The association of NLRP3 p.Q705K and CARD8 p.C10X genetic polymorphisms with rheumatoid arthritis: A review of the literature

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Abstract
Many genetic factors are involved in the pathogenesis of rheumatoid arthritis (RA). This study is aimed to summarize the literature regarding the relationship between variation in the genes encoding NLRP3/CARD8 and RA, using a candidate polymorphism approach. Several investigations have been conducted on the association of the genetic alterations of the NLRP3 p.Q705K and CARD8 p.C10X with the inflammatory diseases. Although there are variable results, the role of inflammatory pathways and genes involved is undeniable. The genetic context of the NLRP3 p.Q705K and CARD8 p.C10X gene variants in the pathogenesis of RA is still unclear to confirm that these variations show a pivotal role in the pathophysiology of RA. More comprehensive studies in this area seem to be needed.

Keywords: rheumatoid arthritis, polymorphism, inflammasome, CARD8, NLRP3

1. Rheumatoid arthritis
Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder associated with chronic arthritis and the progression of joint damage. The general characteristics of RA diseases include synovial inflammation, hyperplasia, rheumatoid factor production (RF), anti-citrullinated protein antibodies (ACPAs), and cartilage and bone destruction, as well as systemic complications including cardiovascular, pulmonary, and skeletal disorders.1 Unlimited growth of disease in the articular and skeletal system has caused major systemic problems for patients, and cardiovascular disorders and other systemic complications are a major challenge for these patients.2 About 0.5-1% of the population are affected by RA. The disease is seen generally between the age of 50-65 years and it is three times more common among women than men.3

Articular and skeletal manifestations of the disease have caused major systemic problems for patients, cardiovascular disorders and other systemic complications are a major challenge for these patients.

2. Genetic and environmental factors in RA
Although no factor has yet been identified as the principal initiator of the disease, several pieces of evidence suggest that environmental factors, along with the genetic and epigenetic background, are the main causes of the disease. In general, the interaction between the environment and genetic factors plays a critical role in the pathophysiology of RA.4 Many hypotheses suggest that infection has a significant role in the onset of the disease. Infectious factors such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), Escherichia coli (E. coli), and Proteus species are linked to the disease. Studies in lad models have shown that digestive tract microbes (stomach and intestines) are involved in the development of RA. It appears that inflammatory reactions caused by infective agents in the joints of the patients can damage the host tissues.5, 6 Besides, the findings show that respiratory stress, including cigarette smoking, increases the risk of the disease among susceptible individuals.7, 8

Most of the studies have stated a significant association between the familial RA and the development of RA. This association is prominent in the first-degree relatives of affected subjects. The relative risks (λ) for RA were estimated to be 3.88 for RA-affected parents and 4.38 for RA-affected siblings.9 Genetic diversity or polymorphisms have shown to be potentially related to various diseases, as many hypotheses suggest that genetic polymorphisms...
are crucial factors in the pathophysiology and susceptibility to the disorder, and play an essential role in the disease progression rate.  

More than 70 different genetic variants associated with RA have been identified, among them, the locus of HLA is the most effective genetic factor accounting for about 50% of the genetic susceptibility to the disease. More than 22 members of the HLADR4 subgroup have been known, of which only a few were associated with RA. Additionally, the association of major histocompatibility complex class II, DR beta 1 (HLADRB1) with the disease has been confirmed in patients who are positive for RF or ACPA. The hypothesis to justify this relation is the presence of a usual sequence of 67-74 amino acids, called the ‘Shared epitope’ in all of the alleles found in HLADRB1 in RA patients who have a particular ability to develop the disease. Gene risk factors for ACPA-negative patients are less important than ACPA- positive, as the known risk alleles in the ACPA-positive are constantly added. With newer techniques such as genome-wide association studies (GWAS) and arrays of polymorphisms, many non-HLA loci have been known to affect RA. Some of the known susceptible loci in RA, along with their functional significance, are listed in Table 1. These gene variants include polymorphisms located in the coding or non-coding regions of the genome. While most of these variants increase the risk or susceptibility to RA, others are considered as markers for the progression of the disease and generally can help in the diagnosis and treatment of the disease.

