REVIEWS

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]

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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. [10, 11]

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.[8] 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.[12-14] 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.[15] 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.[16]

RA creates a complex interaction between the environment and genetics. For example, smoking and other respiratory stress increase the risk of developing HLADR4 susceptibility.[7] 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.[17, 18]

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.[19, 20]

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.[21, 22] 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.[23]

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.[24]

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 arthritis[13]
PTPN22	rs2476601	1p13.2	ACPA-positive RA with worse prognosis[54]
CD40	rs6032662	20q13.12	Increased risk for ACPA-positive RA[55]
TAGAP	rs629326	6q25.3	Increased risk for ACPA-positive RA[55]
IL2	rs6822844	4q27	Cytokine that regulates activation of T cells, particularly regulatory T cells[56]
IL2RA	rs2104286	10p15.1	High-affinity receptor for interleukin-2 on lymphocyte subsets[56]
DNMT3B	rs2228611	283C/T	Influences progression of joint destruction in RA[57]
STAT4	rs7574865	2q32.3	NR[58]
IL6R	rs8192284	1q21.3	Influences disease activity of ACPA-positive RA[59]
CCR6	rs3093023	6q27	Increased RA susceptibility[60]
CTLA4	rs1863800	2q33.2	NR[61]
REL	rs5771069	2p16.1	Proto-oncogene member of the NF-κB family that regulates leukocyte activation and survival[62]
TNFAIP3	rs9376293	6q23.3	Increased rate of joint destruction in ACPA-positive RA[63]
TAGAP	rs629326	6q25.3	Increased rate of joint destruction in ACPA-positive RA[64]
ANKRD55	rs6859219	5q11.2	Increased risk for ACPA-positive RA[65]
CARD8	C10X/rs2043211	19q13.33	Worse disease course in early RA[65]
GATSL3	rs1043099	22q12.2	Increased RA susceptibility[28]
IRAK1	rs13397	Xq28	Increased RA susceptibility[55]
RASGRP1	rs8043085	15q14	Increased risk for ACPA-positive RA[55]
SPRED2	rs934734	2p14	Increased risk for ACPA-positive RA[65]

Abbreviations: NR, not reported

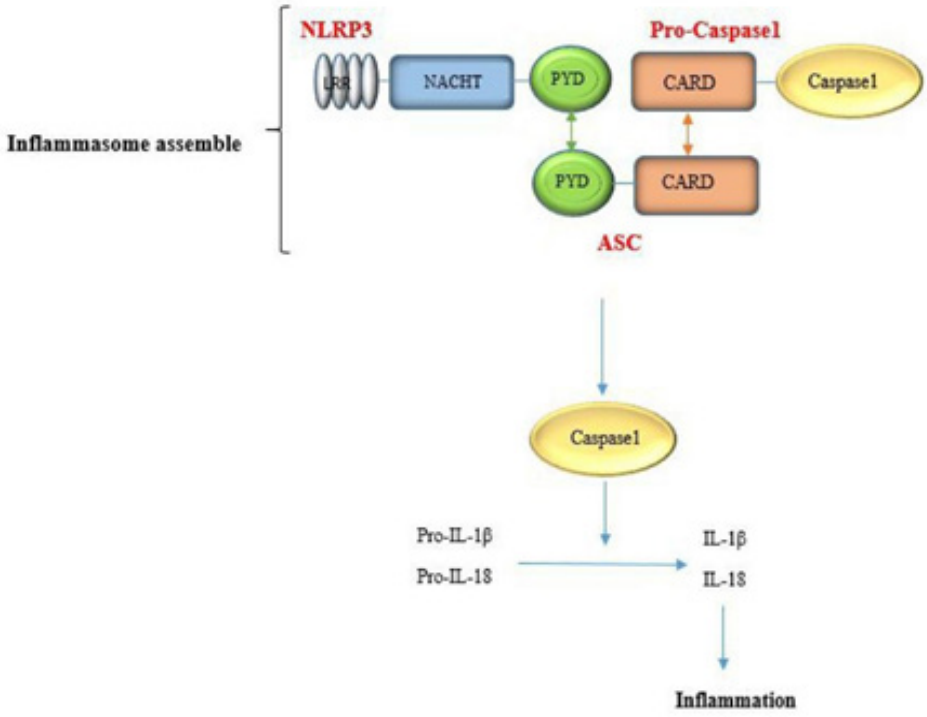
4. The NLRP3 inflammasome and IL-1β activation

