Polymorphisms of COTL1 gene identified by proteomic approach and their association with autoimmune disorders

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Abstract

To select candidate genes, we attempted to comparative analysis of protein levels between rheumatoid arthritis (RA) patients and healthy controls by two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF-MS). We identified 17 proteins that showed up- or down-regulated spots in RA patients. We found that coactosin-like 1 (COTL1) were highly expressed in RA patients compared with healthy controls. We performed a case-control study to determine whether the COTL1 gene polymorphisms were associated with RA and systemic lupus erythematosus (SLE). The genotype frequency of c.-1124G > T and the allelic frequency of c.484G > A in RA patients, and the genotype frequency of c.484G > A in SLE patients were significantly different from healthy controls (P = 0.009, 0.027, and 0.025, respectively). We also investigated the correlation with the levels of rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody in RA patients, and anti-nuclear antibodies (ANA) in SLE patients. The c.484G > A polymorphism in RA patients has significant association with the levels of anti-CCP antibody (P = 0.03). Our findings demonstrated that c.-1124G > T and c.484G > A polymorphisms of the COTL1 gene might be associated with the genetic susceptibility of autoimmune disorders.

Keywords: COTL1 protein, human; electrophoresis, gel, two-dimensional; polymorphism, single nucleotide; proteomics; rheumatoid arthritis; systemic lupus erythematosus

Introduction

Rheumatoid arthritis (RA) and systematic lupus erythematosus (SLE) are the complex autoimmune diseases which are caused by combination of multiple genetic, hormonal, and environmental factors (Gregersen, 1999; Kotzin, 1996). RA is accompanied by the presence of many autoantibodies such as rheumatoid factors (RFs), anti-cyclic citrullinated peptide (anti-CCP) antibody, and antibodies to immunoglobulin binding protein (BiP). RFs and anti-CCP antibody are used in clinical practice (Bläss et al., 1999, 2001; Schellekens et al., 2000). They can be detectable up to 70-80% in RA patients (Mewar et al., 2006; Raptopoulou et al., 2007). SLE is also characterized by the presence of antinuclear antibody (ANA) (Kurien et al., 2006).

Two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) have become a powerful method for comparative proteome analysis (O’Farrell, 1975; Wang et al., 1994; Mann et al., 1996; Lottspeich, 1999). These techniques have been used for studying RA using the samples derived from RA patients such as plasma, synovial fluid, and peripheral blood mononuclear cells. Schulz
and coworkers performed comparative proteome analysis with peripheral blood mononuclear cells (PBMCs) and found 16 proteins showed down-regulated spots in RA patients (Schulz et al., 2007).

Human coactosin-like 1 (COTL1) protein is similar to Dictyostelium discoideum coactosin (de Hostos et al., 1993). The COTL1 gene is located on chromosome 16q24.1. Human COTL1 protein is a small protein composed of 142 amino acid residues with a molecular mass of 17 kDa. Human COTL1 was also identified as a filamentous actin (F-actin) binding protein in transfected CHO and COS-7 cells (de Hostos et al., 1993) and as a 5-lipoxygenase (5LO) binding partner in a yeast two-hybrid screen (Provost et al., 2001). 5LO is the first committed enzyme of leukotrien biosynthesis. The LKKAET-like motif of COTL1 interacts with 5LO involved in leukotriene biosynthesis in the leukocytes (Provost et al., 1999, 2001; Samuelsson 1983).

In this study, we attempted to perform proteome analysis with plasma derived from RA patients and controls to screen RA candidate genes using 2-DE and MALDI-MS techniques. We identified COTL1 protein which is highly expressed in RA patients by comparative proteome analysis, and compared the mRNA expression level of COTL1 between RA patients and healthy controls by RT-PCR. To determine whether the SNPs of COTL1 gene are associated with the susceptibility of autoimmune disorders such as RA and SLE, we compared the allelic and genotypic frequencies between RA or SLE patients and healthy controls without RA and SLE. We further investigated the relationship between genotypes of each polymorphism and RF or anti-CCP antibody levels in RA patients, and ANA levels in SLE patients.