RA creates a complex interaction between the environment and genetics. For example, smoking and other respiratory stress increase the risk of developing HLADR4 susceptibility. The interaction of the gene-environment through the loss of immune tolerance in lymphocytes and dendritic cells, as well as secondary lymphoid tissues and bone marrow, results in the release of autoimmune ACPA antibodies and RF. These results in inflammatory responses on the joints causing cartilage demolition and bone erosion. Indeed, synovitis starts with a positive cycle leading to the induction of systemic disorders in RA syndrome.

3. Inflammasomes
Inflammasomes, which are known as pattern recognition receptors (PRRs), are one of the most important receptors of the innate immune system that can detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). These receptors, which are expressed by innate immune cells, are generally divided into four main families including toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), and NOD-like receptors (NLRs). TLRs/CLRs and NLRs/RLRs are classified as membrane receptors and cytosolic receptors respectively. NLRs, as the largest group of cytosolic receptors in innate immune cells, consists of three domains. The c-terminal domain (leucine-rich repeats,LRRs) acts as recognizer domain and detect microbial molecules or endogenous mediators caused by cellular stress. The central domain (NATCH) plays a key role in the oligomerization and formation of the central structure of the inflammasome complex, and ultimately the functional domain located in the N-terminal end. The N-terminal domain consists of a caspase recruitment domain (CARD), pyrin domain (PYD), and acidic trans activating domain or baculovirus inhibitor repeats (BIRs). Thus, NLR proteins, which include the PYD domain, belongs to the NLRP family, and those containing the CARD domain belongs to the NLRC family, and the next group belongs to the NLRB family. Some members of the NLRs family, accompanied by absent in melanoma 2 (AIM2, the only cytoplasmic member of the PYHIN protein family), are involved in the formation of a cytoplasmic multiprotein oligomers complex called inflammasomes. These are responsible for the activation of inflammatory responses.

There are many genetic investigations concerning the association between inflammasomes and RA pathogenesis. For instance, it has shown that gene expression of NLRP3 inflammasome components, including NLRP3, ASC, and caspase-1, is significantly higher in RA subjects than healthy controls. Genetic analysis regarding the NLRP1 inflammasome and RA pathogenesis was also conducted in many populations. Sui et al. reviewed whether the NLRP1 gene polymorphisms are associated with susceptibility to RA and indicated that these polymorphisms are critical risk factors for RA, as they cause an up-regulation in the NLRP1 gene expression.

These surveys show that the genetic variants encoding inflammasomes are associated with RA pathogenesis. In another study, the function of caspase-1 in RA was analyzed in an animal model. The results showed that inflammation of joints and cartilage degradation was significantly reduced in caspase-1–/– mice induced with chronic arthritis, indicating that caspase-1 plays a significant role in RA pathogenesis.
### Table 1. Candidate genes with single-nucleotide polymorphism (SNPs) associated with RA, and their function in the pathogenesis of the disease

