REVIEW

Rheumatoid arthritis: What have we learned about the causing factors?

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Abstract: Rheumatoid Arthritis (RA) is a common inflammatory autoimmune disease characterized by the synovitis of both small and large joints, which may lead to the destruction of cartilage and bones causing significant disabilities due to erosion of bones surfaces, if left untreated. It is a multifactorial and heterogeneous disease having contribution of both genetic (50-60%) and environmental factors. The unawareness of general public might be a contributing factor in the high prevalence rate of RA world-wide. This review article focuses on the causing factors (genetics and environmental) involved in this devastating disease. We also gave brief overview of the treatment options and animal models of RA. The literature was reviewed using mesh terms in PubMed search "etiology of RA, genetics of RA, environmental factors in RA, Genome Wide Association Studies (GWAS) in RA". The data was thoroughly reviewed and comprehensive information was extracted to help the readers in improving understanding towards the mechanisms, which trigger the outcomes of RA. The more we increase awareness about RA, the better we manage this disease and hence can improve life style and socio-economic status.

Keywords: Rheumatoid Arthritis, etiology, genetic factors, environmental factors.

INTRODUCTION

Rheumatoid Arthritis (RA) is a common, systemic and chronic inflammatory disease characterized by inflammation of synovium of any joint including small joints of hands and feet and large joints of shoulder and knees. The synovitis of joints leads to the destruction of bones and cartilage resulting in the (radiographic) damages (Imboden, 2009). These damages can cause significant disability and even permanent loss of function, due to erosion of bone surface, if left untreated (Silman and Pearson, 2002; Majithia and Geraci, 2007). The etiology of RA is very complex and is yet to be explored properly. It has a wide spectrum of clinical manifestations, variability in disease severity, progression and differences in therapeutic response. These heterogeneous phenotypes of RA may suggest that variety of factors can contribute in the development of this complex trait, which includes environmental, hormonal and genetic factors.

The concordance rate of RA is about 3 to 4% in di-zygotic twins, 12 to 15%in monozygotic twins, 2 to 4% in non-twin siblings and is less than 1%in general population. Thus, RA has a strong genetic basis with estimated heritability ranging from 50% to 60% (Silman *et al*., 1993; Seldin *et al*., 1999; Mac Gregor *et al*., 2000; Bax *et al*., 2011). In other words, siblings of the affected individuals are at high risk to RA than general population (Wandstrat and Wakeland, 2001). Since 2000, large number of studies have been conducted to understand the genetic susceptibility to RA among which Genome Wide Association Studies (GWAS) is considered to be a powerful tool to discover novel variants and loci especially in common complex diseases. Recent GWAS and meta-analysis of GWAS have reported more than 40 RA susceptibility loci/genes in different population. About 30% of genetic susceptibility of RA is contributed by Human Leukocyte Antigen (HLA) region while the non-HLA loci/gene account for about 5% susceptibility (Raychaudhuri *et al*., 2008; Kochi *et al*., 2010; Stahl *et al*., 2010; Craddock *et al*., 2010; deVries, 2011; Okada *et al*., 2012; Eyre *et al*., 2012; Jalil *et al*., 2013), suggesting the role of rare variants and gene-environment interaction in remaining heritability (Asimit and Zeggini 2011; Zuk *et al*., 2012).

Clinical features and ACR criteria of rheumatoid arthritis

Initially, based on the phenotypic characters and clinical presentations of the patients the American College of Rheumatology (ACR) developed an ACR 1987 criteria for the diagnosis of RA (Arnett *et al*., 1988). These include morning stiffness (lasting for up to an hour),

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arthritis of three or more joints, arthritis of hand joints, rheumatic nodules, symmetrical arthritis, radiographic damages, degree of erosion and serum rheumatoid factor. A person would have RA if he or she satisfies at least four out of these criteria. These criteria are accepted worldwide and are used by both basic researcher for inclusion/exclusion of patients in the studies and by clinicians for diagnosis of the RA patients. The American College of Rheumatology Subcommittee on Rheumatoid Arthritis (ACR-SRA) recommends a baseline laboratory evaluations and clinical tests which include a complete blood cell, Rheumatoid Factor (RF), Anti-cyclic Citrullinated Peptide (ACCP) antibody, Erythrocyte Sedimentation Rate (ESR) or C-reactive Protein (CRP) and radiographic findings of involved joints (Ruddy *et al*., 2005). The onset of initial symptoms can be slow insidious (55-65% of cases) i.e. over weeks or months or an explosive sudden (8-15% of cases) which reaches to peak within few days (Fleming *et al*., 1976). The most commonly involved joints are the wrists, elbow, knee, metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) joints, metatarsophalangeal (MTP) joints and toe PIPs and cervical spine and the lumbosacral spine.

Later on, some limitations were noticed in 1987 ACR criteria. Actually, these classification criteria were developed from the patients with established and long-standing RA (Silman and Symmons, 1995). So, these criteria may not be helpful in early diagnosis and in-time treatment of the disease, which is necessary to avoid erosive bone damage and functional loss (van der Heide *et al*., 1996). However, a major goal of the modern therapies is to prevent the erosive bone damages (Emery and Salmon, 1995), which becomes difficult once the bone is deformed.

Therefore, these flaws in 1987 ACR criteria for RA led to the development of new classification criteria. For this purpose a joint collaborative project was held between ACR and European League Against Rheumatism (EULAR) resulted in the development of new purely data-driven classification criteria for RA (Funovits *et al*., 2010). These 2010 ACR/EULAR criteria do consider some markers for inflammatory arthritis from 1987 ACR criteria like swelling and tenderness of small joints, serology and acute phase reactants. Similarly, 1987 ACR criteria excluded some traditional markers such as symmetry and morning stiffness to be less important during diagnosis phase. The major goal of this joint project was to make an early diagnosis possible and prevent erosion of the bones. It was decided that diagnosis should not be based on the sever outcomes of the definite disease and effective and timely therapy should be provided to all patients. Thus, typical features of established RA such as rheumatic nodules formation and erosion of the bone surface or deformities were excluded from the classification criteria during diagnosis.