Table 1. Identification of proteins that exhibit significant differential expression between RA patients and healthy controls using MALDI-TOF/MS.

| Spot No. | Protein identity | Accession No. | Fold change (RA/Control) | Matched peptides No. | Sequence coverage (%) | Theoretical value |
|----------|------------------|---------------|--------------------------|----------------------|----------------------|-------------------|
| 1        | Coactosin-like 1  | gi 27695621   | (+) 12.2                 | 5/24                 | 38                   | 16089 5.76        |
| 2        | Vitamin D-binding protein | gi 455970 | (+) 5.9                 | 8/42                 | 25                   | 54513 5.34        |
| 3        | Leucine-rich alpha-2-glycoprotein 1 | gi 16418467 | (+) 1.6                 | 12/71                | 33                   | 38382 6.45        |
| 4        | Actin, gamma 1   | gi 54696574   | (+) 2.2                 | 26/81                | 57                   | 42108 5.31        |
| 5        | Haptoglobin      | gi 1212947    | (+) 2.4                 | 11/68                | 27                   | 38941 6.27        |
| 6        | Poly (ADP-ribose) polymerase family, member 14 | gi 7023247 | (+) 10.8                | 8/34                 | 23                   | 62781 6.37        |
| 7        | Leucine-rich alpha-2-glycoprotein 1 | gi 16418467 | (+) 1.7                 | 8/47                 | 25                   | 38382 6.45        |
| 8        | Zinc finger protein 582 | gi 21389593 | (+) 3.8                 | 10/53                | 22                   | 61827 8.79        |
| 9        | Serum amyloid A1 | gi 225986     | (+) 40.8                | 6/66                 | 66                   | 11675 5.89        |
| 10       | Transthyretin (prealbumin, amyloidosis type I) | gi 3891561 | (-) 3.2                 | 9/62                 | 92                   | 13840 5.35        |
| 11       | Transthyretin (prealbumin, amyloidosis type I) | gi 3891561 | (-) 2.7                 | 7/59                 | 74                   | 13840 5.35        |
| 12       | Apolipoprotein E | gi 17883      | Only control            | 28/66                | 72                   | 36242 5.81        |
| 13       | Fibrinogen alpha chain | gi 223057 | Only control            | 6/58                 | 47                   | 14443 6.16        |
| 14       | Galactose-1-phosphate uridyl transferase | gi 950381 | Only RA                  | 4/37                 | 97                   | 4884 6.06         |
| 15       | Hemoglobin, beta | gi 999567     | Only RA                  | 7/75                 | 64                   | 15866 6.70        |
| 16       | S100 calcium- binding protein A9 | gi 29126810 | Only RA                  | 8/86                 | 60                   | 13291 5.71        |
| 17       | Keratin 9        | gi 453155     | Only RA                  | 8/67                 | 22                   | 62178 5.14        |

Results

We performed proteome analysis using the plasma isolated from RA patients and healthy controls by 2-DE and MALDI-TOF MS technique to find RA specific proteins. More than 1500 spot images were detected on the 2-DE gel, localized in the ranges of pl 3 to 10 and Mr 10 to 200 kDa (Figure 1). The isolated spots in 2-DE were digested with trypsin and then analyzed by MALDI-TOF MS. We chose and identified 17 proteins as the candidate proteins which showed different expressions between RA patients and healthy controls (Table 1). While the two proteins out of them were down-
Figure 1. Comparative proteome analysis of sera from healthy controls and RA patients. (A) 2-DE gel images of plasma from healthy controls. (B) 2-DE gel images of plasma from RA patients. Each number indicates a protein that was up- or down-regulated in the expression level. Samples were separated on a nonlinear pH 3 to 10 IPG strip followed by a 9 to 10% linear gradient polyacrylamide gel. Each gel was stained with Coomassie blue G250. The data are representatives from five independent experiments.

Figure 2. Expression levels and patterns of COTL1 mRNA. (A) The expression levels of COTL1 mRNA for 4 RA patients and 4 healthy controls. (B) The expression patterns of COTL1 mRNA in various human tissues. Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas; 9, spleen; 10, thymus; 11, prostate; 12, testis; 13, ovary; 14, small intestine; 15, colon; and 16, peripheral leukocytes. The data are representatives from three independent experiments.