| Gene     | Variations | SNP locus | Phenotype                                                                 |
|----------|------------|-----------|---------------------------------------------------------------------------|
| HLA-DRB1 | ---        | 6p21      | HLA DRB1 allele (also known as the shared epitope) involved in MHC molecule–based antigen presentation and responsible for self-peptide selection and T-cell repertoire; first discovered and still by far the strongest genetic link to rheumatoid arthritis13 |
| PTPN22  | rs2476601  | 1p13.2    | ACPA-positive RA with worse prognosis54                                      |
| CD40    | rs6032662  | 20q13.12  | Increased risk for ACPA-positive RA55                                        |
| TAGAP   | rs629326   | 6q25.3    | Increased risk for ACPA-positive RA55                                        |
| IL2     | rs6822844  | 4q27      | Cytokine that regulates activation of T cells, particularly regulatory T cells56 |
| IL2RA   | rs2104286  | 10p15.1   | High-affinity receptor for interleukin-2 on lymphocyte subsets56             |
| DNMT3B  | rs2228611  | 283C/T    | Influences progression of joint destruction in RA57                        |
| STAT4   | rs7574865  | 2q32.3    | NR58                                                                       |
| IL6R    | rs8192284  | 1q21.3    | Influences disease activity of ACPA-positive RA56                          |
| CCR6    | rs3093023  | 6q27      | Increased RA susceptibility60                                               |
| CTLA4   | rs1863800  | 2q33.2    | NR51                                                                       |
| REL     | rs5771069  | 2p16.1    | Proto-oncogene member of the NF-kB family that regulates leukocyte activation and survival62 |
| TNFAIP3 | rs9376293  | 6q23.3    | Increased rate of joint destruction in ACPA-positive RA53                   |
| TAGAP   | rs629326   | 6q25.3    | Increased rate of joint destruction in ACPA-positive RA54                   |
| ANKRD55 | rs6859219  | 5q11.2    | Increased risk for ACPA-positive RA55                                        |
| CARD8   | C10X/rs2043211 | 19q13.33 | Worse disease course in early RA60                                          |
| GATSL3  | rs1043099  | 22q12.2   | Increased RA susceptibility28                                               |
| IRAK1   | rs13397    | Xq28      | Increased RA susceptibility28                                               |
| RASGRP1 | rs8043085  | 15q14     | Increased risk for ACPA-positive RA55                                        |
| SPRED2  | rs934734   | 2p14      | Increased risk for ACPA-positive RA55                                        |

Abbreviations: NR, not reported

### 4. The NLRP3 inflammasome and IL-1β activation

Over the past ten years, families of receptors that drive pro-inflammatory cytokines production in infections and tissue injuries are described. One of the main classes that have studied about the inflammation of joint diseases is NLRs. Meanwhile, the NLR family pyrin domain containing 3 (NLRP3) has been introduced as a key NLR for the production of interleukin 1 beta (IL-1β)25, 26. IL-1β is produced as an inactive cytoplasmic precursor (P35 Pro-IL-1β), which must be cleaved into the mature active form (P17). Cleavage of pro-IL-1β is catalyzed by cysteine protease caspase-1. Therefore, the biological activity of IL-1β is directly dependent on the function of caspase 1, which is activated by inflammasome complexes.27
The best completely characterized inflammasome is the NLRP3 (also known as NALP3, cryopyrin, and PYPAF1) inflammasome, which includes the NACHT LRR PYD domain containing 3 (NALP3) receptor, the adaptor proteins ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), TUCAN (also known as cardinal), and pro-caspase 1. While NLRP3 inflammasome is in an inactive form in the cytoplasm, it can be activated by PAMPs, including bacterial muramyl dipeptide (MDR) and viral or bacterial RNAs, as well as DAMPs such as ATP, intracellular potassium concentration, monosodium urate crystals, and a variety of skin irritants like ultraviolet radiation. Following this activation, the pyrin domain of NLRP3 interacts with the pyrin domain of ASC, and the CARD domain of ASC binds and recruits caspase-1 to the inflammasome. Subsequently, the CARD of caspase-1 interacts with the CARD of cardinal as well. The association of inflammatory proteins activates caspase 1 and then changes Pro-IL-1β to the active form of IL-1β (Fig. 1).