The irreversible erosive damages of the bones in the chronic RA are due to the intra-articular inflammation. Although, RA is clinically very heterogeneous disease and severity of RA varies among the patients ranging from mild and self-limiting to an active and severely progressive disease (Lee and Weinblatt, 2001). However, level of inflammatory markers (C-reactive protein, CRP and Erythrocyte Sedimentation Rate, ESR) and serological markers (Rheumatoid Factor, RF and anti citrullinated protein antibodies, ACCP) are widely used both in diagnosis and as indicators of severity of RA (van Gaalen *et al*., 2004; Lindqvist *et al*., 2005) but still are not very specific to RA (Smolen *et al*., 2008). The genetic predisposition to the severity of joint destruction in RA patients was recently investigated and it was reported that 58% of variation in the progression rate of bone erosion is due to genetics of the patients (P=0.003) (Knevel *et al*., 2012). Thus differences in the genetic makeup of severe disease course than milder disease can predict about the patients with high risk for future bone damages. Several studies have tested correlation of the disease severity with candidate locus/allele. These studies explain the need for genetic predictors of the disease severity, which could be useful biomarkers in future (Marinou *et al*., 2010; Stahl and Raychaudhuri, 2012).

Epidemiology of rheumatoid arthritis

Rheumatoid arthritis is a multifactorial heterogeneous disease with different incidence rate and prevalence across different populations. The variations in epidemiology of RA can be due to exposure of different population to specific environmental triggers and can also be associated with the study design such as statistical methods used, case-ascertainment criteria, and number of cases enrolled etc.

The incidence of RA varies from population to population. Different studies have shown incidence of RA in certain populations like 9/100,000 in France; 25/100,000 in Norfolk, UK; 31/100,000 in Massachusetts, USA; 33/100,000 in Rochesher, Minnesota USA from 1985-1994; 34/100,000 in Finnish population; 35/100,000 from 1995-2001 in southern part of Denmark and 36/100,000 in Finland (Chan *et al*., 1993; Guillemin *et al*., 1994; Symmons *et al*., 1994; Kaipiainen-Seppanen and Aho, 2000; Doran *et al*., 2002; Savolainen *et al*., 2003; Alamanos *et al*., 2006; Pedersen *et al*., 2009). It was summarized that incidence of RA (case per 100,000 population) as 29 (24-36) in Northern Europe, 16.5 (9-24) in Sothern Europe and 38 (31-45) in North America (Tobón *et al*., 2010).

Like incidence, prevalence of RA also vary according to geographical area and population (Costenbader *et al*., 2008) and is more prevalent in developed countries than developing ones. Several studies conducted on European and European derived populations have reported that prevalence of RA in North America and Northern Europe

is 0.5% to 1.1%; while in Southern Europe it is 0.3% to 0.7%. Furthermore, significantly lower prevalence of 0.1% to 0.5% has been reported in studies conducted in developing countries and 0.2% to 0.3% in Asian population (Saraux *et al*., 1999; Guillemin *et al*., 2001; Silman and Pearson, 2002; Symmons *et al*., 2002; Carmona *et al*., 2002; Teng *et al*., 2011). The lower prevalence of RA in developing countries may be due to the fact that limited clinical diagnostic procedures ignore most of the patients from clinical assessment.

Studying the distribution of certain disease or diseases and the factors responsible in causing these conditions across multiple regions and populations of the world is called geo-epidemiology. Thegeo-epidemiologymight uncover population specific or ethnogenetic risk factors; like observing particular HLA types or other genes associated with RA in certain population. These observations can be compared with neighboring population and communities which can help in identifying the environmental triggers involved in pathogenesis of RA in particular region (Shapira *et al*., 2010).

The trends in incidence and prevalence of RA has not been well investigated. However, some studies have suggested declines in both incidence and prevalence of RA after 1960 (Doran*et al*., 2002; Kaipiainen-Seppanen and Kautiainen, 2006). This hypothesis was justified by the observation that three factors may be involved in this decline. Firstly, it was suggested to be due to variations in methodologies and case-enrollment criteria. Several studies conducted before 1987 ACR criteria of RA can be biased; because of difficulties in differentiating RA from other polyarthritis. Secondly, ethnic and geographic factors are also equally important; like higher incidence of RA was reported in Pima Indians than other population of American and Europe. Finally, a true decline in incidence of RA has been noticed specifically in women using oral contraceptives (Doran *et al*., 2002; Savolainen *et al*., 2003; Doran *et al*., 2004).

Molecular genetics of rheumatoid arthritis

Rheumatoid arthritis is the most common multifactorial disease, which depends on the contribution of various factors including genetic, environmental and hormonal factors for the onset and development of clinical manifestation. The genetic heritability and familial susceptibility of RA can be evident from familial clustering. The higher concordance rate of RA in siblings than general population suggested a strong genetic basis with estimated heritability ranging from 50% to 60%. (Silman *et al*., 1993; MacGregor *et al*., 2000; Seldin *et al*., 1999; Bax *et al*., 2011). A wide range of genetic studies including candidate gene approaches, GWAS and their meta analysis have identified more than 35 genetic susceptibility loci/genes in different ethnic groups. We will discuss general function of some important genes below:

Human leukocyte antigen (HLA)

Major histo-compatibility complex (MHC)(HLA region) is responsible for about 30% (One-third) of the genetic susceptibility of RA with most important *HLA-DRB1* gene (MacGregor *et al*., 2000) having DRB1*04:01 and DRB1*04:04major risk alleles in Caucasians and DRB1*04:05in East Asian populations (Newton *et al*., 2004). The human MHC genomic region has been divided in to three main classes which are MHC I, II and III. MHC class I consists of three genes, *HLA-A, -B, -C* and MHC class II consists of-*DR, -DQ, -DP*. The Class I antigens such as HLA-A, -B, -C consist of a β2-micro globulin and a highly polymorphic heavy chain. Similarly, Class II antigens (HLA-DR, -DQ, -DP) have an alpha chain and a highly polymorphic beta chains that is encoded by the *HLA-DRB1, -DQB1, -DPB1* genes.