Regulated, nine proteins were up-regulated in RA patients (Table 1). Apolipoprotein E and fibrinogen alpha chain were spots only in healthy control, whereas the galactose-1-phosphate uridyl transferase, hemoglobin-β, S100 calcium-binding protein A9, and keratin 9 were spots only in RA patient (Table 1). Fibrinogen β-chain degradation products, calgranulin B (MRP14) in synovial fluids (SF), and serum amyloid A protein were identified in the plasma and SF from RA patients (Sinz et al. 2002). The level of serum amyloid A1 (SAA1) in RA patient was significantly higher than that of healthy control (Table 1). We focused on COTL1 whose expression level was more than 12.2 fold higher in RA patients compared with healthy controls. For validation of 2-DE comparison results, the expression levels of COTL1 were compared between four RA patients and four healthy controls by RT-PCR. The result showed that the expression levels of COTL1 were much higher in RA patients (Figure 2A). We also further examined the expression pattern of COTL1 in 15 normal human tissues and peripheral leukocytes. While the expression levels of COTL1 in heart, brain, and skeletal muscle were only barely detected, those of COTL1 gene in kidney, spleen, and peripheral leukocytes were highly detected (Figure 2B). Interestingly, the expression levels of COTL1 were highest in peripheral leukocytes. This result leads us to think that the up-regulated COTL1 expression in peripheral leukocytes might be related to the inflammatory responses of RA pathogenesis.
To determine whether the COTL1 gene variations are associated with the susceptibility of autoimmune disorders such as RA and SLE, we chose four SNPs of COTL1 gene, c.484G > A (rs13521), c.588C > T (rs247862), and c.1050T > A (rs1047121) in the 3' UTR, and c.-1124G > A (KN0013308) in the promoter region, from NCBI SNP database and KSNP database (http://ksnp.ngri.go.kr). We analyzed the genotypes and allele frequencies of these SNPs between healthy controls and RA or SLE patients (Table 2). The genotype frequencies of all loci were in HWE (P > 0.05, data not shown). The genotype frequency of c.-1124G > T and the allelic frequency of c.484G > A in RA patients were significantly different from those of control group (P = 0.009 and 0.027, respectively). The genotype frequency of c.484G > A were also significantly different between healthy controls and SLE patients (P = 0.025). These results indicate that the polymorphisms of COTL1 may be associated with the susceptibility of autoimmune disorders such as RA and SLE.

We further attempted to analyze the possible correlation between the polymorphisms and the clinical features of RA and SLE. Although these polymorphisms in RA patients are shown to have no significant association with RF levels, the c.484G > A polymorphism of COTL1 is significantly associated with the levels of anti-CCP antibody (P = 0.03) in RA patients (Table 3). We also calculated and compared the haplotype frequencies by c.-1124G > A, c.484G > A, c.588C > T, and c.1050T > A SNPs of COTL1 between controls and RA or SLE patients. While five major haplotypes (more than 5%) revealing more than 86.1% of distributions are identified in controls, four major haplotypes (~88.1%) in RA patients and three major haplotypes (~88.2%) in SLE patients are identified out of sixteen possible haplotypes (Table 4). The distribution rate of the haplotype GGCA is significantly different between control group and RA or SLE patients (P = 0.021 and 0.044, respectively). Further the haplotype GGTT also shows a significant association with SLE (P = 0.038).

Discussion

RA and SLE are representative complex autoimmune diseases. RA is a common organ specific disorder with a prevalence 500 per 100,000 and SLE is a rare non organ-specific disorder with a prevalence 50 per 100,000. COTL1 polymorphisms are significantly associated with the levels of anti-CCP antibody (P = 0.03) in RA patients. We further attempted to analyze the possible correlation between the polymorphisms and the clinical features of RA and SLE. Although these polymorphisms in RA patients are shown to have no significant association with RF levels, the c.484G > A polymorphism of COTL1 is significantly associated with the levels of anti-CCP antibody (P = 0.03) in RA patients (Table 3). We also calculated and compared the haplotype frequencies by c.-1124G > A, c.484G > A, c.588C > T, and c.1050T > A SNPs of COTL1 between controls and RA or SLE patients. While five major haplotypes (more than 5%) revealing more than 86.1% of distributions are identified in controls, four major haplotypes (~88.1%) in RA patients and three major haplotypes (~88.2%) in SLE patients are identified out of sixteen possible haplotypes (Table 4). The distribution rate of the haplotype GGCA is significantly different between control group and RA or SLE patients (P = 0.021 and 0.044, respectively). Further the haplotype GGTT also shows a significant association with SLE (P = 0.038).