The pathogenesis of RA has been mediated by chronic inflammation of the joints that can lead to irreparable damage to them. Cytokines have been implicated as essential mediators of inflammation and joint destruction in RA. Interleukin 1 (IL-1) is a 17-kD cytokine primarily produced by monocytes and macrophages. Other cells, including endothelial cells, keratinocytes, mesangial cells, astrocytes, B lymphocytes, and activated T lymphocytes may also create IL-1. Many documents suggest that, among several cytokines, 'IL-1β' released from monocytes and macrophages play a critical role in the propagation of the chronic inflammatory process in the joints of rheumatoid patients. The findings show that in arthritis, different proteases including protease 3, elastase, and mast cell kinase can be secreted by leukocytes, which are effective in the maturation of pro-IL-1β into an active form. On the other hand, in the synovial tissues of RA patients, myeloid and endothelial cells are the main sources of IL-1β and interleukin 1 alpha (IL-1α) production. The increased IL-1 production was not observed in non-rheumatic synovial tissue and was correlated with arthroscopic and radiologic evidence of destructive arthritis in the RA patients. Also, circulating monocytes that have been taken at the initial stage of the disease will spontaneously secrete IL-1 in vitro, a finding that probably reflects monocytopoiesis as immature cells secrete more IL-1. These results are supported by finding of high levels of the IL-1β and IL-1α messenger RNA (mRNA) in the rheumatoid synovial membrane. However, the result of some researches has been variable due to the presence of IL-1 inhibitors in synovial fluids.

Fig.1: Schematic presentation of NLRs inflammasome activation and the production of mature inflammatory cytokines
Lastly, although suggestive evidence exists, further studies are necessary to establish whether disease-modifying drugs used in the treatment of RA may inhibit the production or biologic effects of IL-1. A hypothetical model of cytokine involvement in rheumatoid synovitis is shown in fig. 2. In this figure, the antigen-presenting cells with HLADR stimulate T lymphocytes to release interferon gamma (IFNγ) and other cytokines. Some unknown factors from T lymphocytes and other cells are considered to stimulate macrophages to synthesize and release IL-1, tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6). Following the stimulation of macrophages by these factors, macrophages begin to synthesize and secrete IL-1 and TNF-α. In turn, these cytokines induce the production of collagenase and other proteases in synovial fibroblasts and chondroids, which trigger proteoglycans and collagen, leading to the destruction of articular cartilage. In general, although the etiology of RA is unclear, a key role for IL 1β is well confirmed, therefore it will be expected that the inflammasome to be involved in RA.

5. NLRP3 and CARD8 polymorphisms in inflammatory diseases

There is a considerable amount of literature on responsible genetic variants for the susceptibility to inflammatory diseases. In recent years, there has been growing interest in investigations on the relationship between genetic polymorphisms in the genes encoding NLRP3 and (Caspase Recruitment Domain Family Member 8) CARD8 with complex inflammatory diseases. It has now suggested that mutations in genes that encode the NLRP3 inflammasome components were associated with a vast spectrum of diseases with the profound systemic inflammatory state, such as inflammatory bowel disease (IBD), Crohn’s disease (CD), cardiovascular disease (CVD), diabetes type 1 (T1D), Alzheimer’s disease (AD), gout, celiac disease, ankylosing spondylitis (AS), atherosclerosis, and RA. Some studies on the association of polymorphisms of the NLRP3 and CARD8 genes in various inflammatory diseases are listed in table 2. Particular variants in these genes were considered as a potential susceptible factor for the onset of the disease. The present paper aims to review findings regarding the association of NLRP3 and CARD8 variants to the inflammatory disease of RA, identified with a candidate polymorphism approach. The investigation of genetic basis of disease, which plays a central role in susceptibility will contribute to better knowledge and understanding of the disease progression.