HLA-DR antigen of MHC class II has shared epitope (SE) on beta chain (a five amino acids at positions 70-74), which has significant association with susceptibility and severity of RA (Gregersen *et al*., 1987; du Montcel *et al*., 2005; Gorman *et al*., 2004). These residues forma helical domain and may likely to influence antigen presentation by making an antigen binding site (Newton *et al*., 2004). The MHC class III is present between MHC class I and II. It has been determined that MHC Class III region also contains *AIF1* and *NFKBIL1,* which are important RA-susceptible genes (Mu *et al*., 1999; Mattey *et al*., 1999; Ota *et al*., 2001; Ando *et al*., 2003; Tamiya *et al*., 2005; Lin *et al*., 2006; Yan *et al*., 2007; Harney *et al*., 2008; Yang *et al*., 2009). Six HLA loci which are in strong linkage disequilibrium (LD) can be ordered as *HLA-A, -C, -B, -DRB1, -DQB1* and *-DPB1* (from telomere to centromere) (Geraghty *et al*., 1999). A recent study on Asian population have shown that *HLA-DRB1*SE alleles (DRB1*04:05) have strong interaction with smoking and increasing the risk of RA in anti citrullinated protein antibodies (ACPA) positive individuals (Too *et al*., 2012).

Out of all these HLA loci only *HLA-DRB1* and the SE have been well explored with respect to RA. However, other HLA genes because of their highly polymorphic nature needed to be examined for their possible role in progression or protection of this devastating disease.

Peptidylarginine deiminase 4 (PADI4)

PADI gene is a family of gene present on chromosome 1(1p36). This gene coding for enzyme peptidylarginine deiminase 4 (PADI4), which converts arginine to citrulline with in peptides through posttranslational modification mechanism. Involvement of PADIs in the pathophysiology of RA was suggested after confirming that synovial fluid is the site for citrullination of auto antigenic peptides (Kinloch *et al*., 2008). This idea was further supported by Chang *et al*., (2009) through measuring the expression level of PADI4 in the synovial of RA patients. It was further investigated that autoantibodies to cyclic citrullinated peptides (ACCP) are

highly specific to RA (patients with ACCP have more swollen joints and radiological destructions as compare to those with no ACCP) and can predict about the onset of the disease even couple of years before the symptoms appear (Rantapaa-Dahlqvist *et al*., 2003; Vossenaar *et al*., 2003). Association of *PADI4* with RA has been controversial and inconsistent between Asian and European population base studies, whereas some groups have shown positive association of this gene with RA while others have not. Likewise, studies conducted on Japanese population and Korean population from Asia have shown that *PADI4* is involved in the outcome of RA, while not in Chinese Han population (Suzuki *et al*., 2003; Ikari *et al*., 2005; Kang *et al*., 2006; Chen *et al*., 2011). Similarly, North American population have reported positive association of this gene with RA (Plenge *et al*., 2005). Another large population-based study conducted on Caucasians for the first time reported strong association of PADI4 with RA (Eyre *et al*., 2011). Similarly, Stahl *et al*., 2010 showed modest effect of PADI4 through a GWAS-meta analysis in Europeans populations. However, a recent meta analysis of 27 studies suggested that PADI4 is a significant risk factor of RA in Asian Population than Europeans and Europeans derived populations (Hou *et al*., 2013).

TNF-receptor associated factor 1-complement component (TRAF1-C5)

The *TRAF1-C5* consist of two important parts, one TNF-receptor associated factor 1 (TRAF1) and second complement component 5 (C5). These two are important immune system related genes, which are involved in perpetuation of inflammation. Through GWAS of 1522 RA cases and 1850 controls of European descent *TNFR1-C5* was mapped on 9q33-34 as a novel genetic risk in ACCP positive RA patients (Plenge *et al*., 2007). Association of *TNFR1-C5* gene with RA was replicated and validated by different studies in Caucasian populations (Kurreeman *et al*., 2007; Barton *et al*., 2008; Chang *et al*., 2008; Zervou *et al*., 2008; Kurreeman *et al*., 2008). Nishimoto *et al*., (2012), through a multicenter-case control study reported positive association of *TRAF1* in Japanese RA patients. These results from different groups confirmed the susceptibility of *TRAF1-C5* to RA.

TRAF1 gene encodes intracellular protein, which mediates signal transduction. During this process TRAF1 binds to TNF receptors (mainly 1, 2) and CD40 and regulate cytokine signaling pathways including TNFα by binding several protein kinase and adaptor protein (Lee and Choi, 2007). In addition to its direct role in TNFα signaling *TRAF1*also mediate activation and proliferation of T cells (Sabbagh *et al*., 2006). Through mouse model experiments it has been shown that *TRAF1*konockout mice had more T-cell proliferation and activation in response to TNF, suggesting the role of *TRAF1* as a negative regulator of this signaling pathway (Bradley and Pober, 2001). Tumor necrosis factor (TNF) is an important cytokine with well established role in the patho-physiology and pathogenesis of RA (Firestein, 2003). For this reason, RA is treated with TNF antagonists from decades (Elliott *et al*., 1994; Weinblatt *et al*., 1999). The primary function of complement component to protect against microorganisms, while deregulated activity can play role in inflammation as well. Its pivotal role in the pathogenesis of RA has been recorded. Depletion of complement component from synovial fluid occur during inflammation in RA has been noticed, because cleavage of C5 generates pro-inflammatory anaphylatoxin C5a and C5b; generating membrane-attack complex later (Zvaifler, 1973; Cooke *et al*., 1975; Bao and Quigg, 2007). Furthermore, studies have shown that C5 deficient mice are also resistant to inflammatory arthritis and targeting C5 by antibodies prevent the onset of RA and reduce the clinical severity (Wang *et al*., 1995; Wang *et al*., 2000; Ji *et al*., 2002), which also explains the role of C5 in RA onset and pathogenesis. These observations reveal that two important immune mediator genes*C5* and *TRAF1* found adjacent to each other on chromosome 9 have combine affects in pathogenesis of RA.

