). In addition, an increased risk of rs1412830 variant, as well as a decreased risk of rs944796, was observed under the recessive model (TT vs. CC+CT: P = 0.016; GG vs. CC+GC: P = 0.008, respectively). However, these significant associations were disappeared after multiple testing by FDR correction (all P > 0.05). Comparing the genotype and allele frequencies of the ZFAS1 rs6125607 polymorphism among RA patients and normal controls, we found that TT genotype and T allele frequencies were significantly higher in RA patients than normal controls (TT vs. CC: P = 0.007; T vs. C: P = 0.040, respectively), and an increased risk of rs6125607 polymorphism existed in recessive model (TT vs. CC+TC: P = 0.005). After FDR correction, these differences were not statistically significant (TT vs. CC: P = 0.181; T vs. C: P = 0.496, TT vs. CC+TC: P = 0.080, respectively). Similarly, no significant associations between Inc-DC, MALAT1 genes polymorphism and RA susceptibility were found (Table 1).

To examine the potential genetic association between the genotype, allele frequencies of ANRIL, Inc-DC, MALAT1, ZFAS1 genes and anti-CCP, RF in RA patients, we performed a case-only analysis (Table S1). In ANRIL gene, the rs944796 G allele, rs2518723 T allele frequencies were significantly increased in RA patients with anti-CCP-positive when compared to patients with anti-CCP-negative (all P < 0.05), while the rs3217992 T allele frequency was reduced (P = 0.039) (Table 2). No significant differences existed in allele and genotype frequencies of Inc-DC, MALAT1, ZFAS1 genes.

Haplotype Analysis

Six main haplotypes (CCATC, CCTCC, CCTCT, CCTTC, CGTTC, TCATC) for ANRIL, three main haplotypes (AG, GA, GG) for Inc-DC, six main haplotypes (ACTCT, AGCCT, AGTCC, AGTCT, GGTCT, GGTGT) for MALAT1 and five main haplotypes (CACA, CGCA, CGCG, CGTGA, TGCA) for ZFAS1 were detected by SHEsis software (Table 3). The results demonstrated that the haplotype CGTA frequency was significantly higher in RA patients than normal controls (OR = 1.191, 95% CI: 1.012–1.402, P = 0.036).

LncRNAs Expression Levels in PBMCs From RA Patients and Normal Controls

We further analyzed the association of ANRIL, Inc-DC, MALAT1, ZFAS1 in PBMCs with RA patients by qRT-PCR. As shown in Table 4, the expression levels of ANRIL, Inc-DC, MALAT1, ZFAS1 in PBMCs were significantly reduced in RA patients than normal controls (all P < 0.001). However, the differences in these IncRNAs levels between anti-CCP-positive RA patients and anti-CCP-negative RA patients, as well as RA patients with RF-positive and RF-negative RA patients, were not statistically significant.

The correlation of ANRIL, Inc-DC, MALAT1, ZFAS1 expression levels with clinical parameters, disease activity of RA patients were also analyzed, and the results shown that the expression level of ZFAS1 was negatively associated with CRP in RA patients (P = 0.002). However, there were no significant correlations of these IncRNAs levels with DAS28 of RA patients (Table 5). The potential influence of main medical therapies including glucocorticoids, disease-modifying antirheumatic drugs (DMARDs), biologics on IncRNAs expression levels in RA patients were assessed in this study. Similarly, no significant association was found (Table 6).

Associations Between IncRNAs Genes Polymorphisms With Their Levels in RA Patients

To examine the associations between the respective genotype frequencies of these IncRNAs genes with their expression levels in RA patients, we included 65 patients for analysis. However, there were no significant differences regarding these IncRNAs expression levels between their disparate genotypes of RA patients (Table 7).