6. NLRP3 Q705K and CARD8 C10X polymorphisms

It is fundamental to note that there are various polymorphisms in the NLRP3 gene concerning inflammatory diseases. These variants located in different parts of the gene, for instance, exons, introns, and untranslated regions (5’ UTR (leader sequence) or 3’ UTR (trailer sequence). The most important variants include rs2027432/rs10754558/rs4353135/rs35829419, and rs7512998/rs4266924/rs55646866/rs6672995, which are up-regulation polymorphisms and SNPs with unknown biological function, respectively. The polymorphism of NLRP3 c.2113C>A, p.Q705K (rs35829419) is one of the most significant SNPs associated with several inflammatory diseases, particularly in RA. It is a gain-of-function change in exon 3 of the NLRP3 gene, a low penetrant variant, which increases the production of IL-1β.

| Diseases | Gene | SNP | Study group | Number of cases | Gender (male%) |
|----------|------|-----|-------------|----------------|---------------|
| CVD      | NLRP3| rs35829419 | Sweden       | 555            | NR            |
|          | CARD8| rs2043211   | China        | 2441           | 62.7%         |
|          | NLRP3/CARD8 | rs35829419/ | New Zealand | 1151           | 79.7%         |
| AS       | CARD8| rs2043211   | Sweden       | 492            | 69%           |
| CD       | NLRP3| rs35829419(c.2113C>A) | Italy        | 504            | 39%           |
| T1D      | NLRP3| rs10754558  | Brazil       | 196            | 48%           |
| Gout     | NLRP3| rs7512998   | China        | 480            | NR            |
| AD       | CARD8| rs2043211   | Brazil       | 300            | 33.6%         |

Abbreviations: NR, not reported; CVD, cardiovascular disease; AS, arthritis spinal; CD, celiac disease; T1D, type 1 diabetes; AD, Alzheimer’s disease
Table 3. A summary of the research and the relationship between different variables with rheumatoid arthritis (P-value <0.05 was significant)

| Variables studied | Genetic model | RA group of patients | control group | P-value | OR(95 CI) | Population studied |
|-------------------|---------------|----------------------|---------------|---------|-----------|-------------------|
| CARD8 (rs2043211) 41 | AA/AT+TT      | 100                  | 191           | 0.44    | (2.23-0.73) 1.27 | France            |
| CARD8 (rs2043211) 41 | TT/AT+AA      | 100                  | 191           | 0.894   | (2.43-0.41) 1     | France            |
| NLRP3 (rs35829419) 41 | AA/AC+CC      | 141                  | 191           | 0.353   | (0.03-3.7) 0.33   | France            |
| NLRP3 (rs35829419) 41 | CC/AC+AA      | 141                  | 191           | 0.879   | (0.3-2.48) 0.87   | France            |
| CARD8 (rs2043211) 41 | TT/AT+AA      | 141                  | 191           | 0.155   | (2.7-0.61) 1.12   | Tunisia           |
| CARD8 (rs2043211) 41 | AA/AT+TT      | 141                  | 191           | 0.759   | (0.54-1.29) 0.83  | Tunisia           |
| NLRP3 (rs35829419) 41 | CC/AC+AA      | 141                  | 191           | 0.34    | (3.76-0.68) 1.6   | Tunisia           |
| CARD8 / NLRP3 41 | AA/CA         | 2                    | 1             | 0.62    | (0.15-19.92) 1.74 | France            |
| DAS28 40 | --           | 324 (SNP CARD8-X)    | 219(SNP CARD8-CC) | 0.02    | NR        | Sweden           |
| ESR 40 | --           | 324 (SNP CARD8-X)    | 219(SNP CARD8-CC) | 0.004   | NR        | Sweden           |
| TJC 40 | --           | 324 (SNP CARD8-X)    | 219(SNP CARD8-CC) | 0.02    | NR        | Sweden           |
| NLRP3 (rs35829419) 40 | All genotypes | 543                  | 568           | NC      | NR        | Sweden           |
| NLRP3 (rs4353135) 4 | G             | 96                   | 103           | 0.036   | (4.54-1) 2.127   | Taiwan            |
| CARD8 (rs2043211) 4 | A             | 96                   | 103           | 0.046   | (3.3-0.96) 1.779  | Taiwan            |
| CARD8 (rs2043211) 4 | AA,TT,AT      | 1530                 | 1292          | 0.88    | (1.13-0.9)       | Spain             |
| CARD8 (rs2043211) 45 | AA,TT,AT      | 322(CVD)             | 1292          | 0.67    | (1.16-0.076) 0.96 | Spain             |
| DAS28 45 | --           | 14 (SNP NLRP3CA)     | 114(SNP NLRP3 CC) | 0.003   | NR        | Spain            |
| DAS28 45 | --           | 64(SNP CARD8AT)      | 49 (SNP CARD8 AA) | 0.049   | NR        | Spain            |
| CARD8 (rs2043211) 10 | TT            | 218                  | 307           | 0.044   | (1.23-3.09) 1.77  | Brazil            |
| NLRP3 (rs35829419) 44 | A/C           | 2705                 | 2711          | > 0.05  | (0.706-1.169) 1.77 | Multi country     |
| NLRP3 (rs35829419) 44 | AA/CC         | 2705                 | 2711          | > 0.05  | 0.908     | Multi country     |
| NLRP3 (rs35829419) 44 | AC/CC         | 2705                 | 2711          | > 0.05  | (0.216-2.861)    | Multi country     |
| CARD8 (rs2043211) 44 | AA/AC         | 2705                 | 2711          | > 0.05  | 0.786     | Multi country     |
**Table**: Gene Polymorphisms and Their Impact