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.[27] 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.[28] 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.[29,30] 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.[30] 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.[31]

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.[32] 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.[33]

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.[34] Some studies on the association of polymorphisms of the NLRP3 and CARD8 (TUCAN) genes in several 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.[35] 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

Table 2. The association of polymorphisms of NLRP3 and CARD8 genes with various diseases

Diseases	Gene	SNP	Study group	Number of cases	Gender (male%)
CVD[66-68]	*NLRP3* *CARD8* *NLRP3/CARD8*	rs35829419 rs2043211 rs35829419/ rs2043211	Sweden China New Zealand	555 2441 1151	NR 62.7% 79.7%
AS[69]	*CARD8*	rs2043211	Sweden	492	69%
CD[70]	*NLRP3*	rs35829419 (c.2113C>A)	Italy	504	39%
T1D[71]	*NLRP3*	rs10754558	Brazil	196	48%
Gout[72]	*NLRP3*	rs7512998	China	480	NR
AD[73]	*CARD8*	rs2043211	Brazil	300	33.6%

Abbreviations: NR, not reported; CVD, cardiovascular disease; AS, arthritis spinal; CD, celiac disease; T1D, type 1 diabete; AD, Alzheimer's disease

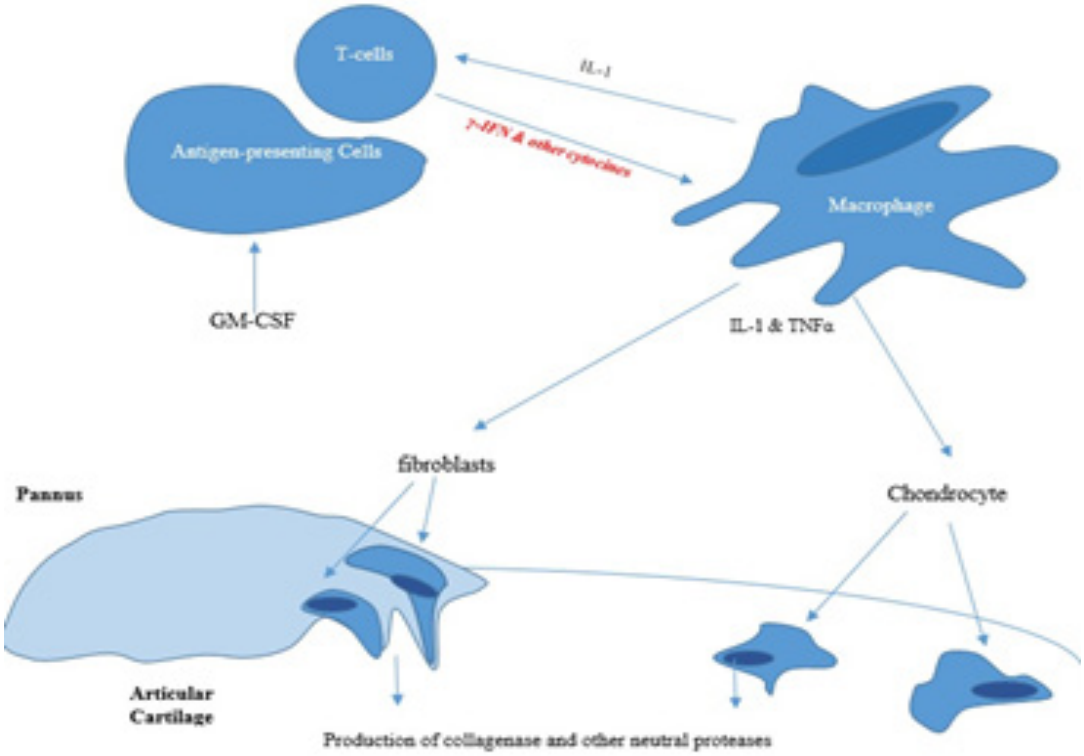
Table 3. A summary of the research and the relationship between different variables with rheumatoid arthritis (P-value <0.05 was significant)