DISCUSSION

To date, the exact pathogenic mechanism of RA remains largely unknown, although several pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), IL-6, IL-1b have been reported to related to the occurrence of RA (25–27). Previous studies have shown that IncRNAs had distinct and specific roles in the activation and differentiation modulating of immune cell, and IncRNAs played an important role in autoimmune diseases (28). A study detected lncRNA transcription in CD14+ monocytes isolated from peripheral blood cells of RA patients before and after anti-IL-6R (tocilizumab) or anti-TNF-α (adalimumab) therapy by a microarray-based experiment. They observed that 7,419 IncRNAs expression levels were altered by either IL-6 or TNF-α, 85 of which exhibited were significant changed (29). These results suggested that IncRNAs were very important in the molecular pathophysiology of RA. In the present study, our results demonstrated that lower expression levels of ANRIL, Inc-DC, MALAT1, ZFAS1 existed in RA patients, and ANRIL, Inc-DC, MALAT1, ZFAS1 IncRNAs genes were not related to RA susceptibility.
**TABLE 4** | Comparison of lncRNAs expression level in PBMCs between different subgroups.

| Group                              | Number | ANRIL level | P value | Lnc-DC level | P value | MALAT1 level | P value | ZFAS1 level | P value |
|------------------------------------|--------|-------------|---------|--------------|---------|--------------|---------|-------------|---------|
| RA patients                        | 120    | 0.605 (0.382, 0.849) | <0.001  | 0.378 (0.269, 0.586) | <0.001  | 0.409 (0.257, 0.533) | <0.001  | 0.458 (0.352, 0.648) | <0.001  |
| Normal controls                    | 120    | 0.853 (0.612, 1.147) | 0.818 (0.537, 1.166) | 0.902 (0.627, 1.228) | 0.870 (0.625, 1.161) | 0.358 (0.284, 0.577) | 0.920 (0.250, 0.543) | 0.681 (0.270, 0.499) | 0.391 (0.337, 0.678) |
| RA patients with anti-CCP-positive | 99     | 0.603 (0.404, 0.868) | 0.387 (0.264, 0.591) | 0.418 (0.250, 0.543) | 0.469 (0.362, 0.633) | 0.387 (0.264, 0.591) | 0.418 (0.250, 0.543) | 0.469 (0.362, 0.633) | 0.926 (0.250, 0.543) |
| RA patients with anti-CCP-negative | 21     | 0.619 (0.350, 0.823) | 0.358 (0.284, 0.577) | 0.372 (0.270, 0.499) | 0.926 (0.250, 0.543) | 0.358 (0.284, 0.577) | 0.372 (0.270, 0.499) | 0.926 (0.250, 0.543) | 0.926 (0.250, 0.543) |
| RA patients with anti-RF-positive  | 101    | 0.629 (0.404, 0.857) | 0.344 (0.268, 0.561) | 0.415 (0.251, 0.575) | 0.681 (0.469, 0.926) | 0.469 (0.352, 0.642) | 0.469 (0.352, 0.642) | 0.997 (0.250, 0.543) | 0.997 (0.250, 0.543) |
| RA patients with anti-RF-negative | 19     | 0.510 (0.360, 0.675) | 0.436 (0.273, 0.614) | 0.379 (0.306, 0.474) | 0.926 (0.250, 0.543) | 0.469 (0.352, 0.642) | 0.469 (0.352, 0.642) | 0.997 (0.250, 0.543) | 0.997 (0.250, 0.543) |