Table 2. Genotype and allele analysis of the SNPs in COTL1 gene between controls and RA or SLE patients.

| Position | Genotype/Allele | Control, n (%) | RA, n (%) | SLE, n (%) | Odds ratio (95% CI) | P value |
|----------|----------------|----------------|-----------|-----------|---------------------|---------|
| c.-1124G > T, (KN0013308) | GG | 442 (78.0) | 146 (78.1) | 1.00 | 1.00 | 0.009 | 0.127 |
|          | GT | 122 (21.5) | 37 (19.8) | 1.09 (0.81-1.47) | 0.918 (0.61-1.39) |
|          | TT | 3 (0.5) | 2 (2.1) | 5.72 (1.62-20.23) | 4.037 (0.89-18.25) |
|          | G  | 1006 (88.7) | 322 (88.0) | 1.00 | 1.00 | 0.059 | 0.64 |
|          | T  | 128 (11.3) | 45 (12.0) | 1.30 (1.00-1.68) | 1.08 (0.75-1.54) |
| c.484G > A, (rs13521) | GG | 322 (56.9) | 103 (56.6) | 1.00 | 1.00 | 0.097 | 0.025 |
|          | GA | 197 (34.8) | 74 (40.7) | 0.82 (0.63-1.08) | 1.17 (0.83-1.66) |
|          | AA | 47 (8.3) | 5 (2.7) | 0.62 (0.37-1.03) | 0.33 (0.13-0.86) |
|          | G  | 841 (74.3) | 280 (76.9) | 1.00 | 1.00 | 0.027 | 0.331 |
|          | A  | 291 (25.7) | 84 (23.1) | 0.79 (0.64-0.97) | 0.87 (0.66-1.15) |
| c.588C > T, (rs247862) | CC | 305 (54.3) | 103 (53.9) | 1.00 | 1.00 | 0.937 | 0.512 |
|          | CT | 218 (38.8) | 70 (36.7) | 0.96 (0.74-1.24) | 0.95 (0.67-1.35) |
|          | TT | 39 (6.9) | 18 (9.4) | 0.96 (0.58-1.59) | 1.37 (0.75-2.49) |
|          | C  | 828 (73.7) | 276 (72.3) | 1.00 | 1.00 | 0.76 | 0.593 |
|          | T  | 296 (26.3) | 106 (27.7) | 0.97 (0.79-1.18) | 1.07 (0.83-1.39) |
| c.1050T > A, (rs1047121) | TT | 509 (89.6) | 177 (93.2) | 1.00 | 1.00 | 0.763 | 0.354 |
|          | TA | 59 (10.4) | 13 (6.8) | 0.85 (0.56-1.30) | 0.73 (0.46-1.18) |
|          | AA | 0 (0) | 0 (0) | - | - | 0.536 | 0.209 |
|          | T  | 1077 (94.8) | 367 (96.6) | 1.00 | 1.00 | 0.86 (0.57-1.30) | 0.65 (0.35-1.19) |

aCalculated from the translation start site; bValue was determined by χ2 test from 2 × 3 contingency table; cLogistic regression analysis were used to estimate odds ratio (95% confidence interval).
Table 3. Analysis of RF and anti-CCP antibody levels in RA patients and ANA levels in SLE patients among the genotypes of COTL1 polymorphisms.

| Position* | Genotype | n | Mean S.D. | n | Mean S.D. | n | Mean S.D. | p*
|-----------|----------|---|-----------|---|-----------|---|-----------|
| c.-1124G>T (rs13521) | GG | 329 | 71.8 73.0 | 0.15 | 184 | 55.0 52.1 | 0.69 | 89 | 775.7 2.46E+03 | 0.54
| GT | 100 | 64.1 75.8 | 51 | 51.4 49.3 | 23 | 452.2 677 |
| TT | 13 | 105.9 94.3 | 5 | 71.7 49.2 | - | - |
| c.484G>A (rs247862) | GG | 273 | 69.8 74.9 | 0.72 | 154 | 49.8 49.4 | 0.03 | 67 | 838.5 2.79E+03 | 0.35
| GA | 137 | 73.4 73.4 | 73 | 66.6 52.9 | 41 | 415.6 786 |
| AA | 25 | 60.7 77.0 | 10 | 35.1 45.9 | - | - |
| c.588C>T (rs1047121) | CC | 247 | 66.7 71.1 | 0.29 | 131 | 55.7 51.4 | 0.80 | 63 | 536.2 1.36E+03 | 0.36
| CT | 170 | 74.8 77.5 | 95 | 54.3 51.2 | 43 | 1036.7 3.17E+03 |
| TT | 31 | 85.6 80.8 | 18 | 47.0 53.6 | 13 | 209.2 76.9 |
| c.1050T>A (rs1047121) | TT | 408 | 71.6 74.3 | 0.65 | 221 | 54.5 50.7 | 0.98 | 109 | 704.8 2.24E+03 | 0.83
| TA | 40 | 66.0 74.9 | 23 | 54.2 58.3 | 7 | 525.7 903 |
| AA | 0 | - | - | 0 | - | - |

*Calculated from the translation start site; **Value were analyzed by ANOVA.