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)[74]	G	96	103	0.036	(4.54-1) 2.127	Taiwan
CARD8 (rs2043211)[74]	A	96	103	0.046	(3.3-0.96) 1.779	Taiwan
CARD8 (rs2043211)[45]	AA,TT,AT	1530	1292	0.88	(1.13-0.9)	Spain
CARD8 (rs2043211)[45]	AA,TT,AT	322 (CVD)	1292	0.67	(1.16-076) 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)[39]	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)	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

CARD8 (rs2043211)[44]	A/T	2705	2711	> 0.05	(0.700- 1.198) 0.916	Multi country
CARD8 (rs2043211)[44]	AA/TT	2705	2711	> 0.05	(0.832–1.196) 0.997	Multi country
CARD8 (rs2043211)[44]	AT/TT	2705	2711	> 0.05	(0.909-1.218) 1.052	Multi country
CARD8 (rs2043211)[44]	AA/ AT	2705	2711	> 0.05	(0.836–1.080) 0.950	Multi country
NLRP3／ *CARD8*[44]	AA+AC/ AA+AT	2705	2711	0.872	(0.545- 2.046) 1.056	Multi country
NLRP3／ *CARD8*[44]	CC/TT	2705	2711	0.872	(0.545- 2.046) 1.056	Multi country

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

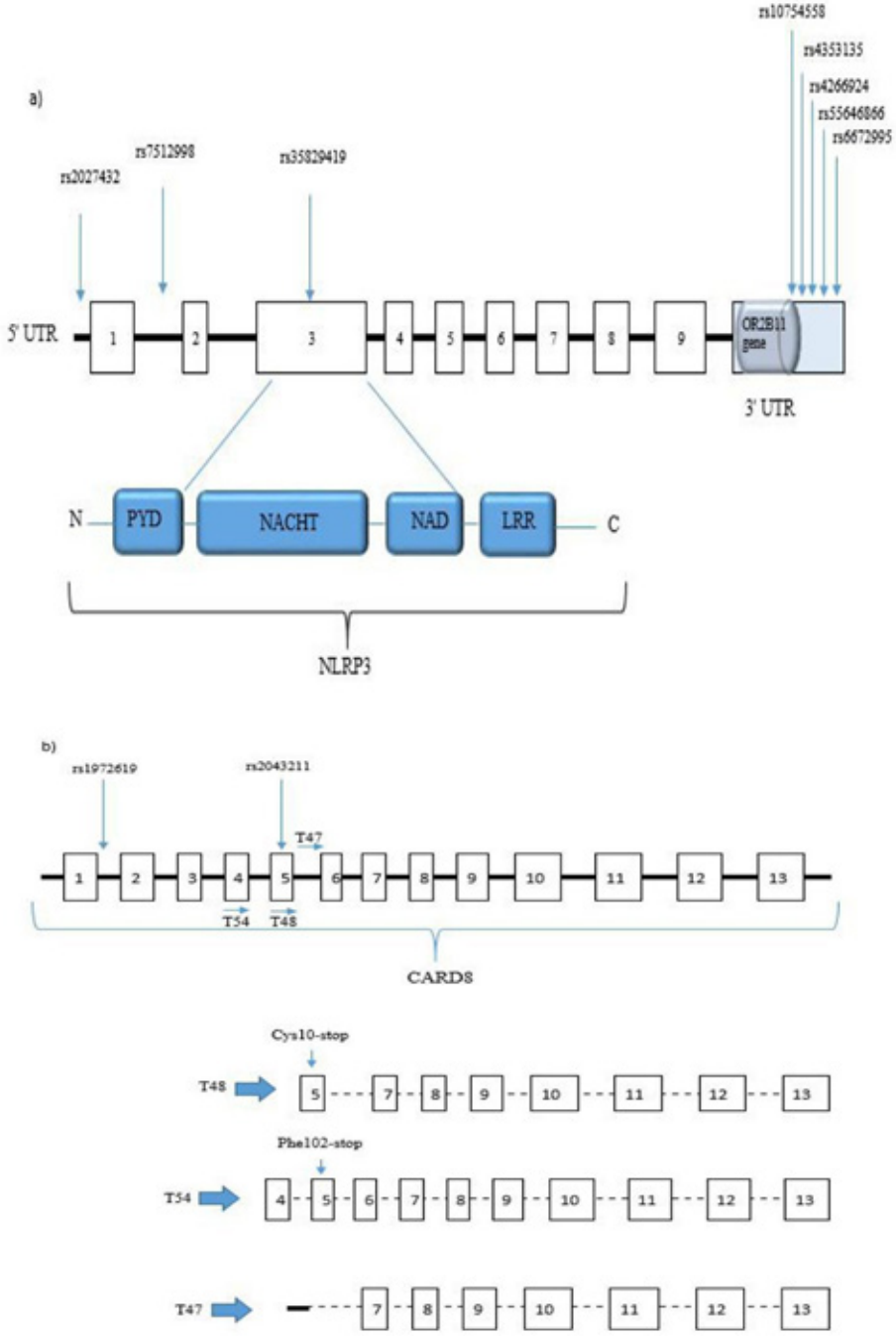
Fig. 2. Stimulatory or agonist effects of cytokines on cell-cell interactions in rheumatoid synovitis