Protein tyrosin phosphatase Non-recptor 22 (PTPN22)

Protein tyrosin phosphatase non-recptor 22 (*PTPN22)* is one of the strongest risk factors of autoimmunity outside major histo-compatibility complex (MHC), located on chromosome 1p13.3-13.1 and is ranked second in term of single-gene contribution to the etiology of RA in Caucasian population (Todd *et al*., 2007; Fiorillo *et al*., 2010). *PTPN22* encodes lymphoid tyrosin phosphatase (Lyp), which form a complex with C-terminal Src tyrosine kinase (Lyp-Csk) and acts as a negative regulator of T-cell receptor (TCR) signaling (Gjörloff-Wingren *et al*., 1999; Clouteir and Veillete, 1999; Hill *et al*., 2002; Begovich *et al*., 2004; Vang *et al*., 2005; Todd *et al*., 2007). A well established non-synonymous C1858T single nucleotide polymorphism (rs2476601) which results in Arg620Trp (R620W) has been reported in number of autoimmune diseases including RA, among many populations especially in ACCP positive RA (Begovich*et al*., 2004; Hasegawa *et al*., 2004; Viken *et al*., 2005; Gregersen, 2005; Rieck *et al*., 2007). There are two types of protein tyrosin phophatase; receptor (membrane bounded-RPTP) and non-receptor (cytoplasmic-NRPTP). The Lyp is a ~105-kDa protein with ~300 amino acid N-terminal domain and ~200 amino acid C-terminal domain, includes four putative polyproline motifs from P1-P4. The C and N-terminus are separated by ~300 amino acid domain called interdomain. Interaction between protein-tyrosin phosphatase (PTP) Lyp and protein-tyrosin kinase (PTK) Csk is mediated by P1 motif of N-terminal domain (Fiorillo *et al*., 2010). The Lyp-Csk complex can inhibit T-cell receptor signaling only when they are physically associated with each other (Cloutier and Veillette, 1999). The Lyp physically bound through SH3 domain to Csk (Cohen *et al*., 1999). The single amino acid change

R620W disrupt this complex formation and hence causes suppression of T-cell activation. It has been shown that T-allele of *PTPN22* bind less efficiently to Csk then C-allele; making cell with T-allele hyper-responsive and hence individuals with this allele show autoimmunity (Bottini *et al*., 2004; Begovich *et al*., 2004). Gregersen, (2005) presented a simple scheme of autoimmunity through R620W resulting in gain of enzymatic function as shown in fig.1. This fig. shows that two mechanisms, either one or both are involved in this reaction. According to first mechanism the shift in signaling threshold could cause positive selection of thymocytes (that normally deleted) and potentially auto reactive T-cells appears in the periphery. While, in second mechanism this shift could cause deficiency in the regulation of auto reactive T-cell, making individual susceptible to autoimmunity.

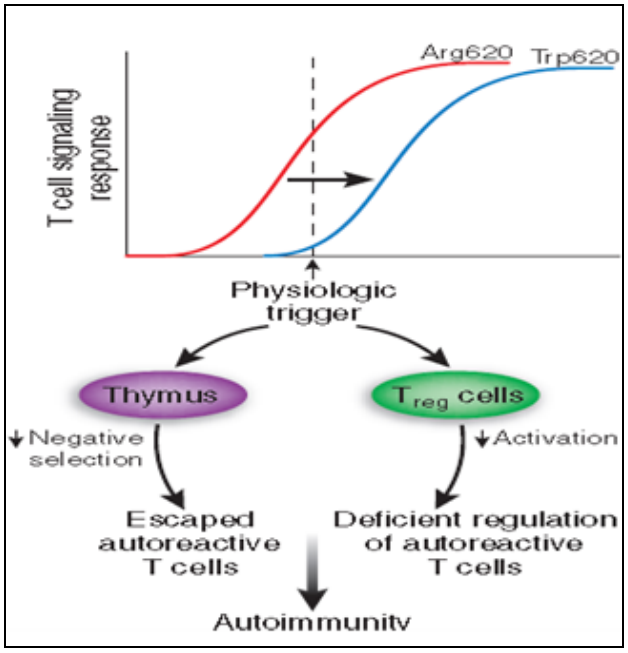

Fig. 1: A general scheme of T-cell signaling and autoimmunity (by Gregersen, 2005).

Signal transducers and activators of transcription 4 (STAT4)

A whole genome-wide SNP linkage scan of 642 Caucasian RA families with Illumina IV SNPs linkage panel containing 5850 SNP markers across the genome, established strong linkage of RA at 2q33 (LOD score 3.52) (Amos *et al*., 2006). Same group of researcher conducted case-control study on both RA and SLE using candidate genes at this region. They tested SNPs in and around 13 candidate genes within 2q33 region and found association of a SNP rs7574865 at *STAT4*gene with both RA (P=2.82E-07; OR=1.32) and SLE (P=1.87E-09; OR=1.55) (Remmers *et al*., 2007). Association of *STAT4*gene with RA has been confirmed in different populations after it was first reported with significant role in RA along with the evidence that antibody status (RF positive or negative; ACCP positive or negative) does not affect susceptibility to RAby *STAT4*/rs7574865 (Lee *et al*., 2007; Martinez *et al*., 2008; Zervou *et al*., 2008; Orozco *et al*., 2008; Kobayashi *et al*., 2008; Barton *et al*., 2008; Palomino-Morales *et al*., 2008). A meta-analysis on T-allele (susceptible allele) of rs7574865 using 15 studies (10 Europeans; 4 Asian; 1 Latin American) containing 16,066 RA patients and 16,509 controls subjects revealed association of RA and *STAT4* (Over all OR=1.271, 95% CI=1.197-1.350, P<0.001). Furthermore, *STAT4* was found significantly associated with RA in both Europeans (OR=1.300, 95% CI=1.195-1.414, P<0.001) and Asian (OR=1.216, 95% CI=1.135-1.303, P<0.001) (Lee *et al*., 2010). Another recent meta-analysis of 40 studies (published before September 2011), confirmed the association of *STAT4* (rs7574865; T-allele) with multiple autoimmune diseases including RA and systemic lupus erythematosus (SLE) (Liang *et al*., 2012).

Signal transducers and activators of transcription (STATs) is a family of proteins including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. Different cytokines, growth factors and hormones are involved in activation of STATs proteins. During activation process these cytokines, growth factors and hormones binds to STATs receptors and phosphory late STAT proteins on either tyrosine or serine residues (Leonard and O'Shea, 1998; Visconti *et al*., 2000).