Table 4. The haplotype frequencies between RA or SLE patients and controls in COTL1 polymorphisms.

| Haplotype | Frequency* | p** |
|-----------|------------|-----|
| c.-112G>T | c. 484G>A | c.588C>T | c.1050T>A |
| G G C T | 0.422 0.428 | 0.431 0.793 | 0.768 |
| G G T T | 0.199 0.217 | 0.251 0.330 | 0.038 |
| G A C T | 0.186 0.177 | 0.168 0.589 | 0.428 |
| T G C T | 0.054 0.059 | 0.032 0.597 | 0.102 |
| G G C A | 0.050 0.029 | 0.025 0.021 | 0.044 |
| T G T T | 0.026 0.038 | 0.024 0.121 | 0.014 |
| T A C T | 0.027 0.034 | 0.054 0.383 | 0.867 |
| Others | 0.036 0.018 | 0.015 - | - |

*Values were constructed by the expectation maximization (EM) algorithm with genotyped SNPs; **Values were analyzed by the permutation test.

prevalence 10 to 50 per 100,000 (Yamada et al., 2005). The pathogenesis of autoimmune diseases comes from epidemiological and genetic evidence for overlapping pathogenesis. The several susceptibility loci are present for multiple autoimmune diseases. The relationship might simply be explained by increased lymphocyte proliferation increasing somatic mutations. However a similarly elevated risk is not seen in other chronic immune responses, such as allergic disease or inflammatory bowel disease. Therefore, when genetic studies are conducted, it would be better to be investigated on several diseases together than only on single disease. The reason why we have selected RA and SLE is that RA is a representative Th1 dominant disease while SLE is a Th2 skewed disease. Therefore we can speculate certain SNPs are associated with Th1 diseases or Th2 diseases. Then further studies on the other diseases that belong to each group could be conducted. We have identified several SNPs which were associated with susceptibility to RA or SLE in Tim-3, Tim-1, Eotaxin-3, and FOXJ1 genes through the case-control association study in Korean population (Chae et al., 2004, 2005a, b; Li et al., 2007).

In this study, we attempted a proteomic approach such as 2-DE and MALDI-TOF MS to find the candidate molecules for RA pathogenesis. We identified the COTL1 as a RA candidate gene that is highly expressed in peripheral leukocytes of RA patients (Figure 2). According to recent studies, Spanbroek and co-workers reported 5LO is expressed in several types of leukocytes, including dendritic cells (Spanbroek et al., 2000). LTC4, LTD4, and LTE4 are called cysteinyl leukotrienes due to their structures. Their receptors (CysLT1 and
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CysLT2) on mast cells can mediate proinflammatory activities such as endothelial cell adhesion and production of chemokine by mast cells, and other inflammatory disorders such as asthma, RA, inflammatory bowel disease (Bailey, 1985; Sharma et al., 2006). Rakonjac and coworkers reported that COTL1 up-regulates Ca2+-induced 5LO activity as a scaffold of 5LO in the absence of phosphatidylycholine by in vitro assays (Rakonjac et al., 2006). Mathis and co-workers demonstrated LTB4 receptors play a critical role in the recruitment of leukocytes to the inflammatory sites in a mouse model of RA (Mathis et al., 2007). Thus, our results suggest that up-regulation of COTL1 may affect the 5LO activity involved in LT biosynthesis, and mediate inflammation in RA.