and interleukin-18 (IL-18).[36]

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-κB) 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.[37]Among the different variations of available polymorphisms in the CARD8 gene, rs1972619 and rs2043 211 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.[35] 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.[38] 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.[39] 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.[40] Although these findings were not confirmed in a similar study in French and Tunisian patients.[41] Recent

studies have reported CARD8 p.C10X is linked to RA, Crohn's disease, and ulcerative colitis.[35] Fontalba *et al.* showed that the CARD8 p.C10X polymorphism affected NF-κB transcription activity and NFκB 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 NKκB inhibitor.[42] CARD8 p.C10X was also associated with the disease activity score across 28 joints (DAS28) in two groups of Spanish patients.[45] 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.[43] A survey, in northern Sweden revealed that the C10X variant influenced the rate of erythrocyte sedimentation, the number of joint defects, and DAS.[40] 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.[44] There was also an epistemic relationship between NLRP3 and CARD8, which increased the susceptibility to the disease in RA patients in southeastern Sweden.[45]

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.[46] 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.[47] *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.[48] Besides, in the THP 1 cells (a human monocyte cell line), the Q705K polymorphism had a gain-of-function impact on the inflammatory responses.[49] 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).[50] 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.[51] 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.[52, 53] 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.

Citation

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References

1. Sayad A, Akbari MT, Mehdizadeh M, Movafagh A, Hajifathali A. The association of HLA-Class I and class II with Hodgkin's Lymphoma in Iranian Patients. BioMed research international. 2014 Jan 1;2014.
2. Salesi M, Boroujeni GT, Salehi M, Farzamnia S. Evaluation of differences in HLA-DR4 gene and its subtypes prevalence among healthy people and RA patients in Isfahan province population. Advanced biomedical research. 2016;5.
3. Jamshidi AR, Tehrani BS, Salari AH, Taghipour R. HLA DR4 subtype relationship with rheumatoid arthritis: a study on Iranian patients. Tehran Univ Med J 2009, 66(11): 804-808
4. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z. Genetics of rheumatoid arthritis—a comprehensive review. Clinical reviews in allergy & immunology. 2013 Oct 1;45(2):170-9.
5. Kamphuis S, Kuis W, De Jager W, Teklenburg G, Massa M, Gordon G, Boerhof M, Rijkers GT, Uiterwaal CS, Otten HG, Sette A. Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. The Lancet. 2005 Jul 2;366(9479):50-6.
6. Auger I, Roudier J. A function for the QKRAA amino acid motif: mediating binding of DnaJ to DnaK. Implications for the association of rheumatoid arthritis with HLA-DR4. The Journal of clinical investigation. 1997 Apr 15;99(8):1818-22.
7. Symmons DP, Bankhead CR, Harrison BJ, Brennan P, Silman AJ, Barrett EM, Scott DG. Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis. Results from a primary care-based incident case□control study in Norfolk, England. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1997 Nov;40(11):1955-61.
8. Barton A, Worthington J. Genetic susceptibility to rheumatoid arthritis: an emerging picture. Arthritis Care & Research. 2009 Oct 15;61(10):1441-6.
9. Sparks JA, Chen CY, Hiraki LT, Malspeis S, Costenbader KH, Karlson EW. Contributions of familial rheumatoid arthritis or lupus and environmental factors to risk of rheumatoid arthritis in women: a prospective cohort study. Arthritis care & research. 2014 Oct;66(10):1438-46.