STAT4 is one of the important members of this family of proteins expressed in peripheral blood monocytes, machrophages and dendritic cells at site of inflammation (Frucht *et al*., 2000) and is involved in regulation of hematopoitic process STAT4 is a latent cytosolic factor which is first phosphorylated and then accumulated in the nucleus after activation by cytokines. *SATA4* is also highly expressed in synovium of RA patients as compare to normal tissue (Walker *et al*., 2006; Walker *et al*., 2007). *STAT4* encodes a transcription factor which transmit signals induced by several key cytokines, including IL-12 and type 1 interferons andIL-23and also play a crucial role in differentiation and proliferation of helper T-cells (Th1 and Th17) (Murphy and Reiner, 2002; Watford *et al*., 2004; Mathur *et al*., 2007). STAT4-dependent signaling byIL-12 receptors results in differentiation of CD4+ T-cells in to interferon-γ producing Th1 cells linage and plays a critical role in the development of Th1-type T-cell response. After activation*STAT4* stimulates transcription of interferon-γ, which is akey indicator of T-cell differentiation into type 1 helper T (Th1) cells. While, signaling by IL-23 receptors helps in developments of IL-17- secreting helper T-cell (Th17), which play critical role in autoimmune diseases such as RA (Morinobu *et al*., 2002; Nishikomori *et al*., 2002; Watford *et al*., 2004; Skapenko *et al*., 2005; Bettell *et al*., 2007; Steinman, 2007). Furthermore, animal model studies have provided many evidences about the role of *STAT4* in pathogenesis of RA and hence suggested it as a possible therapeutic target.

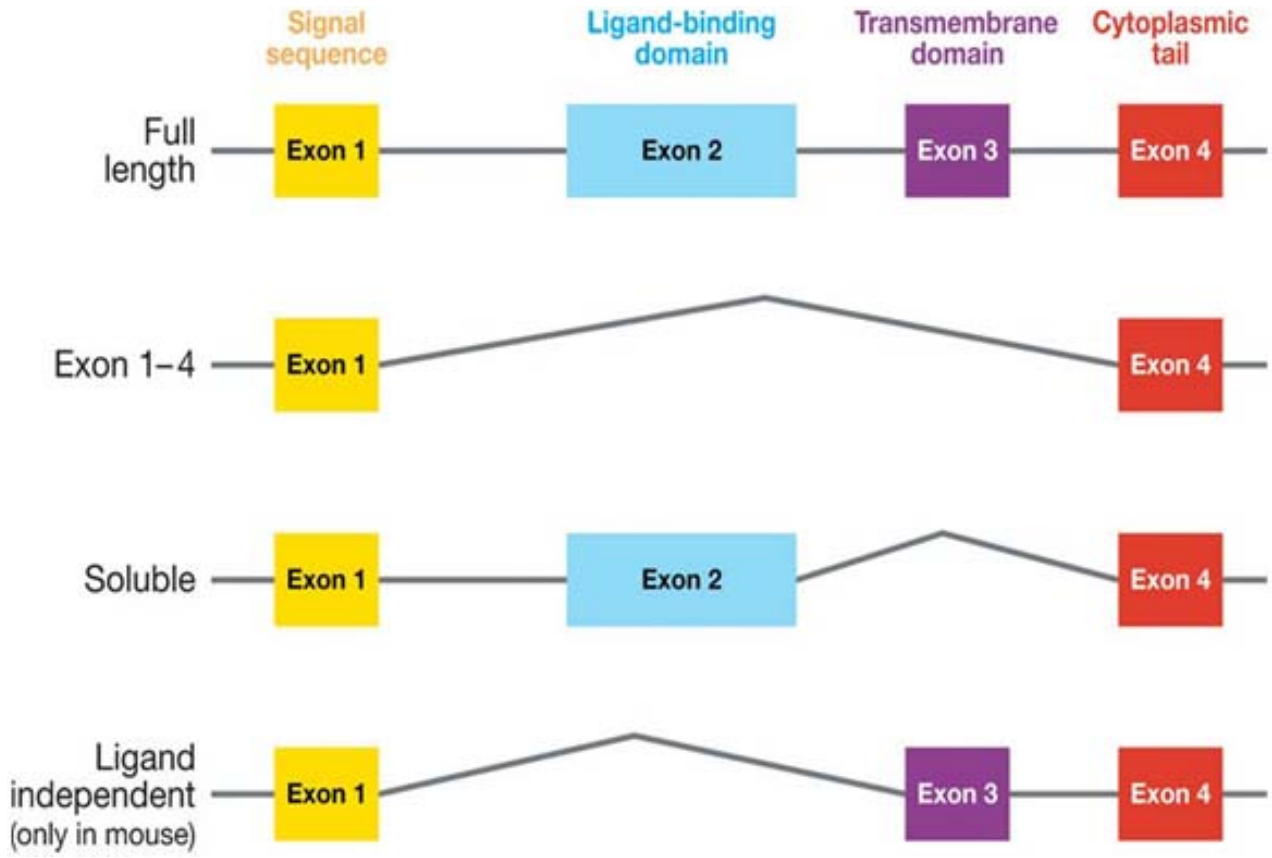

Fig. 2: The *CTLA4* gene splice variants in human and mouse (adapted from Wendy *et al*., 2006)

Kinesin family member 5A (KIF5A)

KIF5A gene encodes a member of kinesin family of protein called kinesin heavy chain is form 5A. This gene was first mapped to 12q13 in Hereditary Spastic Paraplegia (HSP); which is group of inherited disease with progressive stiffness and contraction in the lower limbs because of the dysfunction of the nerves (Hamlin *et al*., 1998; Depienne *et al*., 2007). KIF5A is a motor protein, which facilitates intracellular movement of organelles and microtubules (Wang *et al*., 2007). Some other complications like contracts, ataxia, epilepsy, peripheral neuropathy, and deafness have also been noticed in HSP patients. Studies have shown that HSP patients have defective transport system including protein transport and transportation of other substance in the cell. Recently, it was found that long nerves which transport materials through long distance are the main targets in HSP (DeMatteis and Luini, 2011). Recent genome wide association studies and their meta analysis have confirmed the association of *KIF5A* gene to RA (Plant *et al*., 2010; Stahl *et al*., 2010; Bowes *et al*., 2012).