**ANOVEL** gene was located in the chromosome 9p21 region, and it was the well-defined genetic risk locus related to several diseases such as coronary artery disease (CAD), diabetes, and breast cancer (30–32). Our results implied that ANRIL rs1412830, rs944796 variant might associated with RA susceptibility, while the significant associations were disappeared after multiple testing. However, we found that ANRIL rs944796 G, rs2518723 T, rs3217992 T allele frequencies were significantly associated with RA development. In addition, disease-associated SNPs resided in this region had been reported to change the expression of ANRIL, demonstrating that altered ANRIL expression might be involved in the development of autoimmune diseases. Shaker et al. found that serum level of lnc-DC in multiple sclerosis (MS) patients were significantly increased, and serum lnc-DC level maybe used as a potential novel biomarkers for MS diagnosis (37). One of our recent studies shown that the lnc-DC expression level was significantly decreased in PBMC from RA patients compared with normal controls, ANRIL expression level was significantly decreased in PBMC from RA patients. We further explored the influence of the five SNPs on ANRIL level in PBMC from RA patients, unfortunately, there were no significant differences regarding ANRIL level between disparate genotypes of these SNPs.

In a previous study, the authors discovered a new lncRNA (named lnc-DC) located on chromosome 17 region, which near signal transducer and activator of transcription 3 (STAT3) gene (36). There were increasing researches to explore the contribution of lnc-DC in autoimmune diseases. Shaker et al. found that serum level of lnc-DC in multiple sclerosis (MS) patients were significantly increased, and serum lnc-DC level maybe used as a potential novel biomarkers for MS diagnosis (37). One of our recent studies shown that the lnc-DC expression level was significantly decreased in PBMCs from SLE patients than controls, while lnc-DC rs10515177 variant was not associated with SLE susceptibility (38). Similarly, the lnc-DC expression level was significantly lower in PBMC from
TABLE 7 | Association between lncRNA levels with their respective genotype in RA patients.

| lncRNA SNPs | Genotype | Number | lnc-DC level | P value |
|-------------|----------|--------|--------------|---------|
| rs1412830   | CC       | 49     | 0.542 (0.404, 0.742) | 0.210   |
|             | CT       | 14     | 0.418 (0.267, 0.672) |         |
|             | TT       | 2      | 0.913 (0.504, 1.322) |         |
| rs944796    | CC       | 36     | 0.585 (0.371, 0.798) | 0.437   |
|             | CT       | 14     | 0.418 (0.267, 0.672) |         |
|             | TT       | 2      | 0.913 (0.504, 1.322) |         |
| rs61271866  | TT       | 44     | 0.562 (0.404, 0.764) | 0.251   |
|             | TA       | 19     | 0.497 (0.274, 0.641) |         |
| rs2518723   | CC       | 21     | 0.603 (0.414, 0.975) | 0.329   |
|             | CT       | 28     | 0.520 (0.339, 0.646) |         |
|             | TT       | 16     | 0.476 (0.335, 0.818) |         |
| rs3217992   | CT       | 29     | 0.582 (0.472, 0.839) |         |
|             | TT       | 15     | 0.587 (0.404, 0.955) |         |

(Continued)

RA patients than normal controls in the present study. We also explore the potential association of two SNP (rs7217280, rs10515177) in lnc-DC with RA susceptibility, however, no significant relationship was found. Our study provided the first evidence that lnc-DC might be involved in the development of RA, and the specific roles of lnc-DC genetic variation in pathophysiology of RA need to be further explored.

MALAT1 was expressed on chromosome 11q13, and widely expressed in multiple normal tissues such as reproductive, endocrine and immune systems with an important role in autoimmune diseases including RA, SLE, MS (18, 19, 37, 39). Quercetin is a dietary antioxidant, which has been shown to be effective in the treatment of arthritis in pre-clinical studies, and Pan et al. tried to analyze the mechanisms responsible for the quercetin-induced FLS apoptosis in RA patients (19). Their data indicated that quercetin induced FLS apoptosis in RA patients via upregulating MALAT1, and MALAT1 promoted apoptosis by inhibiting the activation of the phosphoinositide 3-kinase (PI3K)/AKT pathway. In this study, decreased MALAT1 expression level in PBMC from RA patients was firstly reported, while MALAT1 genetic variation was not correlated with RA risk. Our findings provided new evidence that MALAT1 might be involved in RA development.