10. Asnaf SE, Sabetghadam M, Jaafarinejad H, Halabian R, Parvin S, Vahedi E, Salimian J. Is the Inflammasome Pathway Active in the Peripheral Blood of Sulfur Mustard-exposed Patients?. Iranian Journal of Allergy, Asthma and Immunology. 2019 May 19.
11. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. New England Journal of Medicine. 2011 Dec 8;365(23):2205-19.
12. Bax M, van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: what have we learned?. Immunogenetics. 2011 Aug 1;63(8):459-66.
13. Huizinga TW, Amos CI, van der Helm□van Mil AH, Chen W, van Gaalen FA, Jawaheer D, Schreuder GM, Wener M, Breedveld FC, Ahmad N, Lum RF. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA–DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis & Rheumatism. 2005 Nov;52(11):3433-8.
14. Viatte S, Plant D, Raychaudhuri S. Genetics and epigenetics of rheumatoid arthritis. Nature Reviews Rheumatology. 2013 Mar;9(3):141.
15. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. The American Journal of Human Genetics. 2004 Aug 1;75(2):330-7.
16. Karlson EW, Chibnik LB, Kraft P, Cui J, Keenan BT, Ding B, Raychaudhuri S, Klareskog L, Alfredsson L, Plenge RM. Cumulative association of 22 genetic variants with seropositive rheumatoid arthritis risk. Annals of the rheumatic diseases. 2010 Jun 1;69(6):1077-85.
17. Scher JU, Ubeda C, Pillinger MH, Bretz W, Buischi Y, Rosenthal PB, Soumya M. Characteristic oral and intestinal microbiota in rheumatoid arthritis (RA): a trigger for autoimmunity. Arthritis Rheum. 2010;62(10):1390.
18. Capellino S, Cosentino M, Wolff C, Schmidt M, Grifka J, Straub RH. Catecholamine-producing cells in the synovial tissue during arthritis: modulation of sympathetic neurotransmitters as new therapeutic target. Annals of the rheumatic diseases. 2010 Oct 1;69(10):1853-60.
19. Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. Nature immunology. 2012 Apr;13(4):333.
20. Moretti J, Blander JM. Increasing complexity of NLRP3 inflammasome regulation. Journal of Leukocyte Biology. 2020 Jun 12.
21. Yi YS. Role of inflammasomes in inflammatory autoimmune rheumatic diseases. The Korean Journal of Physiology & Pharmacology. 2018 Jan 1;22(1):1-5.
22. Mathews RJ, Robinson JI, Battellino M, Wong C, Taylor JC, Eyre S, Churchman SM, Wilson AG, Isaacs JD, Hyrich K, Barton A. Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA); genetic variants within the NLRP3-inflammasome complex in relation to susceptibility to RA and response to anti-TNF treatment. Annals of the rheumatic diseases. 2014 Jun 1;73(6):1202-10.
23. Sui J, Li H, Fang Y, Liu Y, Li M, Zhong B, Yang F, Zou Q, Wu Y. NLRP1 gene polymorphism influences gene transcription and is a risk factor for rheumatoid arthritis in han chinese. Arthritis & Rheumatism. 2012 Mar;64(3):647-54.
24. Joosten LA, Netea MG, Fantuzzi G, Koenders MI, Helsen MM, Sparrer H, Pham CT, Van Der Meer JW, Dinarello CA, Van Den Berg WB. Inflammatory arthritis in caspase 1 gene–deficient mice: Contribution of proteinase 3 to caspase 1–independent production of bioactive interleukin-1β. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2009 Dec;60(12):3651-62.
25. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunological reviews. 2008 Dec;226(1):10-8.
26. McCormack WJ, Parker AE, O'Neill LA. Toll-like receptors and NOD-like receptors in rheumatic diseases. Arthritis research & therapy. 2009 Oct 1;11(5):243.
27. Walle LV, Van Opdenbosch N, Jacques P, Fossoul A, Verheugen E, Vogel P, Beyaert R, Elewaut D, Kanneganti TD, van Loo G, Lamkanfi M. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature. 2014 Aug;512(7512):69-73.
28. Yamagata N, Kobayashi K, Kasama T, Fukushima T, Tabata M, Yoneya I, Shikama Y, Kaga S, Hashimoto M, Yoshida K. Multiple cytokine activities and loss of interleukin 2 inhibitor in synovial fluids of patients with rheumatoid arthritis. The Journal of rheumatology. 1988 Nov;15(11):1623-7.
29. Hazuda DJ, Strickler J, Kueppers F, Simon PL, Young P. Processing of precursor interleukin 1 beta and inflammatory disease. Journal of Biological Chemistry. 1990 Apr 15;265(11):6318-22.
30. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood, The Journal of the American Society of Hematology. 2011 Apr 7;117(14):3720-32.