Cytotoxic T-lymphocyte antigen-4 (CTLA4)

CTLA4,a 6173 bp (6.17kb) long gene at 2q13, down-regulate T-cell activation and hence protects from T-cell autoimmunity. It encodes a transmembrane 223 amino acid long glycoprotein belongs to immunoglobulin superfamily, having 35 amino acid signal peptides. The extra cellular part of this protein molecule is encoded by exon 1 and 2, containing the B7-1(CD80) and B7-2 (CD 86) ligand binding sites on exon 2 and leader sequences on exon 1 (Brunet *et al*., 1987; Dariavach *et al*., 1988; Lindsten *et al*., 1993; Linsley *et al*., 1995; Metzler *et al*., 1997; Ling *et al*., 1999;Ostrov*et al*., 2000). The transmembrane region of *CTLA4* molecule is encoded by exon 3 and the 36 amino acid cytoplasmic portion (lacking any enzymatic activity) is encoded by exon 4 (Dariavach *et al*., 1988; Ling *et al*., 1999; Baroja *et al*., 2000). It contains proline rich region at position 169, lysin rich motifs and two tyrosine residues at position 165 and 182, which have shown to be involved in modulating its function by variety of signaling molecules (Baroja *et al*., 2000; Baroja *et al*., 2002; Lee *et al*., 1998; Schneider *et al*., 1995; Shiratori *et al*., 1997). Splicing variants identified in human and mice are shown in fig. 2. Human have one full-length mRNA having all four exons, a second transcript without exon 3 and is called soluble *CTLA4* (sCTLA4), and a third transcript containing only exon 1 and exon 4 (Brunet *et al*., 1987; Dariavach *et al*., 1988; Ling *et al*., 1999;Ueda *et al*., 2003;Vijayakrishnan *et al*., 2004; Magistrelli *et al*., 1999; Oaks *et al*., 2000); while mouse have an additional splicing variant which

does not contain exon 3 and 4 and is called ligand-independent *CTLA4* (liCTLA4) (Ueda *et al*., 2003).

Since, the co-stimulatory molecule *CD28* have structural similarities and sequence homology with CTLA4; thus compete for the same ligand (B7-1 and B7-2) (Linsley *et al*., 1990; Freeman *et al*., 1993a; 1993b). However, *CTLA4* have greater affinity for ligand then *CD28* (Linsley *et al*., 1991; Linsley *et al*., 1994).

Although, the *CTLA4* gene is primarily expressed in T-cells; however, expression in other cells like $CD4^+$, CD25, and regulating T-cells have also been determined (Harper *et al*., 1991; Perkins *et al*., 1996; Takahashi *et al*., 2004). Variety of other cells like B-cells, monocytes, granulocytes, CD34+, placental fibroblast and mouse embryonic cell do express this gene for unexplained regulatory function (Pioli *et al*., 2000; Pistillo *et al*., 2003; Wang *et al*., 2002). Several authors have demonstrated the association of *CTLA4* gene with number of autoimmune diseases. Some single nucleotide polymorphisms (SNP) like -1722T/C, -1661A/G, -318C/T and +49A/G are well studied variants. The initial three variants (-1722T/C, -1661A/G, -318C/T) are found in regulatory/promoter region and are thought to be associated with higher promoter activity and hence increase *CTLA4* expression (Wang *et al*., 2002). The transition at +49A/G causes threonine to alanine substitution in the leader peptide of exon 1 (Nistico *et al*., 1996; Deichmann *et al*., 1996; Donner *et al*., 1997; Kristiansen *et al*., 2000; Wang *et al*., 2002) which may affects the inhibitory function of *CTLA4* and may also influence the endocytosis (Wang *et al*., 2001). A recent ethnicity-specific meta-analysis was performed on Caucasian and Asian populations, including 5,752 RA patients and 5,508 controls from 19 studies (9 Caucasian, 8 Asian, 1 Mexican, and 1 Tunisian population) demonstrated that +49A/G polymorphism confer susceptibility to RA in Asian population but not in Caucasians (Lee *et al*., 2012).

Other non-HLA RA susceptibility genes/loci include Solute Carrier Family 22A4, Complement component 5-TNF receptor-associated factor 1(*C5-TRAF1*), Macrophage migration inhibitory factor (*MIF*), Runt-Related Transcription Factor (*RUNX1*), Tumor Necrosis Factor Alpha Receptor 2 (*TNFR2*), Cluster of Differentiation 244 (*CD244*), Corticotropin-releasing hormone (*CRH*) and Angiotensin-Converting Enzyme (*ACE*). These genes either involved in T cell proliferation or cytokines regulatory pathways and hence add to auto-reactivity and autoimmunity in the human body.

Environmental risk factors of RA

The differences in prevalence of RA across different regions and populations of the world have focused the scientists on environmental factors and gene-environment interactions in addition to genetic factors in pathogenesis of RA. Several important environmental determinants involved in the development and severity of RA have been extensively studied. We are discussing them in brief below.

Smoking

In addition to association of cigarette smoking with many diseases like several malignancies, cardiovascular diseases and pulmonary diseases; smoking is also suggested to be the strongest environmental risk factor associated with the development of RA. Vessey *et al*., (1987) reported for the first time that smoking is an important risk factors in RA pathogenesis. After this initial report interaction of smoking and RA was studied and replicated in various populations and higher risk of development of RA was found in heavy smokers as compare to non-smokers or who smoked less (Karlson *et al*., 1993; Heliovaara *et al*., 1993; Symmons *et al*., 1997; Uhlig *et al*., 1999; Criswell *et al*., 2002; Padyukov *et al*., 2004; Karlson *et al*., 2010). A large study conducted on Caucasian women smokers, the Iowa Women's Health Study (IWHS) reported that risk of RA was 18% which mean one in six of new RA cases can be due to smoking and can be prevented if smoking is eliminated (Criswell *et al*., 2002). Studies have also shown that risk of RA further increases with increasing duration and amount of cigarette taken (Stolt*et al*., 2003). A similar linear relation between smoking and risk of RA has been observed in another large prospective, Nurse's Health Study (NHS). According to this study heaviest smokers with more than 40 packs-years have two-fold higher risk of RA as compare to non-smoker controls (Costenbader *et al*., 2006).