According to results of our case-control association study, the genotype frequency of c.-1124G > T and the allelic frequency of c.484G > A in RA patients, and the genotype frequency of c.484G > A in SLE patients were significantly different from healthy controls (P = 0.009, 0.027, and 0.025, respectively). These results suggest that the c.-1124G > T polymorphism of COTL1 gene promoter region might be one of the important genetic factors in RA susceptibility. The polymorphisms within the binding site of the promoter region might have influence on the expression level by regulation of the binding between the specific transcriptional binding site and the transcription factor. Accordingly, these polymorphisms might have an influence on susceptibility to autoimmune responses. Our results might be important in future studies to determine whether or not the c.-1124G > T polymorphism affects the levels of COTL1 gene expression. Our result also showed that the c.484G > A polymorphism of COTL1 is significantly associated with the levels of anti-CCP antibody (P = 0.03) in RA patients (Table 3). This result suggests that the polymorphisms of the COTL1 might be associated with anti-CCP antibody levels in RA patients.

In conclusion, our results demonstrate that c.-1124G > T in the promoter region and c. 484G > A polymorphism in the 3′ UTR of COTL1 gene might be associated with susceptibility to RA and SLE in Korean population.

Methods

Plasma sample preparation for 2-DE

Subjects were selected based on the criteria approved by the outpatient clinic at Eulji University Hospital. Characteristics of RA patient and healthy control subjects are shown in (Supplemental Data Table S1) Blood was collected in tubes with appropriate concentration of lithium heparin from both healthy controls and RA patients, and centrifuged at 2,500 rpm for 15 min. Plasma was separated into 250 μl aliquots and stored at -70°C. Depilation of high-abundance proteins from plasma was performed using a Multiple Affinity Removal Column (Agilent, Wilmington, DE). The 2-DE procedure was performed as described previously (Cho et al., 2005). Protein samples were suspended in sample buffer (7M urea, 2M thiourea, 4.5% Chaps, 100 mM DTE, 40 mM Tris, pH 8.8).

2-DE/ Image analysis/ Trypsin digestion

2-DE, image analysis (spot detection, quantification, and matching), and in-gel trypsin digestion were performed as described (Park et al., 2002a, b). 2-DE gels were scanned by a Bio-Rad GS-710 densitometer (Hercules, CA) and converted into electronic files, which were then analyzed with the ImageMaster 2D Platinum Software version 5.0 (Amersham Bioscience).

Identification of proteins by MS

For 2-D gel mapping of the plasma proteome, proteins were identified by mass fingerprinting or matching with various internal 2-DE maps. Protein spots excised from 2-D gels were destained, reduced, and alkylated and then digested with trypsin (Promega, Madison, WI) as previously described (Shchepchenko et al., 1996). Tryptic peptides were desalted and purified as described (Cho et al., 2002). Recovered peptides were prepared for MALDI-TOF-MS by mixing with alpha-cyano-4-hydroxy cinnamic acid, 1% formic acid in 50% acetonitrile, and droplets were allowed to dry on the MALDI sample plate. Peptide mass fingerprinting was performed using a Voyager DE-PRO MALDI-TOF MS (Applied Biosystems, Foster City, CA), operating in delayed reflector mode. Proteins were identified from the peptide mass maps using MS-FIT (http://prospector.ucsf.edu), MASCOT (http://www.matrixscience.com/search_form_select.html), and ProFound (http://129.85.19.192/profound_bin/WebProFound.exe) to search for the protein databases, SWISS_PROT (Version 44.1) and GenBank.

Subjects and DNA samples

After institutional review board approval and informed consent, blood samples and records were obtained from 568 controls (213 females, 355 males) without RA and SLE, 455 RA patients (371 females, 84 males), and 196 SLE patients (184 females, 12 males). The mean age of controls, RA patients, and SLE patients was 40.3, 53.3, and 34 years, respectively. Genomic DNA was extracted from leucocytes in peripheral blood by a standard phenol-chloroform method or by using the Genomic DNA Extraction kit (iNIRON Biotechnology, Korea) according to the manufacturer’s instructions. The RA and SLE patients were recruited from the outpatient clinic at Eulji University Hospital and Chonnam national University Hospital. RA and SLE were diagnosed according to criteria of the American Rheumatism Association and the American College of Rheumatology (ACR), respectively (Arnett et al.,...
1988; Hochberg, 1997). Anti-CCP antibody and RF levels in RA patients and ANA levels in SLE patients were determined in a routine laboratory at Eulji University Hospital and Chonnam National University Hospital. The non-RA controls were recruited from the general population and had received comprehensive medical testing at Wonkwang University Hospital. All subjects in this study were Korean.