31. Kolly L, Busso N, Palmer G, Talabot-Ayer D, Chobaz V, So A. Expression and function of the NALP3 inflammasome in rheumatoid synovium. Immunology. 2010 Feb;129(2):178-85.
32. Arend WP, Dayer JM. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis and rheumatism. 1990 Mar;33(3):305.
33. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, Silman AJ. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2000 Jan;43(1):30-7.
34. Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, Tschopp J, Endres S, Latz E, Schnurr M. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut. 2010 Sep 1;59(9):1192-9.
35. Paramel GV, Sirsjö A, Fransén K. Role of genetic alterations in the NLRP3 and CARD8 genes in health and disease. Mediators of Inflammation. 2015 Oct;2015.
36. Verma D, Särndahl E, Andersson H, Eriksson P, Fredrikson M, Jönsson JI, Lerm M, Söderkvist P. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1β and IL-18 production. PloS one. 2012 Apr 17;7(4):e34977.
37. Razmara M, Srinivasula SM, Wang L, Poyet JL, Geddes BJ, DiStefano PS, Bertin J, Alnemri ES. CARD-8 protein, a new CARD family member that regulates caspase-1 activation and apoptosis. Journal of Biological Chemistry. 2002 Apr 19;277(16):13952-8.
38. Ko DC, Shukla KP, Fong C, Wasnick M, Brittnacher MJ, Wurfel MM, Holden TD, O'Keefe GE, Van Yserloo B, Akey JM, Miller SI. A genome-wide in vitro bacterial-infection screen reveals human variation in the host response associated with inflammatory disease. The American Journal of Human Genetics. 2009 Aug 14;85(2):214-27.
39. Addobbati C, da Cruz HL, Adelino JE, Ramos AL, Fragoso TS, Domingues A, Duarte ÂL, Oliveira RD, Louzada-Júnior P, Donadi EA, Pontillo A. Polymorphisms and expression of inflammasome genes are associated with the development and severity of rheumatoid arthritis in Brazilian patients. Inflammation Research. 2018 Mar 1;67(3):255-64.
40. Kastbom A, Johansson M, Verma D, Söderkvist P, Rantapää-Dahlqvist S. CARD8 p. C10X polymorphism is associated with inflammatory activity in early rheumatoid arthritis. Annals of the rheumatic diseases. 2010 Apr 1;69(4):723-6.
41. Ben Hamad M, Cornélis F, Marzouk S, Chabchoub G, Bahloul Z, Rebai A, Fakhfakh F, Ayadi H, Petit-Teixeira E, Maalej A. Association study of CARD8 (p. C10X) and NLRP3 (p. Q705K) variants with rheumatoid arthritis in French and Tunisian populations. International journal of immunogenetics. 2012 Apr;39(2):131-6.
42. Fontalba A, Martinez-Taboada V, Gutierrez O, Pipaon C, Benito N, Balsa A, Blanco R, Fernandez-Luna JL. Deficiency of the NF-κB inhibitor caspase activating and recruitment domain 8 in patients with rheumatoid arthritis is associated with disease severity. The Journal of Immunology. 2007 Oct 1;179(7):4867-73.
43. Blomgran R, Brodin VP, Verma D, Bergström I, Söderkvist P, Sjöwall C, Eriksson P, Lerm M, Stendahl O, Särndahl E. Common genetic variations in the NALP3 inflammasome are associated with delayed apoptosis of human neutrophils. PLoS One. 2012 Mar 5;7(3):e31326.
44. Yang Z, Cao J, Yang Q, Zhang Y, Han L. NLRP 3 p. Q705K and CARD 8 p. C10X single nucleotide polymorphisms are not associated with susceptibility to rheumatoid arthritis: a meta-analysis. International journal of rheumatic diseases. 2017 Oct;20(10):1481-91.