Furthermore, interaction of smoking and genetic factors was examined and their co-relation with RA risk was observed. It was suggested that *HLA-DRB1*-shared epitope (SE) is strongly associated with increased risk due to smoking and is more evident in seropositive RA. Smokers having two copies of *HLA-DRB1*-shared epitope (SE) have higher risk of RA than those who never smoke and having no SE allele (Klareskog *et al*., 2006; Karlson *et al*., 2010). A recent study of Bang *et al*., (2010) reported that SE-alleles and smoking are associated with both anti-CCP positive and anti-CCP negative RA. Smokers with two copies of the SE allele have higher risk of both ACCP-positive and ACCP–negative RA, 36.11-fold and12.29-fold, respectively, as compared to nonsmokers not carrying SE alleles.

Klareskog *et al*. (2006) obtained bronchoalveolar lavage specimens from both smokers and nonsmokers and reported citrullinated proteins in smokers but not non-smokers. Later, it was found that smoking upregulate expression of peptidylarginine deiminase (PAD) in the lungs and was concluded that long-term smoking and possibly with interaction of other environmental triggers may convert arginine of peptide antigens to citrulline in the lungs (Makrygiannakis *et al*., 2008).

In addition to cigarette smoking, the role of other environmental pollutants in risk of RA have been explored. The "distance-to-road" was examined in a prospective study of NHS cohort. It was noted that women living less than 50 meters from road had 30% increased risk of RA, than those residing at a distance from the road (Hart *et al*., 2009). Similarly, exposure to silica and silica dust from stone works, mining, glass or ceramics manufacturing, stone drilling and rock crushing might increase RA risk. In a Swedish case-control study of silica exposed individuals had ACPA-positive RA (OR, 1.7; 95% CI, 1.1-2.5) as compared to unexposed individuals. However, no ACPA-negative RA has been reported in silica-exposed individuals (Stolt *et al*., 2010).

Alcohol

The association of alcohol consumption and the risk of RA has been studied and protective effect of moderate alcohol intake on development of RA has been suggested. An inverse association between consumption of alcohol and risk of rheumatoid arthritis has been observed (Hazes *et al*., 1990; Maxwell *et al*., 2010). A Danish study reported lower risk of developing ACPA-positive RA in those who consume alcohol (Pedersen *et al*., 2006). Two independent case-control populations; a Danish CACORA (case-control study on Rheumatoid Arthritis) and a Swedish EIRA (epidemiological investigation of rheumatoid arthritis) demonstrated dose-dependent effect of alcohol and reduction of RA risk. They found higher rate of alcohol consumption in control individuals versus patients. Individuals with highest alcohol consumption (≥ drinks or 80g ethanol per week) was found to have 40% to 50% decreased risk of RA than those with lower to no consumption (<0.5g ethanol per week) (Kallberg *et al*., 2009). A recent study conducted by Di-Giuseppe *et al*., (2012) observed 37% decrease in risk of RA among heavy drinker women (>4 glasses of alcohol (1 glass = 15g of ethanol) per week compared with women who drank <15g per week or who never drank alcohol (RR, 0.63; 95% CI, 0.42 to 0.96; P=0.04). These observations suggested that moderate to low level consumption of alcohol is associated with reduced risk of RA. Further investigations on the biological mechanisms and pathways have shown that alcohol down regulate immune response inanimals and humans (Mandrekar *et al*., 2004; Verma *et al*., 2008; Fan *et al*., 2011) and decrease the production of pro-inflammatory cytokines (Waldschmidt *et al*., 2006). Furthermore, ethanol candelays the onset and may stops the progression of RA in mice by interacting with innate immunity (Jonsson *et al*., 2007).

Dietary factors

Dietary factors play a vital role in the onset and development of inflammatory processes. In examining the relationship between diet and RA, researchers have found some important factors like antioxidants, micronutrients and some vitamins and proteins. Antioxidant are present in serum where it reduces inflammatory products and cease inflammation. Antioxidant have important protective role against oxygen species, which can cause tissues damage. Beside this antioxidant suppresses the expression of certain cytokines and collagenase induced by TNF-α (Sato *et al*., 1996; Li and Micheletti, 2011). Lower concentration of serum-circulation antioxidant including vitamin C, vitamin E, β-carotene and zinc have been shown in RA patients when were compared with normal control individuals (Aaseth *et al*., 1998; Li and Micheletti, 2011).

The Mediterranean diet of southern European part (having lower prevalence and incidence of RA) which is rich in fruits, vegetables, cereals, beans, nuts, seeds, fish, olive oil and low in red meat suggests the importance of plant foods (Rayman and Callaghan, 2006). Two controlled Mediterranean-diet intervention trials were conducted on RA patients and suggested significant results in improvement of morning stiffness and reducing pain (Skoldstam *et al*., 2003; McKellar *et al*., 2007). Increased red meat and protein in diet have been associated with increased risk of RA (Pattison *et al*., 2004). The vegetarian diet rich in fruits and vegetables and lower fats altering amount of antioxidants, arachidonic acid and fatty acid and hence reducing inflammation in response (Adam *et al*., 2003; Smedslund *et al*., 2010).

Vitamin D is an important hormone in development of bones and also exerts anti-inflammatory properties by regulating cells in innate and adaptive immune system through vitamin D receptors (VDR) (Mathieu *et al*., 2001). Insufficiency of vitamin D has been observed in RA patients (Kerr *et al*., 2010) and furthermore vitamin D can increase disease severity in patients with polyarticular inflammatory arthritis (Patel *et al*., 2007).