RNA extraction/RT-PCR/PCR

For total RNA, human peripheral blood mononuclear cells (PBMC) were isolated fromuffy coats by density gradient centrifugation using Ficoll-Paque Plus (Amersham Biosciences, Sweden), washed with PBS (GIBCO BRL, Invitrogen), and lysed in TRIzol reagent (Invitrogen) according to the instructions of the manufacture. Approximately 1 μg of total RNA was used in the first-strand cDNA synthesis with a sequence specific primer using the M-MLV reverse transcriptase (Bioneer, Korea) for RT-PCR. PCR was performed using hot start Taq DNA polymerase (HS Prime Taq, Genet Bio, Korea), and 0.5 μM of each primer (Supplemental Data Table S2) under the following condition: 10 min at 94°C for initial denaturation, 28 cycles of PCR consisting of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, extension for 30 s at 72°C, and a final extension for 5 min at 72°C in a thermocycler (Gene Amp PCR system 9700, PE Applied Biosystems). The expression assay for several tissues was performed using multiple human tissue cDNA (Clontech).

HRM analysis

The SNPs information of COTL1 gene derived from the NCBI SNP database and KSNP database system (http://www.ksnp.ngr.go.kr). Genotyping was performed by high resolution melting (HRM) analysis for c.484G>T, c.588C>T, and c.1050T>A. The 10 μl reaction mixture was made up using the 1 × QuantiTect Probe PCR Kit (Qiagen) and consisted of 50 ng of genomic DNA, 100 nM of each primer (Supplemental Data Table S2), and 1 × EvaGreen solution (Biotium). PCR cycling and HRM analysis was carried out using the Rotor-Gene thermal cycler RG6000 (Corbett Research, Australia). The samples were read and analyzed using the software Rotor-Gene 1.7.40 (Corbett Research, Australia). The reference sequence of COTL1 was based on human chromosome 16 clone RP11-61F12.

Statistical analysis

The healthy controls and RA or SLE patients were compared by case-control association analyses. χ² tests were used to estimate the Hardy-Weinberg equilibrium (HWE). The haplotype frequencies of COTL1 for multiple loci were estimated using the expectation maximization (EM) algorithm with SNPAlyze software (DYNACOM, Japan). The ANOVA was applied to analyze differences among the each genotype, and anti-CCP antibody, RF levels in RA patients or ANA levels in SLE patients. A P value of less than 0.05 was considered to indicate statistical significance.

Supplemental Data

Supplemental Data include two tables and can be found with this article online at http://e-emm.or.kr/article/article_files/SP-41-5-08.pdf.

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References

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24
Bailey JM. Biochemistry, Mechanism of Action, and Clinical Applications. In Prostaglandins Leukotrienes and Lipoxins, 1985, 583-91, Plenum Publishing Co, New York
Bläss S, Engel JM, Burmester GR. The immunologic homunculus in rheumatoid arthritis. Arthritis Rheum 1999;42:2499-506
Bläss S, Union A, Raymackers J, Schumann F, Ungethum U, Müller-Steinbach S, De Keyser F, Engel JM, Burmester GR. The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. Arthritis Rheum 2001; 44:761-71
Chae SC, Park YR, Shim SC, Yoon KS, Chung HT. The polymorphisms of Th1 cell surface gene Tim-3 are associated in a Korean population with rheumatoid arthritis. Immunol Lett 2004;95:91-5
Chae SC, Park YR, Shim SC, Lee IK, Chung HT. Eotaxin-3 gene polymorphisms are associated with rheumatoid
Arthritis in a Korean population. Hum Immunol 2005a; 66:314-20

Chae SC, Park YR, Song JH, Shim SC, Yoon KS, Chung HT. The polymorphisms of Tim-1 promoter region are associated with rheumatoid arthritis in a Korean population. Immunogenetics 2005b;56:696-701

Cho SY, Park KS, Shim JE, Kwon MS, Joo KH, Lee WS, Chang J, Kim H, Chung HC, Kim HO, Paik YK. An integrated proteome database for two-dimensional electrophoresis data analysis and laboratory information management system. Proteomics 2002;2:1104-13

Cho SY, Lee EY, Lee JS, Kim HY, Park JM, Kwon MS, Park YK, Lee HJ, Kang MJ, Kim JY, Yoo JS, Park SJ, Cho JW, Kim HS, Paik YK. Efficient prefractionation of low-abundance proteins in human plasma and construction of a two-dimensional map. Proteomics 2005;5:3386-96

de Hostos EL, Bradtke B, Lottspeich F, Gerisch G. Coactosin, a 17 kDa F-actin binding protein from Dictyostelium discoideum. Cell Motil Cytoskeleton 1993;26:181-91