45. García-Bermúdez M, López-Mejías R, González-Juanatey C, Corrales A, Castaneda S, Ortiz AM, Miranda-Filloy JA, Gómez-Vaquero C, Fernández-Gutiérrez B, Balsa A, Pascual-Salcedo D. CARD8 rs2043211 (p. C10X) polymorphism is not associated with disease susceptibility or cardiovascular events in Spanish rheumatoid arthritis patients. DNA and cell biology. 2013 Jan 1;32(1):28-33.
46. Yang CA, Chiang BL. Inflammasomes and human autoimmunity: a comprehensive review. Journal of autoimmunity. 2015 Jul 1;61:1-8.
47. Alexander T, Klotz O, Feist E, Rüther K, Burmester GR, Pleyer U. Successful treatment of acute visual loss in Muckle-Wells syndrome with interleukin 1 receptor antagonist. Annals of the rheumatic diseases. 2005 Aug 1;64(8):1245-6.
48. Kolly L, Karababa M, Joosten LA, Narayan S, Salvi R, Pétrilli V, Tschopp J, van den Berg WB, So AK, Busso N. Inflammatory role of ASC in antigen-induced arthritis is independent of caspase-1, NALP-3, and IPAF. The Journal of Immunology. 2009 Sep 15;183(6):4003-12.
49. Cordero MD, Alcocer-Gómez E, Ryffel B. Gain of function mutation and inflammasome driven diseases in human and mouse models. Journal of autoimmunity. 2018 Jul 1;91:13-22.
50. Ospelt C, Brentano F, Rengel Y, Stanczyk J, Kolling C, Tak PP, Gay RE, Gay S, Kyburz D. Overexpression of toll□like receptors 3 and 4 in synovial tissue from patients with early rheumatoid arthritis: toll□like receptor expression in early and longstanding arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2008 Dec;58(12):3684-92.
51. Brentano F, Schorr O, Gay RE, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via Toll□like receptor 3. Arthritis & Rheumatism. 2005 Sep;52(9):2656-65.
52. Roelofs MF, Joosten LA, Abdollahi□Roodsaz S, Van Lieshout AW, Sprong T, Van Den Hoogen FH, Van Den Berg WB, Radstake TR. The expression of toll□like receptors 3 and 7 in rheumatoid arthritis synovium is increased and costimulation of toll□like receptors 3, 4, and 7/8 results in synergistic cytokine production by dendritic cells. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2005 Aug;52(8):2313-22.
53. Huang Q, Ma Y, Adebayo A, Pope RM. Increased macrophage activation mediated through toll-like receptors in rheumatoid arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2007 Jul;56(7):2192-201.
54. Majorczyk E, Pawlik A, Kuśnierczyk P. PTPN22 1858C> T polymorphism is strongly associated with rheumatoid arthritis but not with a response to methotrexate therapy. International immunopharmacology. 2010 Dec 1;10(12):1626-9.
55. Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P, Zhernakova A, Stahl E, Viatte S, McAllister K, Amos CI. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. Nature genetics. 2012 Dec;44(12):1336.
56. Saad MN, Mabrouk MS, Eldeib AM, Shaker OG. Identification of rheumatoid arthritis biomarkers based on single nucleotide polymorphisms and haplotype blocks: a systematic review and meta-analysis. Journal of advanced research. 2016 Jan 1;7(1):1-6.
57. Nam EJ, Kim KH, Han SW, Cho CM, Lee J, Park JY, Kang YM. The− 283C/T polymorphism of the DNMT3B gene influences the progression of joint destruction in rheumatoid arthritis. Rheumatology international. 2010 Aug 1;30(10):1299-303.
58. Lee YH, Woo JH, Choi SJ, Ji JD, Song GG. Association between the rs7574865 polymorphism of STAT4 and rheumatoid arthritis: a meta-analysis. Rheumatology international. 2010 Mar 1;30(5):661-6.