ZFAS1, located at chromosomal band 20q13.13, was reported as an important player to regulate the development of human cancers including glioma, lung, ovary, gastric, and breast cancer (40–42). In addition, ZFAS1 was found to promote chondrocytes proliferation, migration, and inhibit apoptosis and matrix synthesis in osteoarthritis (OA), and ZFAS1 expression level was downregulated in OA chondrocytes in comparison to mild chondrocytes (43). Another study by Xiao et al. also found more than five times ZFAS1 level in the healthy appearing area of cartilage compared with the pathology area in human knee osteoarthritis (44). Similarly, our results demonstrated that the expression of ZFAS1 in PBMC was significantly reduced in RA patients than normal controls, and associated with CRP in RA. In the present study, we also analyzed the potential relationship between rs237742, rs73116127, rs6125607, rs6125608 variants in ZFAS1 and genetic susceptibility to RA, and no difference achieved statistical significance. However, haplotype analysis implied that the haplotype CGTA frequency for ZFAS1 was significantly higher in RA patients than normal controls. These founding would help improve our understanding of the roles of ZFAS1 genetic variants in the pathogenesis of RA.
In conclusion, our study provided the first evidence that ANRIL, Inc-DC, MALAT1, ZFAS1 genes polymorphisms might not be associated with RA susceptibility in the Chinese population. However, several SNPs in ANRIL were related to anti-CCP in RA. In addition, alternations of ANRIL, Inc-DC, MALAT1, ZFAS1 levels and significant correlations of ZFAS1 level with CRP in RA patient demonstrated that these lncRNAs might be regarded as an auxiliary biomarker for RA diagnosis, as well as used to distinguish RA serotypes.

However, some limitations existed in our study should be acknowledged. Firstly, this study does not eliminate the potential influence of ethnic background, environmental factor. Secondly, we are not able to assess the associations between these lncRNAs levels and disease severity, clinical variables, and therapeutic schedule of RA patients over a long period in this case-control study. Finally, our sample size may not be sufficient, and lead to the low power of this study. Hence, replication studies with larger sample size, different ethnic populations are awaited to further explore the exact role of these lncRNAs in RA.

ANGLES STATEMENT

This study was approved by the Ethical Committee of Anhui Medical University (Hefei, Anhui, China). All the study subjects provided informed consent to participate in this study. All studies on humans described in the present manuscript were carried out with the approval of the responsible ethics committee and in accordance with national law and the Helsinki Declaration of 1975 (in its current, revised form).

AUTHOR CONTRIBUTIONS

T-PZ, H-FP, and D-QY designed the study. T-PZ and B-QZ conducted the experiment. S-ST and Y-GF performed the statistical analyses. T-PZ drafted the manuscript. X-ML participated in the collection of samples. H-FP and D-QY contributed to manuscript revision. All the authors approved the final submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.02529/full#supplementary-material

REFERENCES

1. Cooles FA, Isaacs JD. Pathophysiology of rheumatoid arthritis. Curr Opin Rheumatol. (2011) 23:233–40. doi: 10.1097/BOR.0b013e32834518a3

2. Hötker U, Henniger M, Rehart S. Perioperative management in patients with rheumatoid arthritis. Aktuelle Rheumatologie. (2016) 41:448–54. doi: 10.1055/s-0042-171538

3. Pan HF, Leng RX, Wu GC, Ye DQ. Advance in epidemiologic studies on major autoimmune diseases. Chin J Dis Control Prev. (2018) 22:1993–5. doi: 10.14642/j.cnki.zjzjkx.2018.11.001

4. Rossini M, Rossi E, Bernardi D, Viapiana O, Gatti D, Idolazzi L, et al. Prevalence and incidence of rheumatoid arthritis in Italy. Rheumatol Int. (2014) 34:659–64. doi: 10.1007/s00296-014-2974-6