| Gene          | Polymorphism | Allele Combination | Frequency (95% CI) | P Value (df) |
|---------------|--------------|--------------------|--------------------|--------------|
| CARD8         | rs2043211    | A/T                | > 0.05             |              |
| NLRP3/CARD8   | rs1972619    | AA/AC/AA+AT        | 0.872 (0.545-2.046) | 1.056 (df) |

Abbreviations: NR, Not Reported; DAS28, disease activity score across 28 joints; ESR, erythrocyte sedimentation rate; TJC, tender joint count.

**Fig. 2.** Stimulatory or agonist effects of cytokines on cell-cell interactions in rheumatoid synovitis

According to numerous studies, the CARD8 (TUCAN) polymorphic allele, a component of the NLRP1 inflammasome, has been associated with cell death in vitro, and plays a role as the nuclear factor kappa B subunit 1 (NF-kB) inhibitor. Although the real function of CARD8 is not clear, studying the role of this protein in the inflammasome construction seems difficult, while this protein has disappeared in the rodents. Among the different variations of available polymorphisms in the CARD8 gene, rs1972619 and rs2043211 are located in intron 2 and exon 5 of the gene, respectively. The evidence from polymorphism studies of the CARD8 gene proposes that the CARD8 c.30T>A, p.C10X (rs2043211) variant (a nonsense mutation), results in the production of a truncated CARD8 protein, correlates with a severe disease course in early RA. Due to the various isoforms of the CARD8 gene, the functional consequences of
Fig. 3A: The most significant polymorphisms of the NLRP3 gene with respect to inflammatory disorders; 3B: The domains of the NLRP3 protein. B, two important SNPs of the CARD8 gene; different isoforms of the CARD8 mRNA. Boxes: exons.

C10X can be different. For example, the transcription of T47 isoform starts downstream of the C10X, so the variant does not affect the isoform. Hence, patients with a homozygous state for the C10X genotype may have a functional CARD8 protein because of T47 isoform. A graphical presentation of the CARD8 and NLRP3 genes can be seen in Fig 3.