59. Lamas JR, Rodríguez-Rodríguez L, Varadé J, López-Romero P, Tornero-Esteban P, Abasolo L, Urcelay E, Fernández-Gutiérrez B. Influence of IL6R rs8192284 polymorphism status in disease activity in rheumatoid arthritis. The Journal of rheumatology. 2010 Aug 1;37(8):1579-81.
60. Kochi Y, Okada Y, Suzuki A, Ikari K, Terao C, Takahashi A, Yamazaki K, Hosono N, Myouzen K, Tsunoda T, Kamatani N. A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. Nature genetics. 2010 Jun;42(6):515.
61. Barton A, Jury F, Eyre S, Bowes J, Hinks A, Ward D, Worthington J. Haplotype analysis in simplex families and novel analytic approaches in a case–control cohort reveal no evidence of association of the CTLA□4 gene with rheumatoid arthritis. Arthritis & Rheumatism. 2004 Mar;50(3):748-52.
62. Eyre S, Hinks A, Flynn E, Martin P, Wilson AG, Maxwell JR, Morgan AW, Emery P, Steer S, Hocking LJ, Reid DM. Confirmation of association of the REL locus with rheumatoid arthritis susceptibility in the UK population. Annals of the rheumatic diseases. 2010 Aug 1;69(8):1572-3.
63. Scherer HU, van der Linden MP, Kurreeman FA, Stoeken-Rijsbergen G, le Cessie S, Huizinga TW, van der Helm-van AH, Toes RE. Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis. Annals of the rheumatic diseases. 2010 Mar 1;69(3):567-70.
64. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, Li Y, Kurreeman FA, Zhernakova A, Hinks A, Guiducci C. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nature genetics. 2010 Jun;42(6):508.
65. Nagai Y, Imanishi T. RAvariome: a genetic risk variants database for rheumatoid arthritis based on assessment of reproducibility between or within human populations. Database. 2013 Jan 1;2013.
66. Varghese GP, Fransén K, Hurtig Wennlöf A, Bengtsson T, Jansson JH, Sirsjö A. Q705K variant in NLRP3 gene confers protection against myocardial infarction in female individuals. Biomedical reports. 2013 Nov 1;1(6):879-82.
67. Bai Y, Nie S, Jiang G, Zhou Y, Zhou M, Zhao Y, Li S, Wang F, Lv Q, Huang Y, Yang Q. Regulation of CARD8 expression by ANRIL and association of CARD8 single nucleotide polymorphism rs2043211 (p. C10X) with ischemic stroke. Stroke. 2014 Feb;45(2):383-8.
68. Roberts RL, Van Rij AM, Phillips LV, Young S, McCormick SP, Merriman TR, Jones GT. Interaction of the inflammasome genes CARD8 and NLRP3 in abdominal aortic aneurysms. Atherosclerosis. 2011 Sep 1;218(1):123-6.
69. Kastbom A, Klingberg E, Verma D, Carlsten H, Forsblad-d'Elia H, Wesamaa J, Cedergren J, Eriksson P, Söderkvist P. Genetic variants in CARD8 but not in NLRP3 are associated with ankylosing spondylitis. Scandinavian journal of rheumatology. 2013 Nov 1;42(6):465-8.
70. Pontillo A, Vendramin A, Catamo E, Fabris A, Crovella S. The Missense VariationQ705KinCIAS1/NALP3/NLRP3Gene and anNLRP1Haplotype Are Associated With Celiac Disease. American Journal of Gastroenterology. 2011 Mar 1;106(3):539-44.
71. Pontillo A, Brandao L, Guimaraes R, Segat L, Araujo J, Crovella S. Two SNPs in NLRP3 gene are involved in the predisposition to type-1 diabetes and celiac disease in a pediatric population from northeast Brazil. Autoimmunity. 2010 Dec 1;43(8):583-9.
72. Meng DM, Zhou YJ, Wang L, Ren W, Cui LL, Han L, Qu ZH, Li CG, Zhao JJ. Polymorphisms in the NLRP3 gene and risk of primary gouty arthritis Corrigendum in/mmr/8/6/1888. Molecular Medicine Reports. 2013 Jun 1;7(6):1761-6.
73. Fontalba A, Gutiérrez O, Llorca J, Mateo I, Berciano J, Fernández-Luna JL, Combarros O. Deficiency of CARD8 is associated with increased Alzheimer's disease risk in women. Dementia and geriatric cognitive disorders. 2008;26(3):247-50.
74. Yang CA, Huang ST, Chiang BL. Association of NLRP3 and CARD8 genetic polymorphisms with juvenile idiopathic arthritis in a Taiwanese population. Scandinavian Journal of Rheumatology. 2014 Mar 1;43(2):146-52.