5. Taneya V. Cytokines pre-determined by genetic factors are involved in pathogenesis of Rheumatoid arthritis. Cytokine. (2015) 75:216–21. doi: 10.1016/j.cyto.2014.11.028

6. Zhang TP, Lv TT, Xu SZ, Pan HF, Ye DQ. Association of interleukin-10 gene single nucleotide polymorphisms with rheumatoid arthritis in a Chinese population. Postgrad Med J. (2018) 94:284–8. doi: 10.1136/postgradmedj-2017-135441

7. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet. (2010) 42:508–14. doi: 10.1038/ng.582

8. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature. (2014) 506:576–81. doi: 10.1038/nature12873

9. Zhao CN, Mao YM, Liu LN, Li XM, Wang DG, Pan HF. Emerging role of lncRNAs in systemic lupus erythematosus. Biomed Pharmacother. (2018) 106:584–92. doi: 10.1016/j.biopha.2018.06.175

10. Özgür E, Mert U, Isin M, Okutan M, Dalay N, Gezer U. Differential expression of long non-coding RNAs during genotoxic stress-induced apoptosis in HeLa and MCF-7 cells. Clin Exp Med. (2013) 13:119–26. doi: 10.1007/s10238-012-0181-x

11. Li Z, Li X, Jiang C, Qian W, Tse G, Chan MTV, et al. Long non-coding RNAs in rheumatoid arthritis. Cell Prolif. (2018) 51:e12404. doi: 10.1111/cpr.12404

12. Xue Z, Cui C, Liao Z, Xia S, Zhang P, Qin J, et al. Identification of LncRNA Linc00513 containing lupus-associated genetic variants as a novel regulator of interferon signaling pathway. Front Immunol. (2018) 9:2967. doi: 10.3389/fimmu.2018.02967

13. Song J, Kim D, Han J, Kim Y, Lee M, Jin EJ. PBMC and exosome-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. Clin Exp Med. (2015) 15:121–6. doi: 10.1007/s10238-015-0271-4

14. Zhang TP, Zhang Q, Wu J, Zhao YL, Wang JR, Leng RX, et al. The expression levels of long non-coding RNAs Inc0640 and Inc5150 and its gene single-nucleotide polymorphisms in rheumatoid arthritis patients. J Cell Biochem. (2018) 119:10095–106. doi: 10.1002/jcb.27346

15. Rezazadeh M, Ghareshourjn J, Moradi M, Noroozi R, Omrani MD, Taheri M, et al. Association study of ANRIL genetic variants and multiple sclerosis. J Mol Neurosci. (2018) 65:54–9. doi: 10.1007/s12031-018-1069-3

16. Harisimendy O, Notani D, Song X, Rahim NG, Tanasa B, Heintzman N, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-y signalling response. Nature. (2011) 470:264–8. doi: 10.1038/nature09753

17. Wehr P, Purvis H, Law SC, Thomas R. Dendritic cells, T cells and their interaction in rheumatoid arthritis. Clin Exp Immunol. (2019)196:12–27. doi: 10.1111/cei.13256

18. Yang H, Liang N, Wang M, Fei Y, Sun J, Li Z, et al. Long non-coding RNA MALAT-1 is a novel inflammatory regulator in human systemic lupus erythematosus. Oncotarget. (2017) 8:77400–6. doi: 10.18632/oncotarget.20490

19. Pan F, Zhu L, Lv H, Pei C. Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating lincRNA MALAT1. Int J Mol Med. (2016) 38:1507–14. doi: 10.3892/ijmm.2016.2755

20. Ye Y, Gao X, Yang N. LncRNA ZFAS1 promotes cell migration and invasion of fibroblast-like synoviocytes by suppression of miR-27a in rheumatoid arthritis. Human Cell. (2018) 31:14–21. doi: 10.1007/s10238-017-0179-5

21. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JE, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the diagnosis of rheumatoid arthritis. Arthritis Rheum. (1988) 31:315–24. doi: 10.1002/art.1780310405
24. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, et al. A partitioning of canine brain tissue using a partitioning algorithm for clustering. *Arthritis Rheum.* (1988) 31:315–24. doi: 10.1002/art.1780310302

25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods.* (2001) 25:402–8. doi: 10.1066/meth.2001.1262

26. Solus JF, Chung CP, Oeser A, Li C, Rho YH, Bradley KM, et al. Different amounts of protein-bound citrulline and homocitrulline in foot joint tissues of a patient with anti-citrullinated protein antibody positive erosive rheumatoid arthritis. *J Transl Med.* (2013) 11:224. doi: 10.1186/1479-5876-11-224

27. Jahid M, Rehan-Ul-Haq, Chawla D, Avasthi R, Ahmed RS. Association of chromosome 9p21 variants linked to atherosclerosis. *PLoS ONE.* (2009) 4:e5027. doi: 10.1371/journal.pone.0005027

28. Wang P, Xue Y, Han Y, Lin L, Wu C, Xu S, et al. The STAT3-binding long non-coding RNA Inc-DC controls human dendritic cell differentiation. *Science.* (2014) 344:310–3. doi: 10.1126/science.1251456

29. Müller N, Döring F, Klapper M, Neumann K, Schulte DM, Türk K, et al. Interleukin-6 and tumour necrosis factor-alpha differentially regulate proliferation, apoptosis and migration of chondrocytes in osteoarthritis. *Biomed Pharmacother.* (2018) 104:825–31. doi: 10.1016/j.biopha.2018.04.124

30. Xie Y, Zhao D, Dong P, Wang H, Li D, Lai L. Effects of ANRIL polymorphisms on the likelihood of coronary artery disease: a meta-analysis. *J Cell Biochem.* (2018) 120:4620–33. doi: 10.1002/jcb.27750

31. Kong Y, Sharma RB, Nwosu BU, Alonso LC. Islet biology, the CDKN2A/B locus and type 2 diabetes risk. *Diabetologia.* (2016) 59:1579–93. doi: 10.1007/s00125-016-3967-7

32. Xie Y, Zhao D, Dong P, Wang H, Li D, Lai L. Effects of ANRIL polymorphisms on the likelihood of coronary artery disease: a meta-analysis. *J Cell Biochem.* (2018) 120:6113–9. doi: 10.1002/jcb.27898

33. Clark MB, Teo WS, Shaper AG, landmark 64 population study, UK. The 10-year incidence of type 2 diabetes in the 64 population of UK. *Diabetologia.* (2018) 61:1889–97. doi: 10.1007/s00125-018-4599-9

34. Dong D, Mu Z, Zhao C, Sun M. ZFAS1: a novel tumor-related long non-coding RNA. *Cytokine.* (2014) 63:1–7. doi: 10.1016/j.cyto.2014.03.004

35. Dong D, Mu Z, Zhao C, Sun M. ZFAS1: a novel tumor-related long non-coding RNA. *Cytokine.* (2014) 63:1–7. doi: 10.1016/j.cyto.2014.03.004

36. Askarian-Amiri ME, Crawford J, French JD, Smart CE, Smith MA, Clark MB, et al. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. *RNA.* (2011) 17:878–91. doi: 10.1261/rna.2528811

37. Ye D, Jian W, Feng J, Liao X. Role of long non-coding RNA ZFAS1 in proliferation, apoptosis and migration of chondrocytes in osteoarthritis. *Biomed Pharmacother.* (2018) 104:825–31. doi: 10.1016/j.biopha.2018.04.124

38. Xiao K, Yang Y, Bian Y, Feng B, Li Z, Wu Z, et al. Identification of differentially expressed long non-coding RNAs in human knee osteoarthritis. *J Cell Biochem.* (2018) 120:4620–33. doi: 10.1002/jcb.27750

Conflict of Interest: 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.

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