Socio-economic status

The social class and socio-economic position are collectively called socio-economic status (SES). The term SES has prominent impacts in many medical conditions. Low SES is associated with higher psychiatric diseases, depression and higher mortality rate (Lorant *et al*., 2003; Stringhini *et al*., 2010). Low SES is more prone to stress exposure and weaker social support. Different criteria are used as measure of SES like occupation is used in Europe (Stansfeld *et al*., 1998; Mackenbach *et al*., 1997; Stringhini *et al*., 2010), education and income is used in USA (Mitchell *et al*., 1988; Hawley and Wolfe, 1988; Criswell and Katz, 1994). Although, a single measure is insufficient to give complete picture of SES of certain population; however still give valuable data for understanding. Researchers have linked SES with depression and RA (Fitzpatrick *et al*., 1991; Berkanovic *et al*., 1996). Bengtsson *et al*. (2005) also reported inverse association between SES (measured in terms of education and occupation class) and risk of RA. A recent report

published by US national survey suggested that SES including low education and low income are associated with poor mental health and arthritis (Furner *et al*., 2011).

Other environmental risk factors

In addition to the above-mentioned factors, development and progression of RA has been associated with many other factors with minor or major affect. These miscellaneous factors include high birth weight, breast feeding, oral contraceptive, sex hormonal factors, infectious agents (including bacteria, viruses, mycoplasma), complex gene-environment interactions, gender and age and other pollutants. These factors have either independently associated with RA or show combined affects by activation of other triggers, enhance risk of RA.

Treatment options of rheumatoid arthritis

Currently, variety of treatment options and drugs are available for RA which are used to reduce inflammation of affected joints and hence the disease activity can be monitored if properly administered. These drugs are prescribed as a combination therapy according to the patient age, activity and course of the disease, and response of the patient to the drug.

Treatment of RA patient is started with analgesics like acetaminophen and aspirin to reduce inflammation and pain of the affected areas and to give instant relief to the patients (Cuzzocrea, 2011). Another group of drugs called nonsteroidal anti-inflammatory drugs (NSAIDs) is also used an effective therapy for RA. The major NSAIDs currently in use are nabumetone, diclofenac, ibuprofen, piroxicam, naproxen, oxaprozin, phenylbutazone, sulindac, tolmetin,diflunisal, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid and meloxicam. These drugs can reduce inflammation and pain but do nothing with the course and progression of disease (McCabe *et al*., 1998; Cuzzocrea, 2011). The progression of disease and damage of the affected joints can be reduced by treating patients with another group of drugs called disease-modifying antirheumatic drugs (DMARDs which include methotrexate (MTX), sulfasalazine (SSZ), leflunomide, Auranofin (oral gold), hydroxychloroquine (HCQ) and gold salts (injectable). These drugs beside reducing progression also decrease pain and swelling in the affected joints, thus should be taken in the earlier stages of the disease rather than later (Arndt *et al*., 2003). Sever inflammation and activity of life threatening disease is also suppressed with corticosteroids/glycocorticosteroids including methylprednisolone, prednisone and inject able corticosteroids. Steroids are mostly prescribed in combination with DMARDs specially MTX and is considered to be the first-line treatment if given in suitable dose (Tłustochowicz, 2006).

A new and effective class of drugs called biologics have significantly improved treatment of RA. Some important frequently used biologics are anti-TNF compounds (adalimumab, etanercept, infliximab, certolizumab, golimumab can suppress inflammation and hence damage of affected joints), IL-1 inhibitor (anakinra is used in cases who do not respond to DMARDs), B-cell-depleting agent (aituximab is given to patients who do not respond to TNF inhibitors), T-cell co-stimulation antagonist (abatacept) and IL-6 antagonist (tocilizumab). The patients who do not respond to DMARDs or have persistent and progressive disease course are treated with a standard combination therapy of biologics and MTX (Lai and Chen, 2008; Cuzzocrea, 2011).

Animal models and rheumatoid arthritis

Large number of rat and mouse models which mimic different characteristics of RA are available. These experimental animal models can be used for evaluation and understanding the pathogenesis and molecular mechanisms implicating in the RA patients. These models are also used for testing new therapeutic options and drugs before going in human trials.

Collagen-induced arthritis (CIA) is a commonly used experimental mouse model for studying pathological mechanisms and for therapeutic testing of newly developanti-inflammatory drugs against RA. In this method college type II (CII), a collagen in the cartilage, is used to induce CIA. A 200µl emulsion of CII and Freund's adjuvant is injected intradermally and followed by a booster dose of 100µlon the other side of the tail. Development of CIA is started in susceptible strains (H-2q or H-2r) within two to three weeks after booster dose (Trentham *et al*., 1977; Courtenay *et al*., 1980; Wooley *et al*., 1981; Jirholt *et al*., 2001; Zhang *et al*., 2008; Seeuws *et al*., 2010).

Proteoglycan-induced arthritis (PGIA) is induced by proteoglycans isolated form cartilage of osteoarthritis patients. The mice are immunized with 100µg of proteoglycan. Emulsion of PG and an adjuvant (dimethyldioctadecylammonium bromide) is prepared in phosphate-buffered saline (pH 7.4). The severity of disease can be determined from swelling and redness in the paws both front and hind, in susceptible strains (C57BL/6J, BALB/c). The female mice of susceptible strains are more prone to develop RA than males (Glant *et al*., 1987; Glant *et al*., 2001; Glant *et al*., 2011).

CIA and PGIA are the most commonly used experimental models. Studies have identified antibodies to both CII and PG, which confirm them as the most relevant animal models of human RA. Furthermore, CIA and PGIA depend on B and T cells and are associated with large number of MHC and non-MHC genes/loci making them polygenic-disease models (Glant *et al*., 1980; Cook *et al*.,

1994; Svensson *et al*., 1998; Corthay *et al*., 1999; Adarichev *et al*., 2003; O'Neill *et al*., 2005).

Large number of different other experimental animal models of RA are currently available which include Avridine-induced arthritis (AIA), Oil-induced arthritis (OIA), Streptococcal cell wall-induced arthritis, Genetically manipulated mouse strains, Pristane-induced arthritis (PIA) and Adjuvant-induced arthritis (AIA) (Cuzzocrea, 2011; Villa-Forte and Mandell, 2011; Adipue *et al*., 2011; Hu *et al*., 2013).

CONCLUSION

This literature survey presents up to-date etiological findings related to RA, which can be helpful in disease cure and management. It would also be beneficial for clinicians, paramedics, RA patients and general public. Increase in public health awareness about RA etiology would ultimately decrease the prevalence rate of RA and thus may improve socio-economic status.

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