7. The role of Q705K and C10X SNPs in RA
In a large cohort of 218 patients with RA, homozygous cases with the loss-of-function polymorphism of CARD8 C10X were found to have a higher disease activity score (DAS) and patient's functional disability (HAQ), compared to homozygous for wild type allele and heterozygous patients. Two studies in Sweden have demonstrated that Q705K polymorphism in the NLRP3 and C10X in the CARD8 gene were associated with increase in severity and susceptibility to RA. Although these findings were not confirmed in a similar study in French and Tunisian patients. Recent
studies have reported CARD8 p.C10X is linked to RA, Crohn’s disease, and ulcerative colitis. Fontalba et al. showed that the CARD8 p.C10X polymorphism affected NF-kB transcription activity and NFkB inhibition significantly decreased the severity of the disease in laboratory models. Therefore, CARD8, in addition to the involvement in the inflammatory pathway, induces the expression of TNF-α, Pro-IL-1β, and metalloproteinase (MMPs) genes, as an NKxB inhibitor. CARD8 p.C10X was also associated with the disease activity score across 28 joints (DAS28) in two groups of Spanish patients. In mouse models with RA, it was detected that inflammasome NLRP3 plays a small role in the pathophysiology of the disease, and IL-1β activity is independent of NLRP3 function. A survey, in northern Sweden revealed that the C10X variant influenced the rate of erythrocyte sedimentation, the number of joint defects, and DAS. In a meta-analysis study, 2705 cases with RA from different countries including Spain, France, and Sweden consisting of French and Tunisian populations were analyzed. The study showed that there was no association between the NLRP3 p.Q705K or CARD8 p.C10X polymorphisms and susceptibility to RA. There was also an epistemic relationship between NLRP3 and CARD8, which increased the susceptibility to the disease in RA patients in southeastern Sweden. In recent studies, the combination of Q705K and C10X polymorphisms has been associated with increase in IL-1β and interleukin 33 (IL-33) in plasma, which indicates the different variants of NLRP3 inflammasome genes can affect the threshold for inflammasome activity, and the balance of immune responses. Hence, these variants play a key role in the basic active state of the innate immune response. On the other hand, the result of IL-1β blocked and/or inhibition of NLRP3 inflammasome for blocking of IL-1β and IL-18 are the first therapeutic purposes for RA treatment. In vitro neutrophils carrying both the NLRP3 Q705K and the CARD8 C10X polymorphisms showed delayed apoptosis, suggesting that these variants have functional consequences, so that they might result in a deregulated innate immune response, and participate in the pathogenesis of RA. Besides, in the THP 1 cells (a human monocyte cell line), the Q705K polymorphism had a gain-of-function impact on the inflammatory responses. In general, the genetic variants of the NLRP3 inflammasome are likely to affect the progression of RA, but it is still too early to confirm the roles of them in RA pathophysiology. Ospelt et al. investigated the expression of toll-like receptors (TLRs) in synovial tissues in the primary and end stages of RA. In this study, higher expression of TLR3 and TLR4 genes was observed in the synovial tissue of RA compared to the samples of synovial osteoarthritis (OS). These results were repeated in the study of Brentano et al., which showed TLR3 levels in the fibroblasts of RA synovial had an increase compared with osteoarthritis (OA) fibroblasts. Similarly, an increase in TLR7 levels in the synovium of RA patients was observed in comparison to healthy volunteers and OA patients. These observations indicate the role of other innate immune receptors (TLRs) in the pathophysiology of RA. Table 3 summarizes the results of various studies obtained upon evaluating the association of CARD8 and NLRP3 polymorphisms with RA.

Conclusion
Some evidence proposes that the NLRP3 p.Q705K and CARD8 p.C10X genetic variants are likely to influence the progression of RA. But human studies on this subject have not been sufficient, and it is still too early to confirm that they play a critical role in the pathophysiology of RA. It is important to note that reviewing of functional characteristics of SNPs in complex diseases with high environmental and genetic factors is a challenge that affects the pathophysiology of the disease.

One limitation of the present study is that the number of involved surveys was limited because only a few investigations were conducted on the association between inflammasome polymorphism and RA. These studies more come from European nations, and their populations were mainly European, while the correlation between genes and disease may be influenced by race. Therefore, carrying out more analyses in Asians is required. Variable results in this area might be solved by doing more meta-analysis studies so that such reviews can help in more accurate conclusions.

Competing interests
The authors declare that they have no competing interests.

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