Gregersen PK. Genetics of rheumatoid arthritis: confronting complexity. Arthritis Res 1999;1:37-44

Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725

Kotzin BL. Systemic lupus erythematosus. Cell 1996;85: 303-6

Kurien BT, Scofield RH. Autoantibody Determination in the Diagnosis of Systemic Lupus Erythematosus. Scand J Immunol 2006;64:227-35

Li CS, Zhang Q, Lim MK, Sheen DH, Shim SC, Kim JY, Lee SS, Yun KJ, Moon HB, Chung HT, Chae SC. Association of FOXJ1 polymorphisms with systemic lupus erythematosus and rheumatoid arthritis in Korean population. Exp Mol Med 2007;51:292-7

Lottspeich F. Proteome Analysis: A Pathway to the Functional Analysis of Proteins. Angew Chem Int Ed Engl 1999;38:2476-92

Mann M, Talbo G. Developments in matrix-assisted laser desorption/ionization peptide mass spectrometry. Curr Opin Biotechnol 1994;7:77-84

Mathis S, Jala VR, Haribabu B. Role of leukotriene B4 receptors in rheumatoid arthritis. Autoimmun Rev 2007;6:12-7

Mewar D, Wilson AG. Autoantibodies in rheumatoid arthritis: a review. Biomed Pharmacother 2006;60:648-55

O’Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem 1975;250:4007-21

Park KS, Cho SY, Kim H, Paik YK. Proteomic alterations of the variants of human aldehyde dehydrogenase isozymes correlate with hepatocellular carcinoma. Int J Cancer 2002a;97:261-5

Park KS, Kim H, Kim NG, Cho SY, Choi KH, Seong JK, Paik YK. Proteomic analysis and molecular characterization of tissue ferritin light chain in hepatocellular carcinoma. Hepatology 2002b;35:1459-66

Provost P, Samuelsson B, Rådmark O. Interaction of 5-lipoxygenase with cellular proteins. Proc Natl Acad Sci USA 1999;96:1881-5

Provost P, Doucet J, Hammarberg T, Gerisch G, Samuelsson B, Rådmark O. 5-Lipoxgenase Interacts with Coactosin-like Protein. J Biol Chem 2001;276:16520-7

Rakonjac M, Fischer L, Provost P, Werz O, Steinhilber D, Samuelsson B, Rådmark O. Coactosin-like protein supports 5-lipoxgenase enzyme activity and up-regulates leukotriene A4 production. Proc Natl Acad Sci USA 2006;103: 13150-5

Raptopoulou A, Sidiropoulos P, Katsourakis M, Boumpas DT. Anti-citrulline antibodies in the diagnosis and prognosis of rheumatoid arthritis: evolving concepts. Crit Rev Clin Lab Sci 2007;44:339-63

Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 1983;220:568-75

Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63

Schulz M, Dotlaw H, Mikkat S, Eggert M, Neecck G. Proteomic analysis of peripheral blood mononuclear cells: selective protein processing observed in patients with rheumatoid arthritis. J Proteome Res 2007;6:3752-9

Sharma JN, Mohammed LA. The role of leukotrienes in the pathophysiology of inflammatory disorders: is there a case for revisiting leukotrienes as therapeutic targets? Inflammopharmacology 2006;14:10-6

Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Shevchenko A, Boucherie H, Mann M. Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad Sci USA 1996;93:14440-5

Singh A, Bantscheff M, Mikkat S, Ringel B, Drynda S, Kekow J, Thiesen HJ, Glocker MO. Mass spectrometric proteome analyses of synovial fluids and plasmas from patients suffering from rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis. Electrophoresis 2002;23: 3445-56

Spanbroek R, Hildner M, Steinhibliner D, Fusenig N, Yoneda K, Rådmark O, Samuelsson B, Habenicht AJ. 5-lipoxygenase expression in dendritic cells generated from CD34(+) hematopoietic progenitors and in lymphoid organs. Blood 2000;96:3857-65

Wang R, Chait BT. High-accuracy mass measurement as a tool for studying proteins. Biotechnol 1994;5:77-84

Yamada R, Ynamoto K. Recent findings on genes associated with inflammatory disease. Mutat Res 2005;573: 136-51